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Robustness testing, using experimental design, of a flow-through dissolution method for a product where the actives have markedly differing solubility properties

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Abstract

The use of experimental design for the robustness testing of a flow-through dissolution method (Ph Eur/USP Apparatus 4) for atovaquone, one of the drug substances in a dual-active anti-malarial tablet formulation, Malarone tablets, is described. This procedure was developed to overcome the suppression of the atovaquone solubility, caused by the presence of the co-drug proguanil hydrochloride and potential imprecision due to the poor solubility of the coating material in the basic dissolution media employed. For this testing a quarter fractional two-level factorial design was applied, assessing six factors in sixteen experiments, with a further six centre points to assess natural experimental variation. Results demonstrate that the method is robust to small changes in all the main factors evaluated at sample times of 30 min or greater. At 15 min, variations in the concentration of sodium hydroxide in the dissolution media, peristaltic pump speed and flow rate were assessed as statistically significant. This observation is a result of the initial steepness of the dissolution release curve and hence these factors are now controlled routinely in the method. Release of this poorly soluble drug is limited at the 45 min time point ($Q = 75\%$) according to pharmacopoeial guidelines. The approach may be applied for other dissolution procedures.

Keywords: Flow-through dissolution; Malarone; Experimental design; Factorial design Atovaquone; Proguanil hydrochloride

1. Introduction

GlaxoWellcome's Malarone[1] tablets are registered and approved for the treatment of *Plasmodium falciparum* malaria. The tablets contain the actives atovaquone and proguanil hydrochloride, and are available in two strengths containing 250 mg atovaquone and 100 mg proguanil hydrochloride or 62.5 and 25 mg of each drug, respectively. The higher strength tablet is manufactured for adult and the lower strength tablet for paediatric use. Dissolution testing for the active ingredients is routinely applied as a control on the rates of drug release. Proguanil hydrochloride is assessed

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[1] Malarone is a trademark of the GlaxoWellcome group of companies.

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by a conventional USP Paddle approach employing water as solvent. Atovaquone is only marginally soluble in water and 0.1 M HCl (< 0.0002 mg/ml) but is soluble in 0.1 M NaOH (4.6 mg/ml at 25°C). Conventional paddle dissolution for atovaquone in 0.1 M NaOH is not possible, however, as the solubility of this drug is suppressed to below sink conditions (typically 40% release after 45 min) by the presence of proguanil hydrochloride. In addition, the hypromellose film coat is not soluble in basic media. A method was hence developed for atovaquone using the Ph Eur flow-through apparatus (USP Apparatus 4) and details of this novel procedure have been reported in a previous paper (Butler and Bateman, 1998).

The tablets are first subjected to a 1 h wash with water, which disperses the tablets and dissolves the interfering proguanil hydrochloride and film coat, followed by a switch to 0.1 M NaOH to dissolute the atovaquone. This approach has been investigated and validated by Butler and Bateman, but the work provides no information on the robustness of the methodology with respect to the various factors associated with both water and caustic washes. It was hence decided to use experimental design (Carlson, 1992) to investigate further this relatively complex procedure and provide an assessment of the method robustness. The use of experimental design has been previously reported for robustness testing of analytical methods (Box et al., 1978) and also method optimisation (Morgan, 1991; Vannecke et al., 1999).

A two-level quarter fractional factorial design was employed to examine six potentially significant factors within a total of 16 experiments, and an additional six centre points (experiments performed using method conditions) to assess natural experiment to experiment variability. Details of this work follow.

2. Experimental

2.1. Apparatus

All work was carried out using the following equipment from Sotax (Basingstoke, UK); a CE6 Dissotest, a CY7 piston pump and an MSV-6 media switcher. In addition, an Ismatec (Weston-Super-Mare, UK) IPS peristaltic pump and a Beckman (High Wycombe, UK) DU7400I diode array UV/Vis spectrophotometer equiped with an eight-position cell holder were used.

2.2. Dissolution media

Sodium hydroxide (0.1 M) solution was prepared from sodium hydroxide pellets obtained from BDH (Poole, UK). Deionised water was used throughout.

2.3. Procedure

The CE6 Dissotest houses six vertically mounted cells maintained at the test temperature by a heated water jacket. Each cell was prepared by placing a 5 mm ruby bead in the apex of the cone to protect the inlet tube and filling the cone with 1 mm glass beads to create laminar media flow. For this test, 22.6 mm diameter flow cells were used. The tablets were positioned in each cell on the layer of glass beads. To carry out the test, water was conveyed to the cells from the media reservoir by the CY-7 piston pump. After 1 h the medium was switched to 0.1 M sodium hydroxide. Both media were pumped at a flow rate of 16 ml/min, or as dictated otherwise by the experimental design experiments. Undissolved solid material was retained in the cells by a series of filters housed in the filter head on the top of the cell. This consisted of Whatman (Maidstone, UK) GF/F, GF/B, GF/C, GF/A and GF/D filters in order, held by a plug of glass wool, to retain the bulk of excipient material. The effluent from the cell was split using a glass T-piece and a representative fraction passed through the spectrophotometer for measurement of the UV absorbance of atovaquone in 5 mm cells at 487 nm. Solvent debubblers (Kontron Instruments, Watford, UK) placed between the peristaltic pump and the spectrophotometer removed air-bubbles from the system. Analysis was carried out against a nominal 0.112 mg/ml atovaquone standard solution prepared in 0.1 M sodium hydroxide. Both the main effluent and split fraction were passed to waste.

2.4. Experimental design software

All experimental design work was carried out using 'Design Expert 5' (DX5) software by Stat-Ease (Minneapolis, USA).

3. Results and discussion

3.1. Previous work and choice of design

In sodium hydroxide solution the weakly acidic –OH group of the atovaquone molecule ionises, ensuring theoretical sink conditions. However, this solubility is suppressed by the presence of the co-drug, proguanil hydrochloride, which is therefore removed using an initial wash with water. This has the added benefit of removing the hypromellose film-coat, which is insoluble in alkaline conditions. The influence of variables such as sodium hydroxide concentration (0.05, 0.1 and 0.15 M) and flow rate (8 and 16 ml/min) on the dissolution rate has been previously studied (Butler and Bateman, 1998). The dissolution of atovaquone in 0.05 M sodium hydroxide was found to be slower than that in 0.1 M sodium hydroxide whilst dissolution in 0.15 M was higher. A total of 0.1 M sodium hydroxide was selected as the most discriminating. The dissolution release for a flow rate of 8 ml/min was slower in 0.1 M NaOH compared to 16 ml/min (64% compared to 96% released after 120 min). A total of 16 ml/min was the preferred flow rate. These limited experiments provide 'a feel' for the importance of factors affecting the dissolution of atovaquone. It was considered, however, that a more comprehensive examination was necessary to assess the robustness of the methodology with respect to a wider range of factors (see below). A quarter fractional two-level factorial design was chosen to gain maximal information from a small and practical number of experiments.

This design allows six factors to be examined within a total of 16 experiments. The small number of experiments imposes some restrictions on the estimation of two and three factor interactions (see below) but the influence of primary factors can be clearly estimated from a quarter fractional two-level factorial design. It was also considered useful to incorporate six centre point experiments (nominal method conditions) to provide a measure of the natural experiment to experiment variability.

The flow-through dissolution method analyses samples continuously, but for the purpose of this investigation it was considered that examination of data taken at typical dissolution time points of 15, 30 and 45 min, would be adequately representative. The time taken for the initial 60 min aqueous wash has been ignored from all quoted sample times. This has been evaluated previously and found to be robust in the work by Butler and Bateman.

The factors chosen and the range over which these were examined are presented in Table 1.

All experimental work was carried out on a single batch of paediatric tablets and the results obtained were taken as the mean of six tablet results examined using the relevant set of conditions. Both paediatric and adult products are manufactured to the same granule formulation and the results are therefore applicable to both products.

The design layout and resulting data, are given below in Table 2.

This quarter fractional two-level factorial design has minor limitations from aliasing of the primary factors with three factor interactions, and also aliasing between sets of two factor and between sets of three factor interactions. This is a reeesolution IV design ($2IV^{6-2}$) and details are given below in Table 3.

These alias restrictions are not substantive however. The three factor interactions are considered

Table 1
Factors and range chosen for the robustness experiment

Factor	−1 level	+1 level
NaOH conc. (M)	0.09	0.11
Peristaltic pump speed (%)	5.0	25.0
Sample interval (s)	50	70
Flow rate (ml/min)	14.0	18.0
Tubing delay (s)	0	50
Degas on-line? (Y/N)	N	Y

Table 2
Design layout and entry data for the robustness experiment[a]

Design variables							Mean % released at given time (min)		
Std no.	NaOH conc. (M)	Peristaltic pump speed (%)	Sample interval (s)	Flow rate (ml/min)	Tubing delay (s)	Degas on-line? (Y/N)	15	30	45
1	0.09	5.00	50.00	14.00	0.00	N	61.0	84.0	89.8
2	0.11	5.00	50.00	14.00	50.00	N	78.1	96.4	100.1
3	0.09	25.00	50.00	14.00	50.00	Y	68.8	85.1	89.0
4	0.11	25.00	50.00	14.00	0.00	Y	79.0	91.1	94.3
5	0.09	5.00	70.00	14.00	50.00	Y	67.0	91.2	95.5
6	0.11	5.00	70.00	14.00	0.00	Y	79.3	95.5	98.8
7	0.09	25.00	70.00	14.00	0.00	N	75.2	88.9	92.3
8	0.11	25.00	70.00	14.00	50.00	N	72.9	90.6	93.9
9	0.09	5.00	50.00	18.00	0.00	Y	77.4	94.4	97.5
10	0.11	5.00	50.00	18.00	50.00	Y	68.0	86.3	90.8
11	0.09	25.00	50.00	18.00	50.00	N	80.1	90.8	94.4
12	0.11	25.00	50.00	18.00	0.00	N	87.1	94.7	95.9
13	0.09	5.00	70.00	18.00	50.00	N	72.1	88.4	93.2
14	0.11	5.00	70.00	18.00	0.00	N	74.6	91.6	95.5
15	0.09	25.00	70.00	18.00	0.00	Y	76.8	88.7	93.0
16	0.11	25.00	70.00	18.00	50.00	Y	86.7	93.4	94.6
17	0.10	15.00	60.00	16.00	0.00	Y[b]	76.9	92.5	96.0
18	0.10	15.00	60.00	16.00	50.00	Y[b]	76.2	86.9	90.8
19	0.10	15.00	60.00	16.00	0.00	Y[b]	80.8	93.5	95.8
20	0.10	15.00	60.00	16.00	50.00	Y[b]	81.4	93.0	95.7
21	0.10	15.00	60.00	16.00	0.00	Y[b]	76.7	91.6	94.4
22	0.10	15.00	60.00	16.00	50.00	Y[b]	78.0	92.2	96.2

[a] Unrounded data was entered into the sofware.

[b] Centre point data. The presence of on- or off-line degassing was entered as a numerical variable for this experiment; entry as a categorial factor would otherwise require an excessive total of 12 centre points. This is of no significance however, since initial work with this design confirmed that the means of degassing was not critical, as long as degassing was performed.

Table 3
Aliases resulting from the use of this quarter fractional two-level factorial design[a]

[A] = A + BCE + DEF, [B] = B + ACE + CDF,
[C] = C + ABE + BDF,
[D] = D + AEF + BCF, [E] = E + ABC + ADF,
[F] = F + ADE + BCD,
[AB] = AB + CE, [AC] = AC + BE, [AD] = AD + EF,
[AE] = AE + BC + DF, [AF] = AF + DE, [BD] = BD + CF,
[BF] = BF + CD, [ABD] = ABD + ACF + BEF + CDE,
[ABF] = ABF + ACD + BDE + CEF

[a] These aliases are applicable to a quarter fractional two-level factorial resolution IV design and were generated using the DX5 software package. For a key to the terms involved see Figs. 1–3.

negligible and we are primarily interested in single factor or two factor interactions which have a large effect. These are clearly evident using this quarter fractional factorial two-level screening design.

3.2. Results and comments

A statistically significant model explaining 75% of the variance in the data was generated for the results at the time point of 15 min. A table of the analysis of variance (ANOVA) statistics for the 15 min time point is given in Table 4 and the resulting model equation in Fig. 1. The factor AB has been included to aid model heiracy, but the interaction ABF is not statistically significant and is therefore not included.

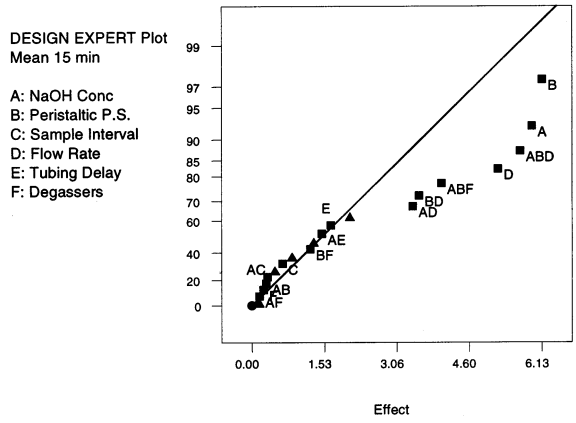

Fig. 1. Half normal plot for the 15 min samples. The 15 min equation in terms of coded factors (the interaction AB is included to ensure model hierarchality): mean 15 min = 3.0A + 3.1B + 2.6D + 0.15AB − 1.7AD + 1.8BD + 2.8ABD + 75.3.

No significant factors were identified at the 30 and 45 min time points and no model was generated. The resulting half normal percent probability plots are given in Figs. 1–3. These plots aid in the identification of the critical factors.

The triangular points indicate centre point data; experiments carried out using the method conditions and indicative of natural experiment to experiment variation. Only those factors which lie away from a line drawn through these points and the majority of experimental data, can be considered statistically significant, i.e. not explained by

Table 4
ANOVA and related statistics for the 15 min time point model

Source	Sum of squares	Degrees of freedom	Mean square	*F* Value	Prob > *F*
Model	623.63	7	89.09	9.36	0.0003
Curvature	40.84	1	40.84	4.29	0.0588
Residual	123.78	13	9.52		
Lack of fit	99.10	9	11.01	1.78	0.3025
Pure error	24.68	4	6.17		
Cor total	788.25	21			
Root MSE	3.09		R^2	0.8344	
Dep mean	76.09		Adj R^2	0.7452	
C.V.	4.06		Pred R^2	0.4529	
Press	431.29		Adeq precision	11.611	

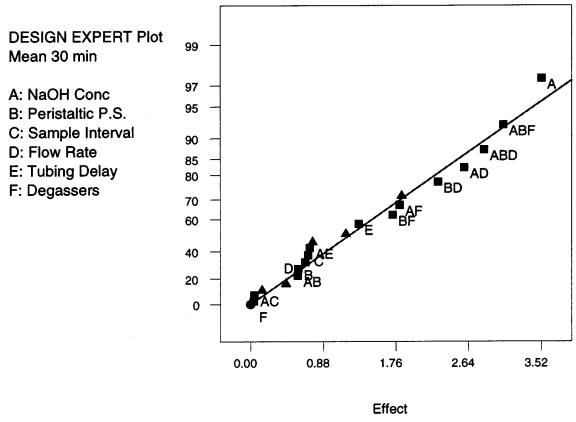

Fig. 2. Half normal plot for the 30 min samples.

natural experimental variation. At the early sample time of 15 min a variety of factors are distant from the line and are hence considered significant, principally the sodium hydroxide concentration, peristaltic pump speed and flow rate. Significant two and three factor combinations of these variables are also evident. This is to be expected early in a dissolution run where small variations in conditions may have an exagerated effect due to the initial steepness of the dissolution release curve. A dissolution profile for a typical batch of Malerone tablets (six individual tablets examined) is given in Fig. 4.

At longer sample times of 30 and 45 min the factors lie close to the line and are primarily

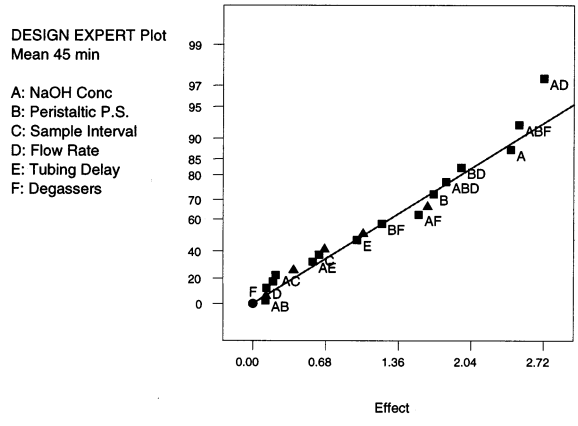

Fig. 3. Half normal plot for the 45 min samples.

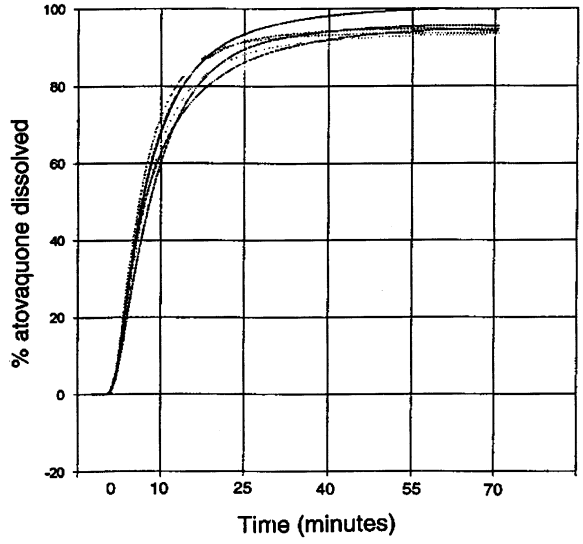

Fig. 4. A dissolution profile for a typical batch of Malerone tablets (six individual tablets examined). The time taken for the initial 60 min wash with water has been excluded from the above.

accountable to natural experimental variation. It is considered that the dissolution method is robust to small variations in conditions at these longer sample times. Dissolution of atovaquone is routinely controlled at $Q = 75\%$ at the 45 min time point.

It must be stressed that in this investigation, the factors of sodium hydroxide concentration, peristaltic pump speed and flow rate have been varied over a wider range (at least $\pm 10\%$) than would normally be expected in normal laboratory variations.

4. Conclusions

The robustness of a flow-through dissolution method for atovaquone in a two component antimalarial tablet formulation has been assessed using experimental design. A quarter fractional two-level factorial design resolution IV was employed, assessing six factors in a total of 16 experiments, with a further six centre points to assess natural experimental variation. Results conclude that the flow-through dissolution method may be

considered robust to changes in all the main parameters evaluated at sample times of 30 min and above. At 15 min, the concentration of sodium hydroxide in the dissolution media, peristaltic pump speed and flow rate were assessed as statistically significant. This observation is considered to be a result of the initial steepness of the dissolution release curve and hence these factors are routinely controlled in the method; release of this poorly soluble drug is limited at the 45 min time point ($Q = 75\%$) according to pharmacopoeial guidelines. The approach may be applied for other dissolution procedures.

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