Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 252 (2003) 27–39
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Evaluating dissolution profiles of an anti-HIV agent using ANOVA and non-linear regression models in JMP software

Sanjive Qazi [a,*], N.K. Peter Samuel [b], T.K. Venkatachalam [c], Fatih M. Uckun [d,e]

[a] *Department of Bioinformatics, Parker Hughes Cancer Center, 2685 Patton Road, St Paul, MN 55113, USA*
[b] *Department of Pharmaceutical Sciences, Parker Hughes Cancer Center, 2685 Patton Road, St Paul, MN 55113, USA*
[c] *Department of Chemistry, Parker Hughes Cancer Center, 2685 Patton Road, St Paul, MN 55113, USA*
[d] *Department of Virology, Parker Hughes Cancer Center, 2685 Patton Road, St Paul, MN 55113, USA*
[e] *Department of Drug Discovery Program, Parker Hughes Cancer Center, 2685 Patton Road, St Paul, MN 55113, USA*

Received 6 June 2002; received in revised form 5 November 2002; accepted 5 November 2002

Abstract

A powerful statistical method was designed using JMP software to detect factors contributing to differences in the dissolution process of an antiviral drug delivered in an oral dosage form. Due to the large number of dissolution media available for solid dosage forms, a statistical method to choose the appropriate medium is critical for testing solid dosage forms. We have developed an analysis of variance model to analyze the overall dissolution profile obtained from the various media. In vitro tests were performed using a standard USP basket apparatus (Vankel Inc., Cary, NC), and the analysis used the restricted/residual maximum likelihood method (JMP software) to partition the variance due to media (pH 1.2 and 6.8, +SDS, water alone and at pH 1.2 with pepsin), time (repeated measure) and capsule (random effect). This allowed correct standard error estimates to be used to compare dissolution in different media using planned linear contrasts. The model provided us with statistically powerful criteria to identify significant differences in capsule dissolution across time and to quantify capsule-to-capsule population variance estimate. The time specific linear contrasts showed the largest sum of square values (SS) occurred at 180 min (SS = 0.268) for the simulated SIF (pH 6.8) versus SGF (pH 1.2) comparison (DF = 166, MSE = 3.92×10^{-3}). The dissolution processes were further characterized using a non-linear regression fit of a power law function to the data for each capsule. This resulted in a method to statistically differentiate between the dissolution processes of the capsules in different media.

Keywords: Dissolution process; Anti-HIV agent; JMP software

1. Introduction

Development of a good therapeutic agent requires both evaluation of activity and design of an appropriate drug delivery method of the active ingredient. We have synthesized a very potent anti-HIV nucleoside analog of d4T (stampidine) (Venkatachalam et al., 1998; Vig et al., 1998; Uckun and Vig, 2002). This novel nucleoside analog is unique because it is significantly more potent than AZT especially for HIV-2 and RT-MDR.

Previous studies have used the design of experiment method in JMP software to validate analytical approaches in which many factors are being considered (Ye et al., 2000). We propose a mixed model analysis of variance method to identify statistically significant factors that influence dissolution profiles.

* Corresponding author. Tel.: +1-651-796-5011; fax: +1-651-796-5480.
E-mail address: sqazi@ih.org (S. Qazi).

0378-5173/02/$ – see front matter
PII: S0378-5173(02)00603-8

In these types of studies there are different sources of variation that need to be considered, and statistical inferences are made from appropriately adjusted degrees of freedom for the effects being investigated. There are two types of effects to consider in the design of an ANOVA model; fixed and random. Fixed effects are generally factors that are under the investigator's control, such as the nature of the dissolution medium (pH, presence of enzyme/presence of surfactants, temperature, spindle speed). Random effects are derived from a selection of levels from a larger population. In a dissolution study, one random effect that has to be considered is the capsule or tablet itself. Each capsule is selected from a larger population of capsules, and the capsule-to-capsule variation in each condition can be used to make inferences about the population variance from which the sample is drawn. In a typical dissolution profile the release of the drug is followed over time by repeatedly taking an aliquot from the medium and assaying for drug content. The time factor in these types of studies is a repeated measure in the ANOVA model. Therefore, the full ANOVA model for dissolution studies will take into account the variance components from fixed effects, random effects and repeated measures to determine significant differences between the factor levels.

Once the significant factors are identified then a more specific non-linear regression model can be used to more fully describe the dissolution process (Brazel and Peppas, 1999; Brazel and Peppas, 2000; Li et al., 2001). Specific differences in the process can then be attributed to different conditions and or physiochemical properties of the drug and its formulation. The data analysis using non-linear regression models will help to determine the relationship between critical variables, and will ensure that future preparations will be within the limits of acceptable dissolution specifications. An attractive feature of using non-linear regression models is that even in cases in which the overall dissolution profiles are similar, differences in the dissolution parameters can be discerned. This is because in the non-linear regression model the dissolution process is a function of two parameters; kinetic constant and diffusion exponent, and certain combinations of the two parameters can result in similar dissolution profiles.

The objective of this study was to identify a dissolution medium for stampidine capsule dosage forms using statistical experimental design and data analysis employing a mixed ANOVA model. In this design, two dissolution profiles were compared and the statistical significance was calculated taking into account the appropriate sources of variance. The two profiles that resulted in the most significant difference identified the pair of media that resulted in the release of the largest and smallest amount of drug from the capsule. Planned linear contrasts were used to determine differences in dissolution of capsules at specific time points. We examined the contribution of the sum of squares (SS) at each time point and statistical significance was calculated using mean square error estimates from the entire data set. The data analyses were performed using the JMP (SAS) statistical software, which has an extensive set of data visualization, advanced ANOVA and non-linear regression techniques. The utility of the JMP software will be discussed with regards to analyzing dissolution profiles (SAS Institute Inc, 1995).

2. Materials and methods

2.1. Materials

Stampidine was synthesized (Venkatachalam et al., 1998; Siddiqui et al., 1999a; Siddiqui et al., 1999b) by condensing D4T with p-bromophenyl alaninyl phosphorochloridate in the anhydrous tetrahydrofuran solvent. The structure of stampidine was confirmed using standard analytical techniques. Avicel 101, a commercially available microcrystaline cellulose (NF) was obtained from FMC corporation, (Wilmington, Delaware), magnesium stearate was obtained from Spectrum Chemicals (Gardena, CA), Hard gelatin capsules, size 4 were purchased from Capsugel (Greenwood, S.C.). Deionized distilled water was purified via the Millipore Milli-Q purification system (Medford, MA). Acetonitrile was purchased from Burdick & Jackson (Muskegon, MI). All other chemicals were purchased from Aldrich (Milwaukee, WI), Sigma Co. (St Louis, MO) or Fisher Scientific (Pittsburgh, PA) and used without further purification.

Hard gelatin stampidine capsules were prepared from using a hand-operated capsule-filling machine. The capsule size was 4 and the unit dose of each capsule was 50 mg of stampidine per capsule.

2.2. In vitro dissolution test

The in vitro dissolution test was performed using apparatus I method (basket, USP) on a Vankel 750 dissolution apparatus (USP, 2000). The dissolution conditions were the following: temperature 37 ± 0.5 °C, volume 900 ml, spindle speed 100 rpm. The dissolution media tested were 0.5% sodium dodecyl sulfate (SDS) in water, deionized water, Simulated gastric fluid (SGF; pH = 1.2) with and without pepsin, and simulated intestinal fluid (SIF, pH = 6.8). Stampidine in SIF with pancreatin could not be detected on HPLC after filtrations through a 0.2 μm filter, presumably due to strong binding to the enzyme and precipitation. Three to six replicate runs were carried out for each condition. Samples (1 ml) were withdrawn at 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min and assayed using HPLC.

2.3. HPLC analysis of stampidine

Chromatographic analysis of stampidine was carried out using a HP 1100 system (Agilent Technologies, formerly HP Corp). The analytical column was Lichrosphere RP (5 μm). The mobile phase was composed of methanol and water in a ratio of 43:57 (v/v).

The column was equilibrated and eluted under isocratic conditions utilizing a flow rate of 1.0 ml/min at 20 °C before injection of 20 ml of sample. The wavelength of detection was set at 265 nm (reference 300 nm) and the run time was 15 min. Peak width, response time and slit were set at >0.03 min, 0.5 s and 4 nm, respectively. The samples obtained during dissolution testing were filtered through a 0.2 μm filter and assayed directly. The retention time for stampidine was between 3.5 and 4.0 min.

2.4. Statistical analysis

Scheme 1 shows the detailed procedure used for determination of significant time and condition effects for capsule dissolution. The linear contrasts were used to calculate overall effects, differences at specific time points and the process of dissolution (from non-linear regression).

2.4.1. Data tranformation (Arcsin, Box–Cox)

Two transformations were performed on the % release values to normalize the data set. Percentage values tend to be heavily skewed at the two extreme ranges, therefore an arcsin transformation converts the asymptotic values at 0 and 100% to values that can range from plus and minus infinity. A Box–Cox family of power transformations using a formalized approach in the JMP software resulted in normalized data set (transformation of $Y = Y^{\lambda} - 1/\lambda \times YY^{\lambda-1}$, where λ is a constant, and YY is the geometric mean). The algorithm in JMP determines the best fit λ by varying λ from −2 to 2 in increments of 0.2 until the sum of squares is minimized (Box and Cox, 1964).

2.4.2. Mixed ANOVA model (REML method)

The ANOVA model contained both fixed (condition) and random effects (time, capsule). ANOVA models were compared with and without the capsule effect in order to assess the importance of capsule-to-capsule variation. Five conditions were tested: water, (SGF) pH = 1.2, (SIF) pH = 6.8, and pH = 1.2 + enzyme (condition factor) and 0.5% SDS. Release from each capsule was measured over a period ranging from 5 to 240 min (time factor).

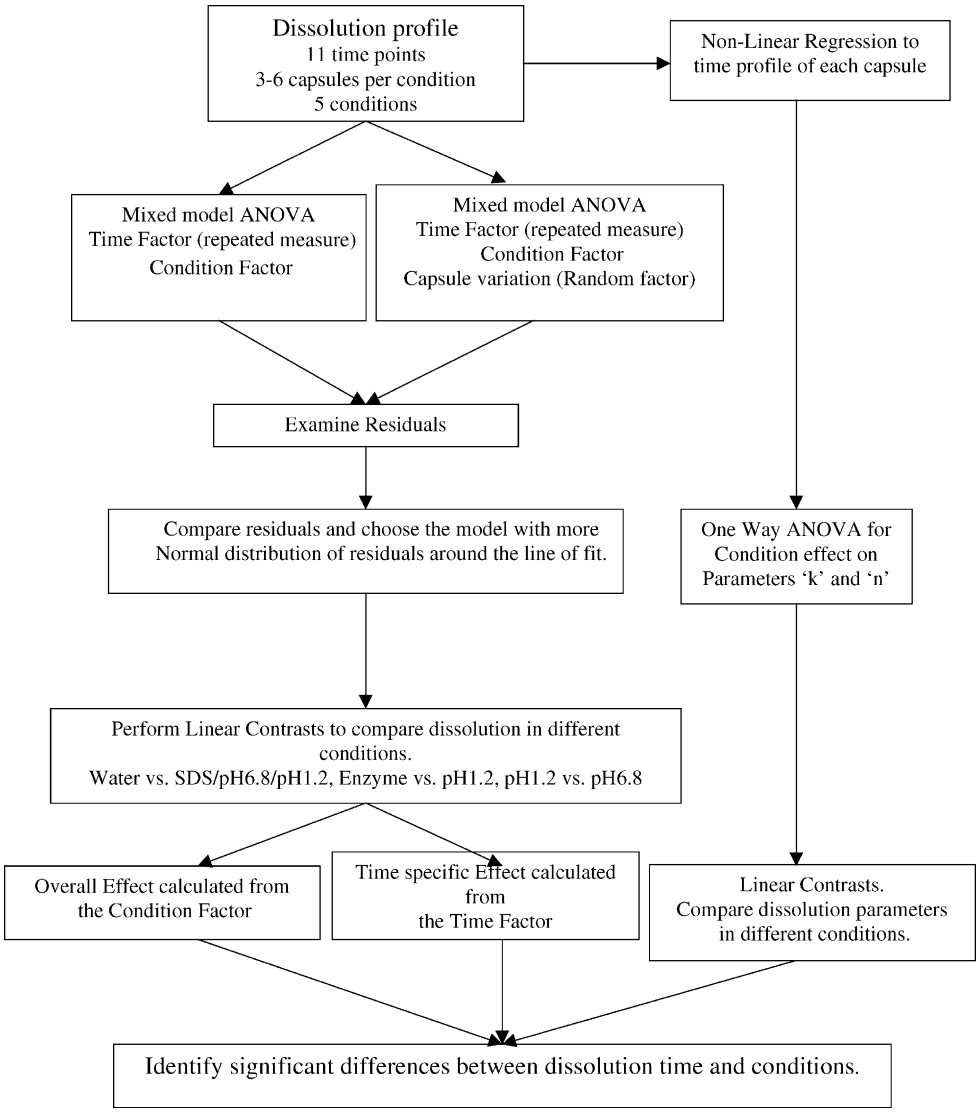
The JMP software offers the REML method (Restricted or residual maximum Likelihood) to solving ANOVA models with random effects. This method uses the unrestricted method to obtain the parameters for the variance components. The REML method correctly determines these variance components by balancing the values on each level with the values across levels and by taking into account the number of levels in the random effect and the standard error of the estimate.

To test for significant differences in dissolution between different conditions, planned comparisons using linear contrasts were performed.

Linear contrasts;

Water versus SDS
Water versus pH 6.8 (SIF)
Water versus pH 1.2 (SGF)
(SGF) pH 1.2 + enzyme versus pH 1.2
(SGF) pH 1.2 versus pH 6.8.(SIF)

The contrasts were calculated using the condition effect and the time effect to obtain the significance values for the overall model (means pooled from all time points) and the specified contrasts at (5–240 min). The JMP software performs a least square means contrast to test null hypothesis that a linear combination



Scheme 1. Flow chart to show implementation of the ANOVA.

of parameters is equal to zero. The parameter is the product of the mean of the group to be tested multiplied by the coefficient, which is set by the user (e.g. Water versus SDS the coefficients are 1, −1). The absolute sum of the coefficients is equal to two. To calculate the mean square error (MSE) used in the linear contrast calculation all the data points were evaluated. The sum of square (SS) and *P*-value of the *F*-test were also reported for overall and specific time comparisons using the MSE.

2.4.3. Non-linear regression model to obtain dissolution exponent 'n' and dissolution rate constant 'k'

Physical parameters of the dissolution process were obtained from fitting the release profiles to the power law (fraction released $= k \times t^{\wedge n}$, where k is the dissolution rate constant and n is the release exponent) (Brazel and Peppas, 1999; Brazel and Peppas, 2000; Li et al., 2001). The two parameters '*n*' and '*k*' were determined for each capsule in each condition.

Convergence to a solution was obtained for all the release profiles using the standard least squares method.

2.4.4. Linear contrasts of the dissolution parameters in different conditions

The capsule-to-capsule variation in the dissolution rate constant (k) and the release exponent (n) for each condition were analyzed using the planned comparisons outlined above. Calculating the R^2 value for each capsule assessed the proportion of the variation explained by the non-linear fit. Linear contrasts were used to test for differences between conditions.

3. Results and discussion

3.1. Dissolution of STAMPIDINE in different media

A well-designed in vitro dissolution method is an important quality control test for oral solid dosage forms (tablets, hard and soft gelatin capsules) (Bonferoni et al., 2000). We chose five different dissolution media for in-vitro studies as an important step in establishing an ideal dissolution time that distinguishes between the conditions used. Choosing an appropriate dissolution medium is an important component in answering the relevance of the dissolution test. The dissolution testing was carried out to mimic physiological conditions (pH 1.2 (SGF), pH 6.8 (SIF), in the presence of enzyme at pH 1.2 or water with or without surfactant) allowing interpretation of the dissolution data with regard to in vivo performance of the product. The testing conditions were based on the physicochemical characteristics of the drug substance (permeability, pK_a, octanol/water partition coefficient, pH stability profile, etc.) and the environmental conditions the dosage form is expected to encounter after oral administration.

In our studies we followed the dissolution of a potent anti-HIV drug, stampidine, in capsule form using the Basket apparatus and assayed using the HPLC technique described earlier. The appearance of

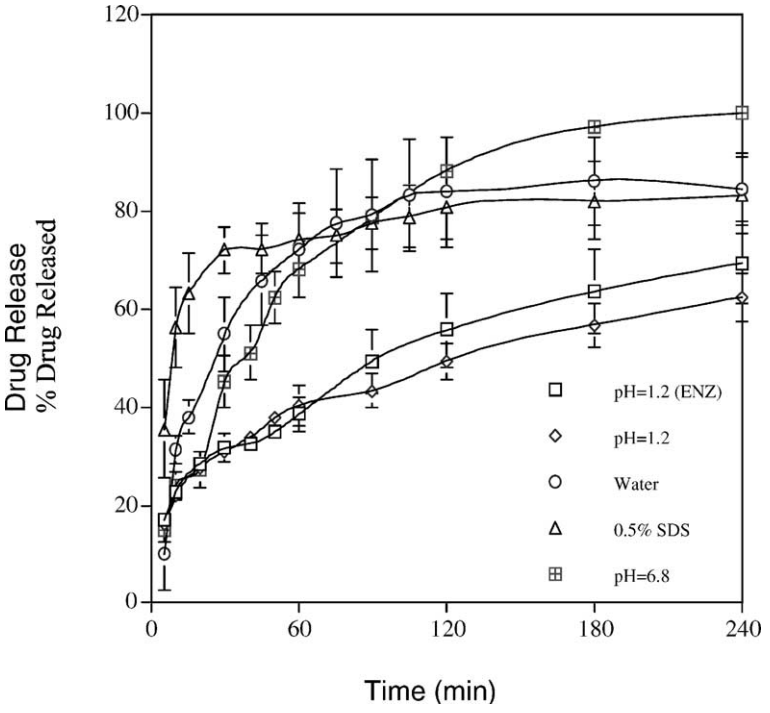


Fig. 1. Dissolution profile of Stampidine capsules in various dissolution media. Dissolution volume 900 ml, USP method I (basket), speed 100 rpm, temperature 37.5 °C, analysis by HPLC (isocratic method).

the compound in five different dissolution media was followed over time (Fig. 1), and the error represents values pooled from 3 to 6 capsules. The five curves suggest that there are differences in the dissolution profiles in the five different conditions. An ANOVA technique was implemented to determine the effect of pH, enzyme and surfactant. The distribution of the data points are skewed as the percentage release values can only range from 0 to 100%. An arc sin transformation was followed by a Box–Cox Transformation to normalize the data set ($\lambda = 0.8$ gave the best fit). The Box–Cox transformation is a very useful feature implemented in JMP software that can normalize many types of datasets for analysis of variance.

3.2. Determination of the capsule variance component

The JMP software provides a statistical method to solve for complex mixed model ANOVA. In our design

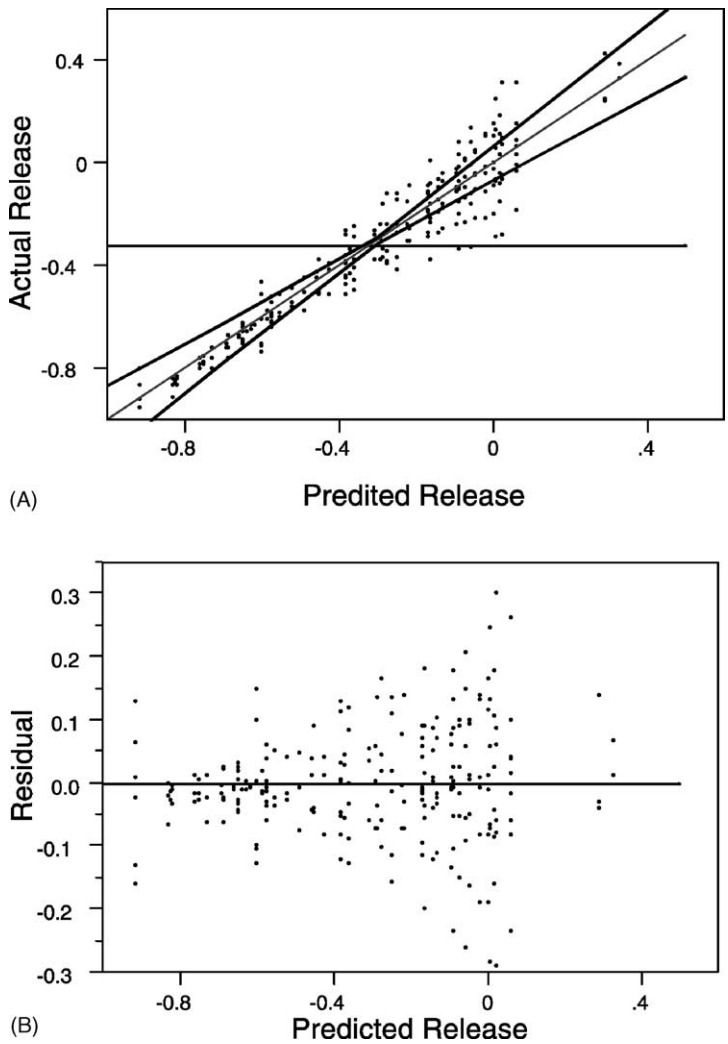


Fig. 2. ANOVA model with time and condition effects. The data set was transformed using an arcsin and Box–Cox functions and fitted to an ANOVA model with condition as the fixed effect and time as a repeated measure. The model accounted for 90% of the variance (A, $P < 0.0001$) of which 87.6% of the variance component was due to the time effect. However, plot of the residuals showed telescoping of the values (B), suggesting that the ANOVA model is inappropriate.

the condition factor was a fixed effect, the time factor was a repeated measure and the capsule-to-capsule variation was a random effect. An appropriate model should explain the total variance based upon these three components, with the variance measured for the capsule effect reflecting the population variance.

An ANOVA model with dissolution media as a fixed factor and time as a random repeated factor shows a good fit of the model (Fig. 2A, $r^2 = 0.90$, $F_{56,182} = 36.06$, $P < 0.0001$), but examination of the residuals shows telescoping, suggesting that the errors are not normally distributed (Fig. 2B). This situation is corrected by adding a capsule effect as a random factor nested in condition. This results in an improved model (Fig. 3A, $r^2 = 0.96$, $F_{72,166} = 78.4$, $P < 0.0001$) with more normal distributed residuals (Fig. 3B). The effect tests showed that the differences between the condition ($P = 0.016$), time ($P < 0.0001$) and capsule ($P < 0.0001$) were highly significant taking into account the fixed (condition), random (capsule) and repeated (time) factors of the model.

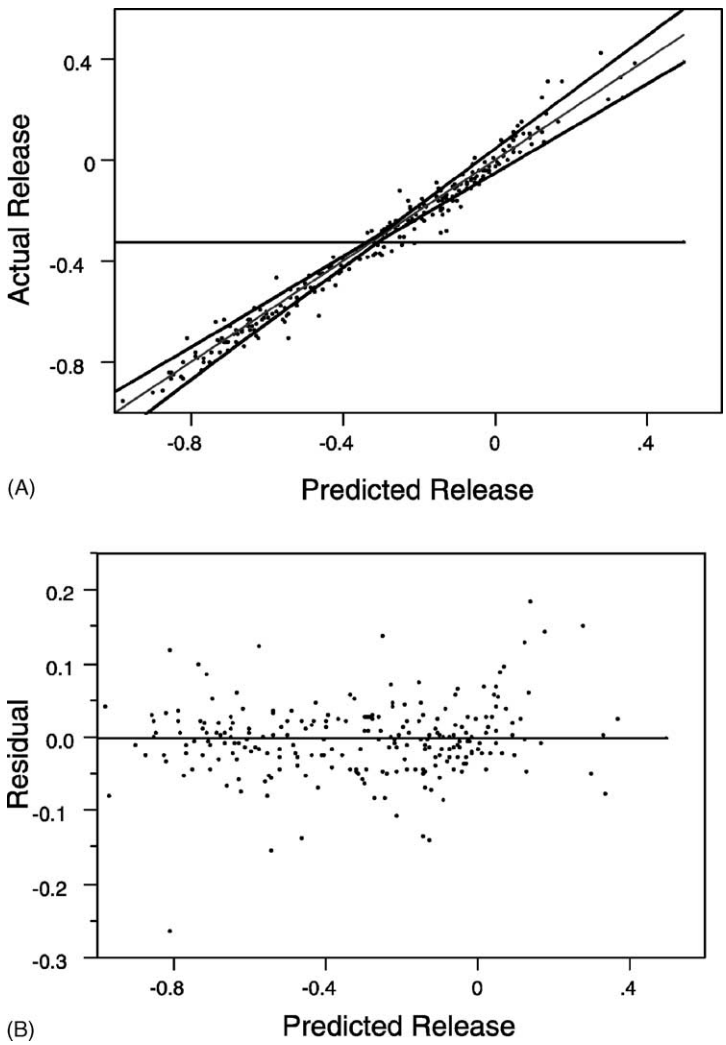


Fig. 3. ANOVA model with time, condition and capsule effects. An ANOVA model that included a capsule effect (random effect) resulted in a better fit of the transformed data (A, 96% of the variance accounted for by the model), and the residual plot (B) shows a more normal distribution of data points.

The REML method in the JMP software correctly determines the variance components for each of the effects being investigated and reports the proportion of each component to the total variance. The time component makes up 88.1% of the variance component, the capsule effect 7.2% and the residual variance is 4.7% of the total variance component.

3.3. Using the ANOVA model to compare dissolution in different media and specific time points

For a drug to be absorbed it must first be dissolved in the stomach or intestine. The drug molecules on the surface are the first to enter into solution and pass throughout the dissolving fluid. We performed the dissolution tests over a period of 240 min because under normal circumstances a drug is expected to remain in the stomach for 2–4 h and in the small intestine for 4–10 h (Ansel et al., 1995). The in vitro dissolution of a drug is dependent on the surface area of the drug particles and the concentration gradient of the drug in the medium. The dissolution of the drug increases with decrease in particle size, increase in the solubility of the drug and increasing the temperature of the medium. Furthermore, changes in the pH or the composition of the medium will influence the solubility and therefore the dissolution of the drug, so we compared dissolution at pH 1.2 (SGF) and pH 6.8 (SIF).

A mixed ANOVA model that included the capsule effect was used to test for significant differences in stampidine dissolution using different media conditions. Linear contrasts were performed comparing the effect of a pair of dissolution media at all time points (Table 1, overall model) and at specific time

Table 1
Linear contrasts to measure the effect of pH, enzyme and SDS

Comparison	Overall model	Mixed model ANOVA								One-way ANOVA	
		Minutes									
		5	10	30	60	90	120	180	240	*k*	*n*
Water vs. SDS											
SS	1.52	120.15	99.90	53.00	0.67	1.63	11.13	13.12	1.57	522.93	86.67
P-value	0.542	<0.001	<0.001	<0.001	0.679	0.520	0.094	0.069	0.528	<0.001	<0.001
Water vs. pH 6.8											
SS	0.25	4.83	5.50	10.03	2.49	0.59	1.53	47.52	70.86	705.90	89.00
P-value	0.803	0.269	0.238	0.112	0.426	0.698	0.533	<0.001	<0.001	<0.001	<0.001
Water vs. pH 1.2											
SS	26.68	4.61	9.70	61.90	119.68	169.74	180.05	144.41	79.13	471.65	8.20
P-value	0.019	0.280	0.118	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.013
Enzyme vs. pH 1.2											
SS	0.19	0.15	0.06	0.05	0.28	2.77	3.21	4.22	4.66	14.73	3.40
P-value	0.830	0.844	0.897	0.910	0.789	0.402	0.367	0.301	0.278	0.250	0.093
pH 1.2 vs. pH 6.8											
SS	19.73	0.01	0.44	16.59	65.78	112.84	161.26	268.47	217.35	17.65	32.40
P-value	0.039	0.968	0.737	0.041	<0.001	<0.001	<0.001	<0.001	<0.001	0.210	<0.001
MSE ($\times 10^3$)	3.92	3.92	3.92	3.92	3.92	3.92	3.92	3.92	3.92	10.34	1.07
Denominator DF	16	166	166	166	166	166	166	166	166	16	16

The specified linear contrasts were performed for the overall model (means pooled from all time points for each condition) and at specific time points using the mixed model ANOVA (time, condition and capsule effect). The mean square error (MSE) was obtained from the entire data set (MSE = 3.92×10^{-3}). Linear contrasts for 5 comparisons are shown for the overall model and 8 time points (5–240 min) for which the sum of squares (SS) and the *P*-values were calculated (*F*-test with Numerator degrees of freedom = 1, Denominator degrees of freedom as shown and *F*-statistic = SS/MSE). Linear contrasts were also performed for the two parameters ('*n*' and '*k*') using the one-way ANOVA model. Significant contrasts are in bold. The overall model with biggest SS value was with the water vs. pH 1.2 comparison ($P = 0.019$) suggesting that the capsule dissolution was most significantly distinguished in these two conditions. The time specific contrasts showed the largest SS values occurred at 180 min (SS = 268.47) for pH 1.2 vs. pH 6.8 comparison.

points (Table 1, 5–240 min). For the overall model, the means were evaluated under the condition effect, and the means for the specific time points were obtained from the time effect. The REML method ensured that the correct standard error estimates were used for the comparisons.

The statistical model showed that there were no significant differences between the overall dissolution profiles of stampidine capsules comparing water to SDS ($P = 0.5$), water to pH 6.8 ($P = 0.8$) and comparing pH 1.2 with and without enzyme ($P = 0.8$). Most significant differences were observed in the dissolution profiles comparing water to pH 1.2 (sum of squares (SS) = 19.7, $P = 0.019$) and pH 6.8 to pH 1.2 (SS = 26.7, $P = 0.039$). Examination of capsule dissolution at individual time points showed how the contribution of the SS varied across time for each of the five comparisons. Even though there is no overall effect in the water versus SDS comparison, there were highly significant effects early in the dissolution process (5–30 min, all $P < 0.001$), which then reduced at later time points (30–240 min). Comparison of pH 1.2 versus pH 6.8 showed differences between 30 ($P = 0.041$) and 240 min ($P < 0.001$ for all time points longer than 60 min). The most similar time profiles occurred for the Enzyme (pH 1.2) and the pH 1.2 comparison (no differences in dissolutions across all time points). The greatest number of differences across time points (6 out of 8) occurred when comparing water with pH 1.2 and between pH 6.8 and pH 1.2. The biggest difference in capsule dissolution was observed at 180 min in the pH 1.2 versus pH 6.8 comparison (SS = 0.268), suggesting that the drug showed significant differences in dissolution between simulated SGF and SIF. The observed differences in the SS contribution to the ANOVA model for each of the comparisons suggested that there were differences in the dissolution process in the different media.

3.4. Non-linear regression analysis using the power law to differentiate the dissolution process in different media

To better understand the dissolution process the data at each condition were fitted using non-linear regression to the power law. The results from the mixed ANOVA model suggested that the dissolution profile should be fitted for each individual capsule and then compiled according to the condition in which the capsule was dissolved. A representative fit of one capsule under each of the conditions ((Fig. 4A–E), values ranged from 0.889 to 0.995 for 21 capsules tested) showed that the power law fitted the data very well. All the dissolution exponent values are low for all conditions tested (i.e. <0.5) suggesting that the release of compound is not a controlling event. A value of 0.5 for 'n' suggests a Fickian diffusion model (this diffusion model has an equation in which the fraction of drug released is proportional to $k \times t^n$, where k represents the dissolution constant, t, the time of dissolution and n the dissolution exponent), whereas a value of 1 suggests a Zipf distribution (release is proportional to $k \times t$). The parameter values were compared using planned linear contrasts after transformation of the data using the Box–Cox algorithm. Use of the ANOVA model to compare the parameter values showed highly significant effect of dissolution condition on the dissolution rate constant 'k' (Fig. 5A, $r^2 = 0.96$, $F_{4,16} = 85.6$, $P < 0.0001$) and release exponent 'n' (Fig. 5B, $r^2 = 0.95$, $F_{4,16} = 82.82$, $P < 0.0001$).

This resulted in a very sensitive method to detect differences in the dissolution process in different media. In our studies we could not detect differences in the overall dissolution profile at water and SDS using the mixed model ANOVA. However, the mixed model ANOVA suggested that adding a capsule effect improved the model and therefore suggested that the non-linear fits should be performed on each of the capsules. The differences observed at early time points in the mixed ANOVA model (5–30 min) can be explained by significant differences in the release exponent and the dissolution rate constants for the water versus SDS linear contrast ($P < 0.0001$ for both k and n). However, the difference in the dissolution profile between pH 1.2 and 6.8 was explained by the increase only in the 'n' parameter for pH 6.8 (Fig. 5B, $n = 0.33$ for pH 1.2 and 0.48 for pH 6.8). Comparison of water versus pH 6.8 showed significant differences in dissolution at later time points (180–240 min, Table 1) because of the differences in both the dissolution parameters. Differences in the dissolution process explained the differences in capsule dissolution at different time points identified by the mixed model ANOVA.

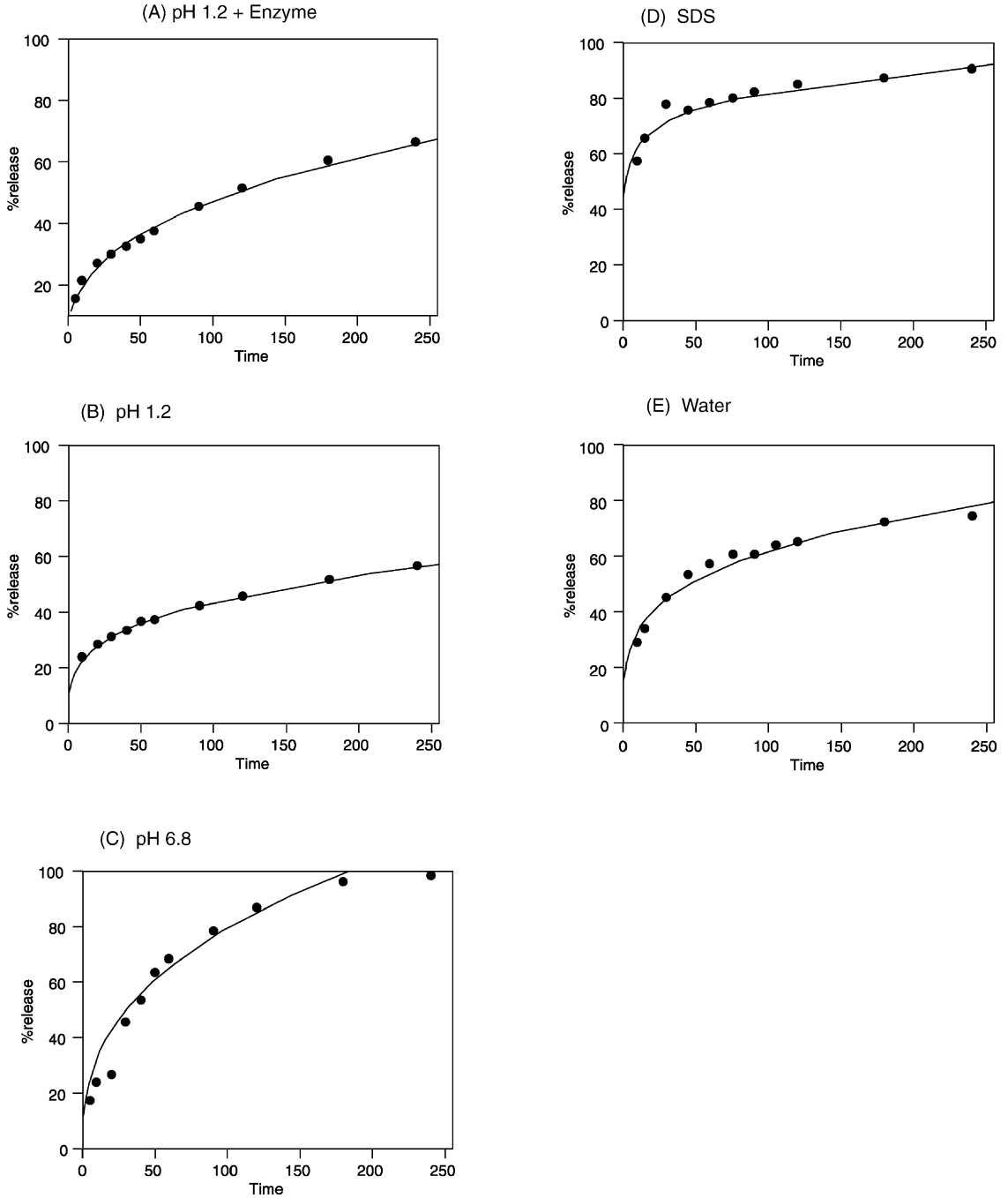
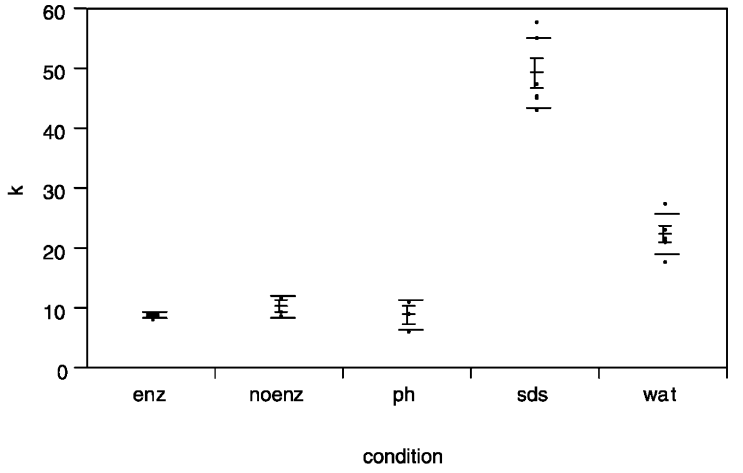


Fig. 4. Non-linear fit of the power law function to the release profile of each capsule. The amount of drug released across time was fitted using the power law function (solid line through the data points). Plots are shown for a single capsule in each of the conditions tested (A–E). The dissolution rate constant '*k*' and release exponent '*n*' were obtained for each capsule and the means for each condition were analyzed using one-way ANOVA. R^2 values for all the fits were greater than 0.889.

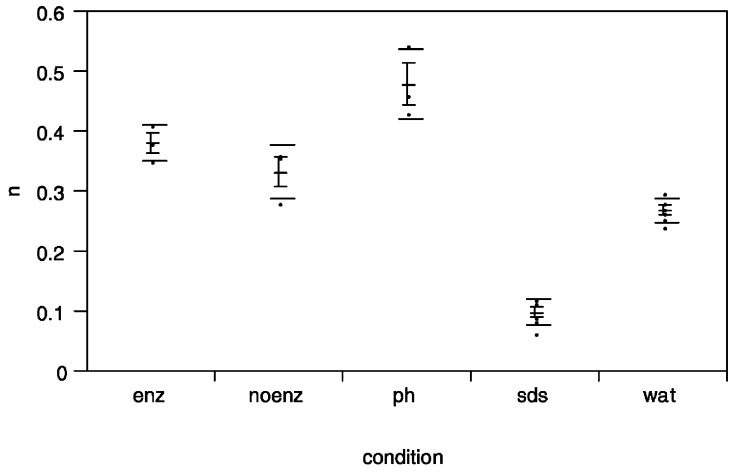
(A)



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
enz	3	8.7310	0.50219	0.2899	7.483	9.979
noenz	3	10.2013	1.70918	0.9868	5.955	14.447
ph	3	8.9167	2.54097	1.4670	2.605	15.229
sds	6	49.1962	5.95890	2.4327	42.943	55.450
wat	6	22.3715	3.20671	1.3091	19.006	25.737

(B)



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
enz	3	0.379000	0.030000	0.01732	0.30448	0.45352
noenz	3	0.331333	0.045347	0.02618	0.21869	0.44398
ph	3	0.478267	0.059026	0.03408	0.33164	0.62489
sds	6	0.097333	0.021708	0.00886	0.07455	0.12011
wat	6	0.267300	0.019701	0.00804	0.24662	0.28798

Fig. 5. Dissolution parameters for the five conditions. Means are shown with standard error bars (standard deviation shown by the outer lines) for capsules in each of the five conditions (enz = pH 1.2 + enzyme. noenz = pH 1.2, pH = pH 6.8, SDS, wat = water) for dissolution rate constant '*k*' (A) and release exponent '*n*' (B).

3.5. Release properties of the drug in different media as measured by the release exponent

Power laws are often used to analyze systems with different scaling properties. The dimensionless numbers and exponents associated with the power law can be used to extract the characteristics for a particular condition. Both '*k*' and '*n*' are dimensionless and characteristic of the dissolution medium. When the value of *n* equals 0 then the rate of release is proportional to the dissolution rate constant. However when the value *n* approaches 0.5 the system approaches Fickian diffusion model. In this study, the value of *n* varied from 0.098 to 0.478 while that of *k* varied from 8.73 to 49.2 and the fit to the power law equation gave a high correlation coefficient ($r^2 = 0.9$).

The highest value for *n* ($n = 0.478$) was observed when the dissolution medium pH was 6.8 and is closest to the Fickian diffusion model. The lowest value for *n* ($n = 0.097$) and the highest value for *k* ($k = 49.2$) were observed with SDS, suggesting that the SDS provides a significant effect on the dissolution profile due to wetting of the drug particles.

For the remaining three dissolution media the dissolution profiles lie between these limits dictated by the *n* and *k* values, suggesting that a combination of Fickian diffusion and the wetting of the drug particles dictate the drug dissolution by the dissolution medium. The values of '*n*' and '*k*' are dimensionless and characteristic of each system, so it should be possible to compare their values with values reported for other dissolution studies. In addition, the knowledge derived from the above exercise may shed more light on the application of the model to clinical relevance for a particular dosage form.

4. Conclusion

The present study was designed to identify the dissolution media and time point that showed the greatest difference in drug release from the capsules. A statistical model based on analysis of variances was used to discern the effect of media in dissolution taking into account different sources of variance. It was found that the use of ANOVA for the entire dissolution profile is a valid technique for detecting statistically significant differences in dissolution profiles. Planned linear contrasts provided us with a statistically powerful method of describing the dissolution at specific time points using the mean square estimate from the entire data set. From the ANOVA analysis of the dissolution data it was concluded that the presence of surfactant SDS did not have an overall significant effect on the dissolution of stampidine in water. Significant differences in release profiles were observed between water and pH 1.2 and between pH 6.8 and pH 1.2. Based on these results we conclude that analysis of variances is a useful statistical tool for identifying differences between media and time for investigating stampidine capsule dissolution. Examination of SS for each comparison across 8 time points showed that the most significant difference in capsule dissolution occurred at 180 min ($SS = 0.268$, $P < 0.001$) between simulated SGF (pH 1.2) and SIF (pH 6.8). Measuring the dissolution rate constant, *k*, and release exponent, *n*, for each capsule can be used as a sensitive method to determine capsule variation within a specific dissolution medium. In summary, drug dissolution in pH 6.8 or water resulted in significantly greater release compared to pH 1.2 at time points longer than 120 min. Under these conditions the drug showed significant release >75%. Therefore, 120 min was chosen as a test point to overcome inconsistencies in the dissolution profiles observed at earlier time points and permitted efficient processing of samples for HPLC analysis. Furthermore, the stability of the drug in water for more than 2 days enables us to confirm its release into the media using HPLC analysis.

References

Ansel, H.C., Popovich, N.G., Allen, Jr., L.V., 1995. Pharmaceutical dosage forms and drug delivery systems, 6th ed. Williams and Wilkins, Philadelphia, PA.

Bonferoni, M.C., Rossi, S., Ferrari, F., Stavik, E., Pena-Romero, A., 2000. Factorial analysis of the influence of dissolution medium on drug release from Carrageenan-Dilitizem complexes. AAS Pharm. Sci. Tech. 1.

Box, G., Cox, D., 1964. An analysis of transformations. JRSS B26, 211.

Brazel, C.S., Peppas, N.A., 1999. Dimensionless analysis of swelling of hydrophilic glassy polymers with subsequent drug release from relaxing structures. Biomaterials 20, 721–732.

Brazel, C.S., Peppas, N.A., 2000. Modeling of drug release from swellable polymers. Eur. J. Pharm. Biopharm. 49, 47–58.

Li, S., Lin, S., Chien, W.Y., Daggy, P.B., Mirchandani, L.H., 2001. Statistical optimization of floating system for oral controlled delivery of calcium. AAS Pharm. Sci. Tech. 2.

SAS Institute Inc., 1995. JMP statistics and graphic guide.

Siddiqui, A.Q., McGuigan, C., Ballatore, C., Wedgwood, O., De Clercq, E., Balzarini, J., 1999a. Simple mono-derivatisation of the aryl moiety of D4A and DDA-based phosphoramidate prodrugs significantly enhances their anti-HIV potency in cell culture. Bioorg. Med. Chem. Lett. 9, 2555–2560.

Siddiqui, A.Q., McGuigan, C., Ballatore, C., Zuccotto, F., Gilbert, I.H., De Clercq, E., Balzarini, J., 1999b. Design and synthesis of lipophilic phosphoramidate d4T-MP prodrugs expressing high potency against HIV in cell culture: structural determinants for in vitro activity and QSAR. J. Med. Chem. 42, 4122–4128.

Uckun, F.M., Vig, R., 2002. Aryl phosphate derivatives of d4T having anti-HIV activity. US Patent 6,350,736, 26.

USP., 2000. United States Pharmacopea 19.

Venkatachalam, T.K., Tai, H.L., Vig, R., Chen, C.L., Jan, S.T., Uckun, F.M., 1998. Enhancing effects of a mono-bromo substitution at the para position of the phenyl moiety on the metabolism and anti-HIV activity of d4T-phenyl methoxyalaninyl phosphate derivatives. Bioorg. Med. Chem. Lett. 8, 3121–3126.

Vig, R., Venkatachalam, T., Jan, S.-T., Uckun, F., 1998. Aryl phosphate derivatives of 3′-deoxythymidine are not potent ant-human immunodeiciency virus agents. Antiviral Chem. Chemother. 9, 439–443.

Ye, C., Liu, J., Ren, F., Okafo, N., 2000. Design of experiment and data analysis by JMP (SAS institute) in analytical method validation. J. Pharm. Biomed. Anal. 23, 581–589.