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Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 883–890
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

An HPLC chromatographic reactor approach for investigating the hydrolytic stability of a pharmaceutical compound

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Received 9 December 2005; received in revised form 30 January 2006; accepted 2 February 2006
Available online 9 March 2006

Abstract

The solution-phase hydrolysis kinetics of the Aprepitant (Emend™) prodrug, Fosaprepitant Dimeglumine, were investigated using an HPLC chromatographic reactor approach. The term 'chromatographic reactor' refers to the use of an analytical-scale column as both a flow-through reactor and, simultaneously, as separation medium for the reactant(s) and product(s). Recently, we reported a novel mathematical treatment for the kinetic data obtained from chromatographic reactors, which we believe is superior to other treatments in terms of its accuracy, robustness and ease of implementation. In this work, we demonstrate that our treatment may be applied equally well to HPLC reactors, as previously we studied only GC reactors. It is found that the hydrolysis of Fosaprepitant Dimeglumine (FD) has an apparent activation energy of 107 kJ/mol when the reaction is investigated on-column, using the gradient elution conditions of the validated HPLC impurity profile method for this compound. For comparison, the activation energy determined for the same reaction occurring in a quiescent solution consisting of a fixed ratio of acetonitrile–0.1% v/v aqueous H_3PO_4 (50:50, v/v) is 91 kJ/mol, calculated using direct application of the Arrhenius equation. The data presented show that, when used as a screening tool, chromatographic reactors may be feasible for use in the pharmaceutical industry to quickly gauge the relative stabilities of various compounds with similar degradation pathways.

Keywords: On-column reaction; Degradation kinetics; HPLC; Chromatographic reactor; Aprepitant; Fosaprepitant Dimeglumine; Emend; Stability testing

1. Introduction

Areas in drug development where high-throughput HPLC analyses are typically performed include column screening for impurity profile method development, catalyst screening for process development, as well as solubility and dissolution determinations. However, much instrument time is also currently devoted to the evaluation of the stability of active pharmaceutical ingredients (APIs) and their formulated drug product (DP) counterparts. For APIs, often this involves the storage of samples under a given set of conditions (i.e. temperature, humidity), inside specific storage containers (e.g. polyethylene bags placed inside either fiberboard or stainless steel drums), for set periods of time. At regular time intervals (e.g. 3, 6, 9, ... months), the purity of the material in each container is assayed chromatographically. Unfortunately, typically many months pass until sufficient data can be collected to adequately characterize the stability of each material. However, at least during early (i.e. preclinical) development, only a cursory knowledge of the degradation kinetics may be needed (note also that API supplies may be limited to only tens or hundreds of milligrams in early development, thus 'formal/GMP' stability studies may not even be possible until additional material is manufactured at a later time). In these cases, stressed stability regimes have recently gained popularity in the industry [1]. For example, the API may be exposed to acid, base and peroxide chemical stresses, as well as thermal and light stresses, to gauge its sensitivity to these conditions. We point out, however, that only oxidative and thermal stresses can routinely be encountered during storage (to potentially affect API quality). And, of these two stresses, only temperature plays a universal role in all decomposition reactions, regardless of the mechanism. Thus, we propose an alternate strategy for evaluating the stability of pharmaceutical compounds: characterization of the temperature-dependent degradation kinetics. This approach involves using the Arrhenius equation [2] to determine the activation energy barrier

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doi:10.1016/j.jpba.2006.02.005

and frequency factor for a given compound/reaction. Having obtained values for these two parameters, one can compare them with a database of other such parameters for various drug candidates with similar degradation pathways to estimate the relative stability of the test compound. Alternatively, these parameters may also be used to predict the rate of the degradation at different temperatures.

The problem with kinetic characterizations is that the experiments are often numerous and tedious to perform (note that unlike thermodynamics, kinetics are empirical in nature). Typically, such analyses involve monitoring (in this case, using HPLC) the progress of a given reaction at several different reagent concentrations, then repeating the experiments at different temperatures (T). Collecting sufficient data, under a fixed set of experimental conditions, to construct concentration-time profiles at each T allows one to extract the rate constants, k. Using the Arrhenius equation, one can plot $\ln(k)$ versus $1/T$ to obtain the activation energy, E_a (from the slope of the linear regression fit of the data points), and frequency factor, A (from the y-intercept of the fit). If we consider that a minimum of four data points is sufficient to construct each plot (in this case we assume that the concentration-time profiles obtained by plotting the log of the concentration versus time are linear, i.e. as per a first or pseudo-first order process), then a total of sixteen chromatograms would need to be collected to extract an estimate for E_a and A, for a single reaction. In sharp contrast, using a chromatographic reactor approach (e.g. [3,4]) one must collect only four 'reaction chromatograms' (each at a different column temperature) to obtain similar information: an *apparent* activation energy, E'_a, and an *apparent* frequency factor, A' (Note that here we denote the Arrhenius parameters as 'apparent' because every on-column conversion occurs under the specific conditions of the chromatographic reactor; i.e. inside a pressurized tube containing flowing liquid, in the presence of a mobile phase and a stationary phase. Such conditions are very different from solution-phase reactions performed near STP.). Therefore, the chromatographic reactor approach may provide a significant time/effort-savings over traditional kinetic analyses.

Chromatographic columns have the potential to provide an efficient means of acquiring kinetic data for certain chemical reactions due to the fact that these columns can be used as simultaneous flow-through reactors, in which the chemical reaction occurs (in the mobile and/or stationary phase) at the same time as the chromatographic separation of the product(s) from the reagent(s). Previously, Langer showed that for HPLC reactors, reaction rates should ideally be between 10^{-2} and 10^{-4} s^{-1} in order to use the column as a reactor for studying the kinetics [3]. Unfortunately, while various mathematical treatments have been used to-date to describe the kinetic data obtained from different types of chromatographic reactors, we have found that many of these approaches may not be optimal, at least in the case of GC applications [5]. Recently, we outlined the development of a new treatment for use with various chromatographic reactors which we hope will facilitate the popularization of the technique [5]. This treatment is described below in Section 2 of the paper, as it applies to the chemical system of interest in this work. Examples of recent works that we have published in which we have implemented this treatment are given in Refs. [6–8].

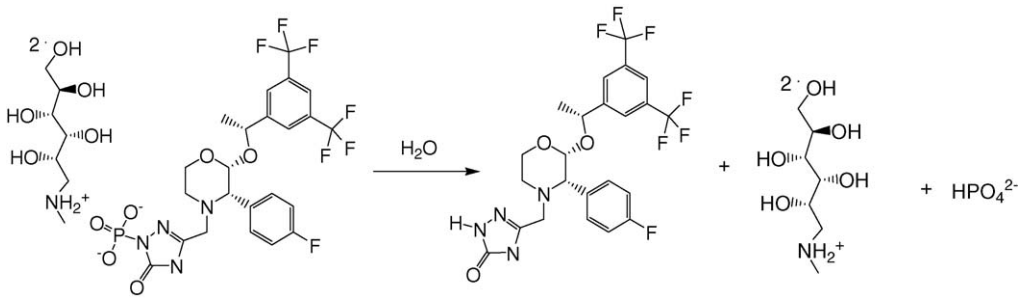
In this work, we utilize our recently developed kinetic treatment (originally intended for use with gas chromatographic reactors, GCRs) to model the HPLC reactor data obtained from studies of the on-column hydrolysis of the Aprepitant prodrug, Fosaprepitant Dimeglumine (FD). Using the combination of a chromatographic reactor approach for collecting the kinetic data and our mathematical treatment to effectively extract the (apparent) Arrhenius parameters from the data, we are able to relate an E'_a value for the process. We then compare this value to the 'true activation energy' as calculated from data collected by studying the hydrolysis of FD in quiescent solutions of acetonitrile–0.1% (v/v) aqueous H_3PO_4 (50:50, v/v). Our work demonstrates that it may be possible to quickly obtain a reasonable estimate of the relative stability of a labile API using an HPLC reactor approach. This finding may have a potential impact on the efficiency of API probe stability determinations conducted during early development in the pharmaceutical industry.

2. Theory

2.1. Mathematical treatment of reaction chromatogram data for the on-column conversion of FD to Aprepitant

Assuming a first order (or pseudo-first-order; refer to Scheme 1) process, the rate equation for the hydrolysis of FD can be written as follows:

$$X_P = 1 - \exp(-k' t_R) \quad (1)$$



Scheme 1. Hydrolysis of the prodrug salt, Fosaprepitant Dimeglumine (FD), yielding Aprepitant.

where X_P represents the mole fraction of product (Aprepitant) formed after a period, t_R, of reaction time and the *apparent* rate constant for the reaction is denoted by k'. Coincidentally, t_R is also equal to the retention time of the FD peak; i.e. the total amount of time the compound spends in both the mobile and stationary phases while on-column (we assume here that the reaction can occur in both phases and that the value of k' represents an 'average rate constant' for the conversion occurring in both phases; i.e. that $k't_R = k'_M t_M + k'_S t_S$, where the subscripts M and S denote the mobile phase and stationary phase, respectively). At a column temperature of 20 °C, minimal degradation of FD is observed during the time spent on-column (t_R), hence $X_P \approx 0$ at this temperature (we will revisit this point in Section 4). Rearranging Eq. (1), one obtains an expression for the apparent rate constant:

$$k' = -\ln[1 - X_P]/t_R \quad (2)$$

The Arrhenius equation may be written as shown below so that the apparent activation energy, E'_a, is expressed as a function of the apparent frequency factor, A', the temperature, T, the gas constant, R, and the apparent rate constant:

$$E'_a = -RT\ \ln(k'/A') \quad (3)$$

Substituting Eq. (2) into Eq. (3), one obtains the following equation:

$$E'_a = -RT\ \ln[\ln(1 - X_P)^{-1}/(A't_R)] \quad (4)$$

Dividing both sides of Eq. (4) by the term $E'_a T$ yields the desired result for fitting chromatographic reactor kinetic data:

$$1/T = -R/E'_a\{\ln[\ln((1 - X_P)^{-1})/t_R]\} + R/E'_a[\ln(A')] \quad (5)$$

From Eq. (5), one can see that by simply constructing a plot of $1/T$ versus $\ln[\ln((1 - X_P)^{-1})/t_R]$, it is possible to determine the values of E'_a and A' from the slope and y-intercept, respectively, of a linear regression fit of the data, i.e. (X_P, t_R, T) triplets.

3. Experimental

3.1. Reagents and materials

HPLC grade water, phosphoric acid (85 wt.% in aqueous, 99.999% purity) and HPLC grade acetonitrile were purchased from commercial sources. The Aprepitant (molecular weight = 534.44 g/mol) and Fosaprepitant Dimeglumine (molecular weight = 1004.85 g/mol) samples used in this work were manufactured in-house by Chemical Engineering Research & Development, Merck & Co., Inc. (Rahway, NJ). Both compounds had purities >99.5%. The major impurity in FD is the pharmaceutically active compound, Aprepitant.

3.2. Apparatus

All chromatograms were collected on an Agilent 1100 series HPLC system equipped with a variable wavelength UV–vis detector monitoring the 215 nm wavelength. Quiescent solution reactions were carried out using a Neslab RTE-111 thermostated recirculator with both heating and cooling capabilities.

3.3. HPLC impurity profile assay for Fosaprepitant Dimeglumine (FD)

The separation of FD and Aprepitant was achieved on a MacMod Ace™ C18(2) Ultra-Inert Base-Deactivated HPLC column (25 cm × 4.6 mm i.d., 5 μm particles) utilizing a mobile phase consisting of two solvents: Solvent A – aqueous phosphoric acid (0.1% v/v) and Solvent B – HPLC grade acetonitrile. The gradient program started at 75A:25B, v/v, and achieved 55A:45B, v/v, over 7 min, then reached 10A:90B, v/v, over an additional 10 min. Finally, a 3 min hold at 10A:90B, v/v, was employed to flush the column (total run-time: 20 min). A 10 min re-equilibration period (at 75A:25B, v/v) was performed between sample injections. The flow rate was maintained at 1.5 ml/min in all experiments/assays. The approximate retention times for FD and Aprepitant are 10 and 14 min, respectively, using this method (with a column temperature of 20 °C).

3.4. On-column hydrolysis of FD

HPLC 'reaction chromatograms' were collected in duplicate using the above impurity profile method with column temperatures in the range 20–60 °C, by injecting a standard solution of Fosaprepitant Dimeglumine onto the column. To prepare the standard solution, the FD sample was dissolved in acetonitrile–0.1% aqueous H_3PO_4 (50:50, v/v) at a concentration of 0.6 mg/ml. An injection volume of 10 μl was used to introduce the sample to the column. The sample tray was maintained at 5 °C to minimize the hydrolysis of the FD sample between injections. With each incremental 10 °C change of the column temperature, the column was allowed to equilibrate for 60 min prior to injection of the standard. For each reaction chromatogram, the total peak area of the Aprepitant product was integrated (including the 'fronting portion') and recorded. The retention time of FD was also recorded for each chromatogram.

3.5. Kinetics of FD hydrolysis in quiescent solution

Quiescent solutions containing between 0.5 and 0.6 mg/ml of FD, dissolved in acetonitrile–0.1% aqueous H_3PO_4 (50:50, v/v), were placed in thermostated baths maintained at 30, 40 and 50 °C. Periodically (e.g. every ∼30 min), samples were withdrawn from the solutions and analyzed using the HPLC impurity profile method described above (with the column temperature fixed at 20 °C) to determine the change in the concentrations of both FD and Aprepitant, over time.

4. Results and discussion

FD is a readily water-soluble compound. This property facilitates its use in an injectable formulation for the experimental treatment of chemotherapy induced nausea and vomiting (CINV). As shown in Scheme 1, the phosphate moiety of FD can be hydrolyzed (under physiological conditions in the

bloodstream) to yield the pharmaceutically active component of the molecule, Aprepitant [9,10]. However, the lability of FD also makes chromatographic analysis of the compound quite challenging. During method development of the gradient impurity profile assay for this compound (see Section 3), it was noticed that higher temperatures (of either the sample tray or column) resulted in elevated levels of Aprepitant in the assay results.

Upon finalization of the method, the impurity profile assay for FD was validated to meet the requirements of Merck & Co., Inc., appropriate to the current stage of development. Specifically, the method validation included demonstration of linear response (concentration-peak area, for both FD and Aprepitant), precision, specificity, solution stability, accuracy, robustness, and determination of a limit of detection (LOD) and a limit of quantitation (LOQ). The detector response was found to be linear (i.e. $R^2 \geq 0.999$) for FD, from ~130% of the target concentration (0.6 mg/ml) down to the LOQ (0.05% of the target concentration). The LOD for the compound was established to be 0.02% of the target concentration. Good linearity was also demonstrated for Aprepitant, over a similar range of concentrations. The relative response factor for Aprepitant/FD was established as 1.82, at 215 nm. The solution stability study showed that the FD sample solution was stable at 5 °C for at least 19 h. Good method precision was demonstrated by comparing three independent sets of impurity profile data, determined for a single FD sample, collected by three analysts using three different HPLC systems on three different days. Finally, the robustness of the method was evaluated by deliberately introducing small changes in the method parameters, including flow rate, column temperature, mobile phase composition, etc.

Based on the validation results, a tray temperature of 5 °C and a column temperature of 20 °C were selected for the final method. Our studies showed that <0.0002 mg/ml of Aprepitant (i.e. less than the limit of quantitation for the method) forms in a typical FD sample preparation that is stored for a period of 19 h at 5 °C. Similarly, it was found that chromatograms collected using column temperatures ≤20 °C showed a consistent level of Aprepitant (and no 'peak fronting', see Fig. 1), supporting the idea that no Aprepitant (i.e. beyond the amount which is present in the FD sample at the outset of each experiment) is formed via the on-column degradation of FD at these column temperatures.

To gauge the stability of FD with respect to solution-phase hydrolysis, it was of interest to perform a kinetic characterization of the conversion. To do this most efficiently, we set out to study the on-column decomposition of FD, using the validated impurity profile method and various column temperatures to regulate the driving force for the degradation. Ultimately, using Eq. (5), we were able to determine the apparent activation energy and apparent frequency factor for the conversion. For comparison purposes, we also used a classical approach to determine the activation energy barrier and frequency factor for this process, i.e. via direct application of the Arrhenius equation. In the latter experiments, quiescent solutions of FD, each equilibrated at a different temperature, were periodically sampled and analyzed by HPLC to obtain the concentration-time profiles of both FD and Aprepitant (see Section 3). The rate constants extracted from this data were subsequently plotted to obtain the activation energy and frequency factor.

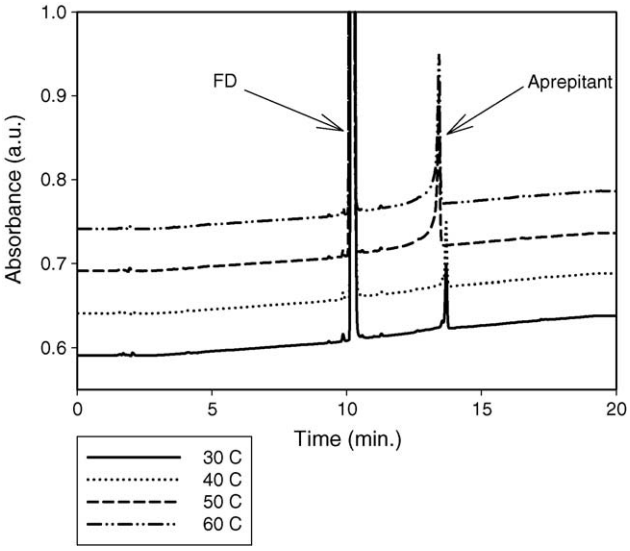


Fig. 1. Overlay of reaction chromatograms for the on-column conversion of FD to Aprepitant. Column temperatures are shown in the legend. Note the elevated baseline between the two peaks, which is indicative of an on-column reaction.

To date, Eq. (5) has previously been utilized only for GCR applications [5–8]. This is because the derivation of the equation was originally based on assumptions pertaining to an ideal column reactor (ICR). A column reactor may be treated as an ICR if several important assumptions are made [11]. These assumptions include: (1) the reactant pulse and resulting product(s) are well separated, (2) the column is completely isothermal and allows for excellent heat transfer to the chemical system inside, (3) reaction rates are controlled chemically, thus mass transfer effects are minimal, (4) the height of a theoretical plate approaches zero, allowing diffusional peak broadening to be ignored (note that sometimes diffusional broadening effects can be minimized through careful peak integration, as in this case, where the reactant and product peaks are well separated) and (5) the column is homogeneous in composition throughout. In addition to GC systems, we believe that the performance of many HPLCs/packed LC columns available today is sufficiently good to allow the majority of these assumptions to hold, in many cases. Finally, note that in the case of liquid chromatographic reactors (LCRs), we can assume that pressure changes over a small temperature range (i.e. ≤50 °C) are negligible due to the limited compressibility of liquids.

Fig. 1 shows an overlay of 'reaction chromatograms' for the on-column decomposition of FD. Generally, as the column temperature is increased, the Aprepitant peak area becomes larger and the 'peak fronting', which grows in the direction of the FD peak, becomes more pronounced. This peak fronting represents Aprepitant molecules that form at different times during the travel of the FD band down the column (i.e. while some form immediately, the majority of the Aprepitant molecules are continuously produced until the FD peak elutes). The data extracted from these chromatograms is summarized in Table 1; the key results are plotted in Fig. 2 (note that since the chromatographic

Table 1
HPLC chromatographic data for the on-column conversion of Fosaprepitant Dimeglumine (FD) to Aprepitant

t_R (min)	t_{void} (min)	T (K)	A_{Ap} (a.u.)	X_P	$1/T$ (K^{-1})	$\ln\{\ln[(1-X_P)^{-1}]/t_R\}$
9.89	2.20	293.2	2.855×10^4[a]	0	3.41×10^{-3}	–
10.1	2.12	303.2	3.256×10^4	6.92×10^{-4}	3.30×10^{-3}	−13.5
10.2	2.06	313.2	4.887×10^4	3.55×10^{-3}	3.19×10^{-3}	−11.8
10.2	2.01	323.2	9.096×10^4	1.10×10^{-2}	3.10×10^{-3}	−10.7
10.1	1.96	333.2	2.106×10^5	3.23×10^{-2}	3.00×10^{-3}	−9.61

The term 't_{void}' represents the elution time of components that are not retained on the column (i.e. the void time); it also represents the reaction time of FD in the mobile phase (only). A_{Ap} represents the integrated peak area of Aprepitant. All other terms are defined in Section 2 of the text.
[a] This peak area corresponds to the amount of Aprepitant present in the FD sample; i.e. at $t = 0$.

peak areas of each component are directly proportional to their respective concentrations, as can be seen from the good linearity results obtained during the method validation, the peak area of Aprepitant, A_{Ap}, can be used directly to determine the mole fraction, X_P, at each T and t_R, with the aid of the validated relative response factor of 1.82 for Aprepitant/FD). From Fig. 2, one can see that Eq. (5) fits the kinetic data very well, thus supporting the use of this equation, and the assumptions on which it is based, for HPLC applications. The apparent activation energy for the hydrolysis of FD (i.e. under the given column conditions), extracted from the linear regression fit of Eq. (5), is 107 kJ/mol. The corresponding apparent frequency factor is $7.3 \times 10^{12}\ s^{-1}$.

For comparison, from the 'quiescent solution' (i.e. unstirred batch reactor) kinetic study, the conversion of FD to Aprepitant was found to have an activation energy of 91 kJ/mol and a frequency factor of $9.1 \times 10^9\ s^{-1}$, using a traditional Arrhenius fit to the chromatographic data for FD (see Table 2 and Fig. 3). However, using the Aprepitant data (see Table 3 and Fig. 4), which represents the much smaller of the two components, the error in the rate constants was found to be sufficiently large to make it impossible to obtain a reasonable estimate for the Arrhenius parameters. Nonetheless, the values of 107 and 91 kJ/mol, representing the activation energies obtained from the two approaches (using different data sets), seem to be in fairly good agreement, especially given that the quiescent solution and on-column experiments differed in at least two major ways. Firstly, the former experiment used an unstirred solution of fixed composition whereas the column reactor approach utilized a continuous flow (which subjected the reagent molecules to higher pressures than in the bulk solutions, but, since the compressibility of liquids is limited, we believe that there is a minimal impact of this variable on the determination of E'_a) with a gradient elution scheme. The gradient elution produced a varying solvent composition profile during the course of reaction, which can impact the hydrolysis conditions (in a time-dependent manner). Secondly, the column reactor introduced a stationary phase to the reaction, which was absent from the quiescent solution reactors. Consideration of stationary phase effects on the reaction rate may be important as the hydrolysis of FD has the opportunity to occur simultaneously in both phases as this solute travels down the column (ideally, for the apparent activation energy to have the best chance of matching the activation energy of the solution experiments, we would

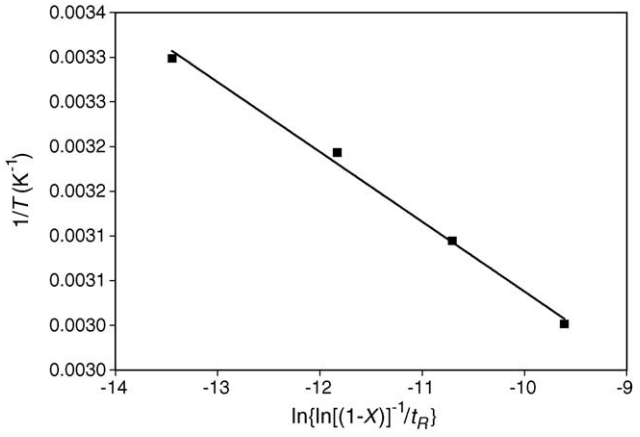


Fig. 2. Plot of $1/T$ vs. $\ln[\ln(1-X_P)^{-1}/t_R]$ for the on-column hydrolysis of FD, using data presented in Table 1. The linear regression fit ($R^2 = 0.995$) has a slope of $-7.8 \times 10^{-5}\ K^{-1}$ and a y-intercept of $2.3 \times 10^{-3}\ K^{-1}$, implying that $E'_a = 107$ kJ/mol and $A' = 7.3 \times 10^{12}\ s^{-1}$.

Table 2
Concentration of FD as a function of time in a quiescent solution containing ~0.6 mg/ml FD in acetonitrile–0.1% aqueous H_3PO_4 (50:50, v/v) at (A) 30 °C, (B) 40 °C and (C) 50 °C

t (min)	[FD] (mg/ml)	$\ln([FD]/[FD]_0)$
(A) $T = 30$ °C (~328 K)		
0	0.6126	–
30	0.6097	−0.004841
60	0.6081	−0.007422
90	0.6060	−0.01086
120	0.6042	−0.01382
169	0.6015	−0.01838
(B) $T = 40$ °C (~338 K)		
0	0.6126	–
30	0.6060	−0.01090
60	0.6001	−0.02066
90	0.5944	−0.03011
120	0.5902	−0.03721
150	0.5837	−0.04843
(C) $T = 50$ °C (~348 K)		
0	0.5536	–
34	0.5363	−0.03176
62	0.5200	−0.06251
96	0.5028	−0.09621
123	0.4922	−0.11754
181	0.4669	−0.17035

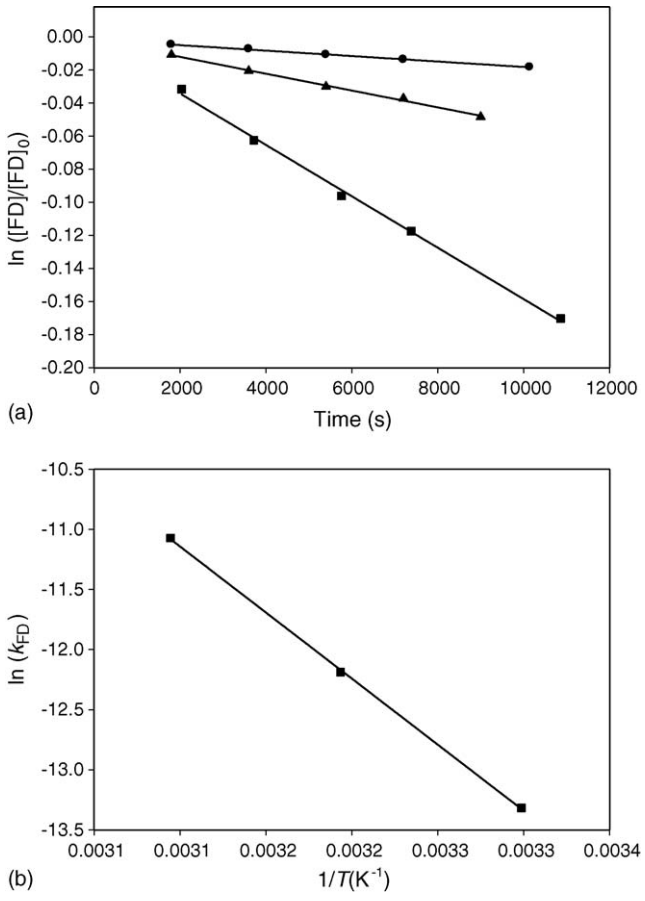


Fig. 3. (a) Log-concentration vs. time plot for FD in the quiescent solution study (see Table 2). (b) Arrhenius plot of the data from (a): the linear regression fit ($R^2 = 0.999$) has a slope of -1.1×10^4 K and a y-intercept of 2.3×10^1, implying that $E_a = 91$ kJ/mol and $A = 9.1 \times 10^9$ s^{-1}.

like either that both k_M and k_S have similar magnitudes and exhibit similar T-dependences, or that $k_M \gg k_S$). In light of these differences, we advocate the use of HPLC column reactor approaches, such as the one we describe here, mainly for the determination of *apparent* Arrhenius parameters. As mentioned earlier, these values are most informative when they are used to compare the relative stabilities of various molecules undergoing similar reactions. Note that an important feature of Eq. (5), highlighted by this work, is that only a ~3% conversion allows one to extract a reasonable estimate for the activation energy from the kinetic data (using the integrated Aprepitant peak areas, which proved problematic in the quiescent solution experiment described earlier—see Fig. 4), which represents a significant improvement over other chromatographic reactor treatments, especially those which model the peak shapes instead of peak areas [5].

Although we are reluctant to compare the two frequency factors directly, due to the fact that the parameter is known to be highly dependent on collision geometry (and hence reactor design), it may be of interest to point out that while the chromatographic reactor achieves a higher probability of collision between the reactant molecules (FD and water) than the quiescent solution reactor, based on the experimentally determined

Table 3
Concentration of Aprepitant as a function of time in a quiescent solution containing ~0.6 mg/ml FD in acetonitrile–0.1% aqueous H_3PO_4 (50:50, v/v) at (A) 30 °C, (B) 40 °C and (C) 50 °C

t (min)	[Ap] (mg/ml)	ln([Ap]/[Ap]$_0$)
(A) $T = 30$ °C (~328 K)		
0	0.00169	–
30	0.00296	0.5605
60	0.00453	0.9860
90	0.00662	1.365
120	0.00841	1.605
169	0.01115	1.887
(B) $T = 40$ °C (~338 K)		
0	0.00169	–
30	0.00664	1.368
60	0.01253	2.003
90	0.01817	2.375
120	0.02238	2.583
150	0.02896	2.841
(C) $T = 50$ °C (~348 K)		
0	0.00101	–
34	0.01730	2.841
62	0.03354	3.503
96	0.05078	3.918
123	0.06139	4.107
181	0.08671	4.453

'Ap' is used as an abbreviation for 'Aprepitant'.

A' and A values, it is clearly more difficult for molecules to overcome the activation energy barrier (i.e. to have a collision that is *successful* in producing product) in the case of the chromatographic reactor. The net result may be that, for a certain set of temperatures, the rate constants predicted using either set of Arrhenius parameters (one having a higher activation energy and a lower frequency factor, the other having the opposite case) may be quite similar. To investigate the validity of this hypothesis, to a first approximation, we arbitrarily selected the temperature of 25 °C (i.e. ~298 K), which is not too far from the range of most of our experimental data. The predicted rate constants (using the Arrhenius equation) are 1.28×10^{-6} and 1.02×10^{-6} s^{-1} at this temperature, based on the Arrhenius parameters for our chromatographic reactor and quiescent solution reactor, respectively. These values differ by an amount which is comparable to the difference between the two activation energies (E'_a and E_a); a much smaller amount than if we compare the two frequency factors directly. Also, at this particular temperature, the higher rate constant for the hydrolysis of FD corresponds to the reaction with the higher activation energy. While these findings lend support to our hypothesis, in order to obtain a more precise estimate of the temperature where the rate constants for each set of Arrhenius parameters become equivalent, one must solve the simultaneous non-linear equations (containing the two 'unknowns', k and T):

$$\ln(k) = -E'_a/RT + \ln(A') = -E_a/RT + \ln(A) \quad (6)$$

Doing so, we find that for the two rate constants to be exactly the same:

$$T = \{E'_a - E_a\}/\{R[\ln(A'/A)]\} \approx 288\,\text{K}(\sim 15\,^\circ\text{C}) \quad (7)$$

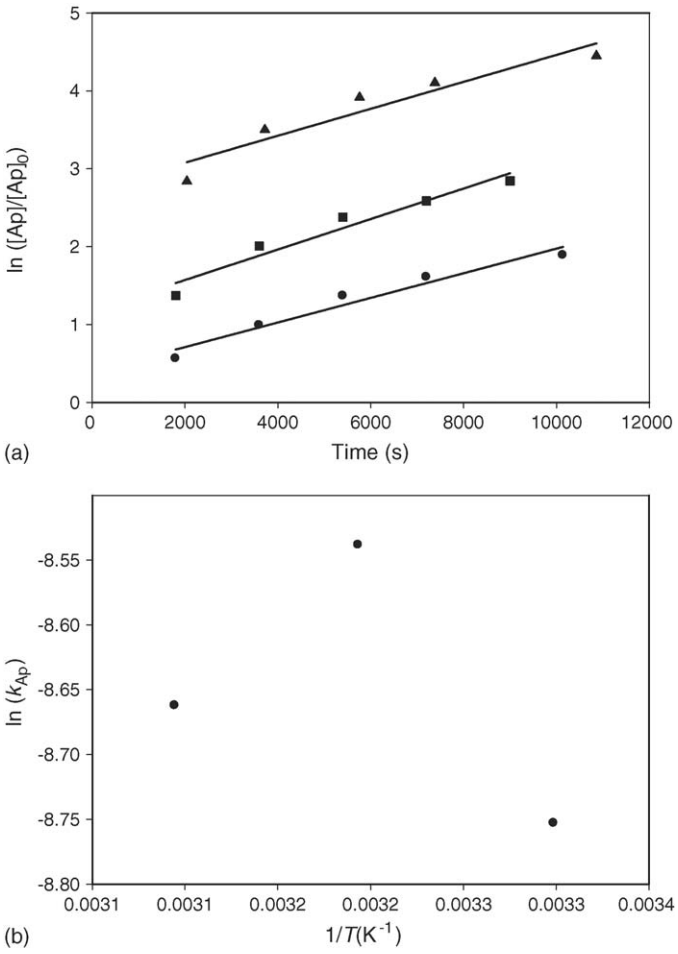


Fig. 4. (a) Log-concentration vs. time plot for Aprepitant (Ap) in the quiescent solution study (see Table 3). (b) Attempt at constructing an Arrhenius plot using data from (a).

Thus, at a temperature of 15 °C, $k = 2.86 \times 10^{-7}\ s^{-1}$ for the hydrolysis of FD in either reactor. This exercise demonstrates two points: (1) the importance of considering both the frequency factor and the activation energy in determining the reactivity of a compound and (2) that it is possible (in certain cases) for the reactivity of a compound, measured independently using two different reactor types (and different experimental conditions), to yield similar results.

5. Conclusions

In the early development stages of some labile APIs, it appears that the rapid 'kinetic screening' of certain degradation pathways (e.g. hydrolysis/solvolysis) using HPLC chromatographic reactors may be a viable approach for establishing the relative stabilities of the compounds. Provided a database of apparent activation energies and apparent frequency factors for similar reactions, obtained under similar chromatographic conditions, it may be possible to assign relative stabilities to different molecules on the basis of these parameters. For future work, it would be of interest to us to determine whether or not trends in these predominantly solution-phase Arrhenius parameters correlate well with the values determined for the complementary reactions occurring in the solid-state, since most APIs are stored as solids. However, solution-phase stability data is also of interest, mainly with regard to its potential impact on manufacturing; i.e. the quality/yield of an API.

While chromatographic reactors can provide an efficient means for collecting kinetic data, our mathematical treatment of the reaction chromatograms has been shown to work well (for both HPLC and GC-based reactors and various chemical systems), even at low conversions (~3%). We attribute some of the success (e.g. robustness and versatility) of our treatment to improvements made over the last few decades in chromatographic technology which have allowed many ICR assumptions to hold, thus helping us to simplify the treatment of the kinetic data. Ultimately, we believe that the combination of a chromatographic reactor approach for collecting kinetic data and our mathematical treatment for extracting the (apparent) Arrhenius parameters can provide a highly efficient and effective means of performing the kinetic characterizations of some common degradation pathways for various drug candidates in development.

The apparent activation energy for the conversion of Fosaprepitant Dimeglumine to Aprepitant was found to be 107 kJ/mol, under the conditions of our HPLC reactor experiments (using gradient elution), which is comparable, to a first approximation, to the activation energy obtained for the same reaction occurring in a quiescent solution containing a fixed composition of acetonitrile–0.1% aqueous H_3PO_4 (50:50, v/v): 91 kJ/mol. While the use of an isocratic mobile phase elution scheme may have helped to address one of the potential causes for the difference in the two activation energy values (it is impossible to completely eliminate the stationary phase and pressure effects in a chromatographic reactor), with the isocratic methods screened prior to performing the work discussed in this article, it was not possible to use acetonitrile–0.1% aqueous H_3PO_4 (50:50, v/v) elution to provide an adequate separation of FD and Aprepitant. With the final isocratic method we tested (using acetonitrile–0.1% aqueous H_3PO_4, 40:60 v/v, elution and a Waters Xterra RP8 column), the Aprepitant peak profiles showed significant coalescence with the FD peak at higher column temperatures, making accurate integration difficult. Thus, we believe that the gradient method presented in this work was the superior method for studying the on-column hydrolysis of FD.

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