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On The Factors That Affect Concentration Variations in A Veterinary Drug Using Backward Stepwise Regression Analysis

Omar Bataineh*, Musaab Abu-Fares, Murad Al-Jdayeh

Department of Industrial Engineering, Jordan University of Science and Technology, Irbid, Jordan

***Corresponding author:** Bataineh O, Department of Industrial Engineering, Jordan University of Science and Technology, Irbid, Jordan; Tel: 96 2798701091; E-Mail: omarmdb@just.edu.jo

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ABSTRACT

This work investigates the case of controlling the variability in the concentration of Citrus oil, a key ingredient used in the manufacturing of an oral solution veterinary drug produced by the Arab Company for Manufacturing Veterinary and Agricultural Products Ltd. (ARVET) in Ramtha, Jordan. The problem occurs as the drug is produced in large batches that comprise a number of ingredients and mixed together. Factors such as batch size, mixing time, and pH of the pure solution are thought to have an effect on homogeneity of the mixture and thus concentration variability in the final product. Although numerous ingredients are used in the drug of interest such as Bromhexine HCl, Eucalyptus oil, Thymol and Camphor, the focus in this study is restricted to the Citrus oil ingredient as a first-stage investigation. The study will employ the power of statistical techniques such as backward stepwise regression (BSR) analysis to identify the most influential factors. Besides, the best setting of the confirmed influential factors will then be determined.

Keywords: Backward stepwise regression; Test for equal variances; Citrus oil concentration; Veterinary drug

INTRODUCTION

Drug development and validation is a lengthy, complex and expensive process [1]. The use of the right approach including statistical methods is vital to achieve goals in a timely manner with optimal quality and at lower costs. Given the nature of drug research and development, which heavily relies on experimental work, uncertainty is inherent in its outcomes. This requires the use of statistical methods in dealing with such scenarios, where randomness is, to some extent, an important aspect of the results [2],[4]. Thus, the literature is rich in studies that deal with drug research and development relying on statistical tools such as regression methods [5]–[7].

For example, Robert et al. [8] employed multiple linear regression to predict the efficacy of an anticancer drug based on molecular, cellular and clinical data. Damte et al. [9] evaluated different linear regression statistical approaches for withdrawal time estimation of veterinary drugs. Yang et al. [10] applied cross validation to stepwise regression algorithms to predict critical quality attributes (CQAs) distributions on a fed-batch cell culture process that was designed to produce mono-clonal antibody using a stable clonal cell line.

Moreover, Yabuta et al. [11] developed regression models for designing rapid and effective H2O2 decontamination processes in the manufacturing of sterile drug products. Goodarzi et al. [12] proposed a Quantitative Structure-Retention Relationship (QSRR) to estimate the chromatographic retention of 83 diverse drugs based on linear and nonlinear regression models.

In this study, the aim is to perform backward stepwise regression analysis for a veterinary drug to minimize variability in the concentration of its ingredients. Test for equal variances will also be utilized to further assess the findings from the regression analysis.

METHODOLOGY

Backward stepwise regression method is based on the assumption that a statistical linear relationship exists between a dependent variable y and a set of independent variables x1, x2, …, xk. A multiple linear regression model is then defined to describe such relationship and to explain the variability that exists in a random sample of n data points. As the xj (j = 1, 2, …, k) variables are not equally important or correlated to y, the aim is to verify which of these variables is least significant in terms of its effect on y and thus should be dropped from the initial model. The method then proceeds as follows:

Form a multiple linear regression model with the full list of independent variables according to

$$Y = \beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k + \epsilon$$

Where βj (j = 0, 1, …, k) are unknown regression coefficients and is a random error term which is assumed as normally and independently distributed with zero mean and constant variance.

Calculate the partial F–statistic value for each independent variable according to

$$F_j = \frac{SS_R(\beta_j \mid \beta_k, \ldots, \beta_0 \text{except} \beta_j)/1}{MS_E(x_1, x_2, \ldots, x_k)}$$

Then remove the one with the smallest partial F–statistic value if the corresponding P–value is greater than the probability of type I error (α).

Fit the new (k – 1) model and repeat the previous step.

The procedure continues stepwise until no further variables could be removed from the regression model.

The variables that stay in the model represent the ones that have a significant effect on the dependent variable y. These variables can then be optimized to achieve the best outcome for the response. The above procedure is programmed in various statistical software packages, such as Minitab®, which was used in this study.

THE CASE STUDY

The Arab Company for Manufacturing Veterinary and Agricultural Products Ltd. (ARVET) in Ramtha, Jordan; is specialized in the area of veterinary drugs such as antibiotics, vitamins, amino acids, feed additives, and disinfectants. Arsilvon®, an oral solution, is their best-selling drug, which helps improve the immunoglobulin level in airway secretions. Thus, it is prescribed as an Ancillary Therapy (AT) for the management of Bronchopneumonia in cattle and poultry, as well as for the treatment of amniotic fluid aspiration in newborn calves [13]. However, the effectiveness of this drug largely depends on the concentration control of its key ingredients such as Citrus oil, Bromhexine HCl, Eucalyptus oil, Thymol and Camphor. This requires determining the best settings for the variables that have an effect on the mixing process of these ingredients. Variables of potential influence such as batch size, mixing time, and pH of the pure solution were studied. As a first-stage investigation, this work covers the case of the Citrus oil ingredient. The study relies on the use of backward stepwise regression analysis as the primary method for reaching the goals of this study.

DATA COLLECTION

All experiments were conducted at the Quality Control Lab of ARVET over a period of four months. During that time, Arsilvon® mixes were prepared in batch sizes ranging between 100 and 550 Liters, depending on forecasted demand. Accordingly, mixing time (hour), pH of the pure solution, and concentration of all ingredients including Citrus oil were all reported. Forty six of collected data points are summarized in Table 1. It should be noted that concentrations in this table are reported as percentages of the target value, rather than the actual concentrations. Thus, a 100% concentration is considered ideal. Based on the quality guidelines for Arsilvon®, if any concentration of an ingredient deviates from its target by more than ±10%, then the mix is rejected.

Reviewing the data in Table 1, it can be noticed that all mixes are very acidic, since pH ranges between 3.02 and 4.68. Also, mixing time is, to a large extent, proportional to the batch size. The most common ratio of batch size to mixing time, although not fixed, is equal to 100 L/hour. But this value can be as high as 125 L/hour or as low as 50 L/hour.

Table 1: PRODUCTION INFORMATION ON THE PAJAMA LINE

Citrus Oil Concentration	Batch size	Mixing Time	Solution
	(Liter)	(hour)	pH
102.40%	450	5	3.5
101.70%	500	5	4.23
101.20%	500	4	3.42
101.20%	350	3.5	3.02
101.20%	100	2	3.4
100.70%	300	3	3.38
101.20%	500	5	4.45
101.60%	500	5	3.02
100.10%	550	5	4.68
101.50%	450	5	3.45
100.00%	500	5	3.4
101.10%	450	5	3.5
101.50%	300	3.5	3.45
100.00%	450	5	3.4
101.70%	500	5	3.56
103.40%	300	3	4.1
101.40%	350	4	3.6
101.00%	300	3.5	3.4
103.80%	450	5	3.42
101.20%	450	5	3.7
101.80%	400	4.5	3.62
100.30%	500	5	4.1
100.90%	300	3	3.4
99.90%	350	3	4.6
100.50%	350	3.5	4.2
102.60%	300	3	3.8
103.00%	500	5	3.61
101.50%	300	3.5	4.5
100.80%	450	4.5	4.1
100.60%	350	3.5	3.7
100.40%	350	3.5	3.51
100.70%	350	3.5	3.9
101.80%	400	4	4

102.70%	450	4.5	3.7
102.00%	300	3	4.6
101.59%	500	5.5	4.66
100.42%	250	2.5	3.85
100.36%	300	2.5	3.42
100.89%	450	4	3.62
100.93%	400	3.5	3.25
101.54%	550	5.5	4.22
100.61%	350	3	3.37
100.47%	500	4.5	4.67
101.12%	550	5	3.98
101.08%	200	2.5	3.23

ANALYSIS OF RESULTS

Backward Stepwise Regression Analysis

Backward stepwise regression procedure outlined above requires that data and associated errors be normally and independently distributed and have a constant variance. This is usually assessed by examining the residuals that result from the difference between the actual observations (citrus oil concentration data of Table 1) and fitted observations (based on the linear model in Eq. (1)). Thus, a frequency histogram of the residuals and a plot of these residuals against observation order were generated with the aid of Minitab®, as shown in Figures 1 and 2, respectively. The histogram reveals that the residuals are approximately bell-shaped, which is indicative of a normal distribution. Plot of the residuals against the observation order shows no signs of unusual patterns or non-constant variability. Therefore, the basic assumptions for the backward stepwise regression procedure can be considered valid.

The results of backward stepwise regression procedure, which were obtained using Minitab®, are shown in Table 2. This table lists the estimated regression coefficients against each regressor variable included in a current step of the stepwise procedure and the corresponding P-value.

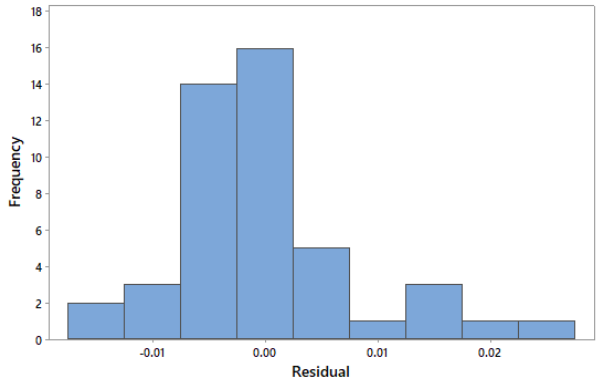

Figure 1: Histogram of the residuals obtained from regression analysis.

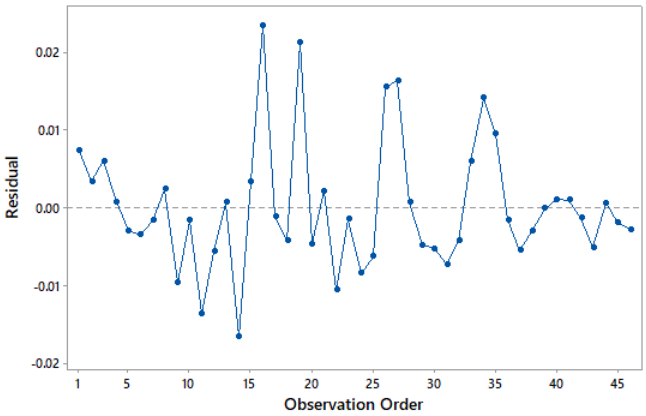

Figure 2: Plot of the residuals against the observation order.

TABLE 2: BACKWARD STEPWISE REGRESSION RESULTS

	Step 1		Step 2	
	Coef	P	Coef	P
Constant	1.0077		1.00577	
Batch size(L)	-5.9E-05	0.075	-6.1E-05	0.059
Mixing time(Hrs.)	0.00755	0.033	0.00763	0.028
pH of pure solution	-0.0006	0.835		

Any P-value that is greater than 0.1 corresponds to a regressor variable that is not significant and has negligible effect on the response variable, i.e. the citrus oil concentration. Therefore, step 1 of the backward stepwise procedure reveals that pH of the pure solution (P-value equals 0.835) is not influential and should be dropped from the regression model. In step 2, both batch size and mixing time are confirmed significant and should stay in the regression model. Accordingly, the citrus oil concentration (denoted COC) can be estimated as

$$COC = 1.00577 - 0.000061BS + 0.00763MT$$

Where BS denotes the batch size and MT denotes the mixing time. Equation (3) can be very beneficial for predicting the required mixing time for a given batch size to achieve the desired concentration of citrus oil. For instance, if a batch size of 400 Liters is to be used, and the target concentration of citrus oil is ideally at 100%, substituting the two values into Eq. (3) gives 2.442 hours for mixing time. However, this does not mean that a 100% concentration of citrus oil will be achieved in a given test run, but the average of actual concentrations will be around 100% if a batch size of 400 Litres is used with mixing time of 2.442 hours.

Test for Equal Variances

One important question arises from the previous discussion, which is whether the proportionality that exists in the test runs

for Arsilvon® mixes between the batch size and mixing time matters to the concentration of citrus oil or not. One possible approach to look into this is to examine the variability in citrus oil concentrations for various ratios of batch size to mixing time. Thus, three ratios of batch size to mixing time were chosen based on the data collected and presented in Table 1. These are 90, 100, and 110 L/hour. A test for equal variances can then be conducted on data points for each of the three ratios. The results obtained with the aid of Minitab® are summarized in Table 3 and Fig. 3.

TABLE 3: STANDARD DEVIATION AND CONFIDENCE INTERVAL FOR THE CITRUS OIL CONCENTRATION

Ratio	N	St. Dev	CI
90	7	0.011859	(0.0040155, 0.0532777)
100	22	0.009424	(0.0066873, 0.0149026)
110	4	0.004521	(0.0010196, 0.0499398)

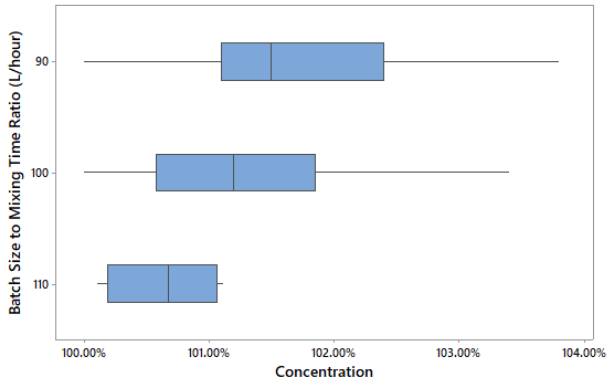

Figure 3: Boxplot of citrus oil concentration for different ratios of batch size to mixing time.

Table 3 lists the standard deviation and confidence interval for the citrus oil concentration at each of the three ratios of batch size to mixing time. It can be concluded from this table that the standard deviation of the citrus oil concentration is minimum when the batch size to mixing time ratio is maximum (110 L/hour). The same conclusion can be reached from the boxplot of Fig. 3. This figure, however, suggests that the difference in variability between the citrus oil concentrations at the three batch size to mixing time ratios is statistically insignificant. But the difference in variability between the 90 and 110 L/hour is quite noticeable.

CONCLUSIONS

The results of this study suggest that both batch size and mixing time have significant effect on citrus oil concentration, whereas pH of the pure solution has a negligible effect. This indicates that controlling citrus oil concentration in the shipped Arsilvon® drug requires to properly setup the mixing time for a given batch size. This was illustrated through an example based on the use of the developed regression equation, as given by Eq. (3). In addition, the difference in variability between the citrus oil concentrations at different batch size to mixing time ratio was found statistically insignificant. However, this is only valid for the tested range of batch size to mixing time ratio, i.e. 90 to 110 L/hour.

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