ELSEVIER

International Journal of Pharmaceutics 173 (1998) 211–219

international journal of pharmaceutics

A flow-through dissolution method for a two component drug formulation where the actives have markedly differing solubility properties

W.C.G. Butler *, S.R. Bateman

Pharmaceutical Development Sciences, GlaxoWellcome, Temple Hill, Dartford, Kent, DA1 5AH, UK

Received 19 June 1998; accepted 8 July 1998

Abstract

A flow-through dissolution method has been developed for the routine testing of an anti-malarial tablet formulation containing two actives having markedly differing solubility properties. The flow-through apparatus was used because the dissolution rate of one of the components in the selected dissolution fluid using the EP/USP paddle apparatus was found to be poor. This was found to result from the formation of non-sink conditions caused by the presence of the other component in the formulation. We describe a test in which the component shown to exert the adverse affect is removed prior to measuring the dissolution rate of the affected component. This was achieved by taking advantage of the markedly differing solubility properties of each component in different dissolution fluids (water and 0.1 M sodium hydroxide). After a specified time in the test, the dissolution fluid is switched from water to sodium hydroxide to allow the dissolution of the affected component to occur. The results obtained were found to be satisfactory with dissolution rates of over 90% obtained. The effect of flow rate and sodium hydroxide concentration was investigated. The results showed that both factors had a significant effect on the dissolution rate of the component of interest.

Keywords: Flow-through dissolution; Malarone®; Tablet; Atovaquone; Proguanil hydrochloride

1. Introduction

GlaxoWellcome's Malarone®[1] tablets are registered and approved for the treatment of *Plasmod-*

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

* Corresponding author.

0378-5173/98/$ - see front matter
PII S0378-5173(98)00238-5

ium 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.

The constituents of Malarone® tablets interfere with two different pathways involved in the biosynthesis of pyrimidines required for nucleic acid replication. Atovaquone is a selective and potent inhibitor of parasite mitochondrial electron transport. Proguanil hydrochloride primarily exerts its effect by means of the metabolite cycloguanil, a dihydrofolate reductase inhibitor.

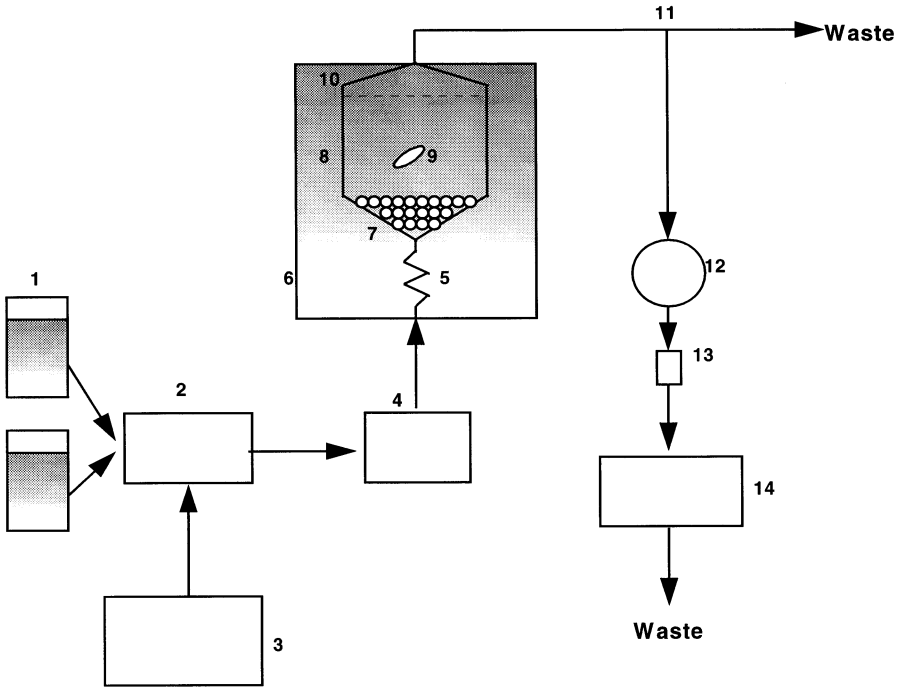
The work in this publication concentrates on the development of a suitable dissolution method for atovaquone using the Ph Eur flow-through apparatus (USP Apparatus 4). This was required because the dissolution rate for atovaquone obtained using the EP/USP paddle apparatus was found to be poor, with only 40% being released after 45 min in 0.1 M sodium hydroxide. Investigation has shown that the release rate of atovaquone is adversely affected by the presence of proguanil hydrochloride which causes the formation of non-sink conditions, making the use of the EP/USP paddle apparatus as a routine method of testing for atovaquone in this product unsatisfactory.

The advantages of the flow-through apparatus have been emphasised repeatedly (Post and Speiser, 1980; Talukdar and Plaizier-Vercammen, 1992). Of these, one significant advantage is that sink conditions are maintained which are independent of drug solubility, and therefore the apparatus is particularly suited as a test for drugs with low solubility. Another advantage is the ability to change pH conditions easily during the dissolution test. With these properties the flow-through apparatus presents a useful alternative to other compendial dissolution methods.

Applications of the flow-through apparatus have been reported for a variety of pharmaceutical dosage forms. These include the study of the influence of the amount of hard fat in suppositories on the in vitro release rate and bioavailability of paracetamol and codeine (Gjellan et al., 1994) and the evaluation of the potential use of pectin/ethylcellulose combinations as suitable film-coating agents for colonic delivery (Wakerly et al., 1994). The flow-through apparatus has been shown to be particularly useful in the design and evaluation of solid oral sustained release formulations (Möller and Langenbucher, 1982; Chaudhary et al., 1994). Critical evaluation (Nicklasson et al., 1991) of the flow-through apparatus against the EP/USP paddle apparatus has been made by comparing the in vitro dissolution of phenacetin crystals. It was found that the flow-through apparatus showed less variation compared to the EP/USP paddle apparatus and to be less dependent on the hydrodynamics and amount of substance tested. It has also been reported (Aiache et al., 1987; Phillips et al., 1989) that good correlation between in vitro drug release and in vivo drug absorption can be established.

The factors affecting drug dissolution using the flow-through cell apparatus have been investigated (Zhang et al., 1994). From the work reported it has been shown that the linear flow velocity of the dissolution medium is a major parameter defining the hydrodynamic 'agitation' of the dissolution fluid. In general, it has been shown that the greater the flow rate of media through the flow cell the faster is the dissolution rate. It has also been shown that the position of the drug dosage form in the flow cell is important and it has been found that drug release from horizontally positioned tablets is different from vertically positioned tablets. The study also looked at the use of glass beads and found that their use made the flow pattern of fluid more laminar, helping to avoid agitation which could have an impact on drug dissolution.

The flow-through apparatus can be operated in either an 'open-loop' or 'closed-loop' mode, and the choice of system configuration used will invariably be dependent on the dosage form. For example, a low strength dosage form can be tested using a 'closed-loop' system in which a small volume of media is re-circulated through the system to provide sample concentration levels sufficient for assay. This approach has been applied to the development of a potential dissolution test for



Key

1.	Media reservoirs (deaerated)
2.	Media selector
3.	PD29 Technical interface
4.	CY 7 Piston Pump
5.	Heater (37°C)
6.	Heated waterjacket
7.	Media flow dispersal beads
8.	Flow-through sample cell
9.	Dosage form
10.	Filter housing
11.	Glass 'T' piece
12.	Peristaltic pump
13.	De-bubblers
14	Perkin-Elmer Lambda 7 spectrophotometer

Fig. 1. Schematic of USP4 test apparatus.

a low dose tablet formulation within GlaxoWellcome. The effect of each type of system configuration upon release characteristics has been studied by investigating the release profiles obtained for adinazolam sustained release formulations in pH 1.2 simulated gastric fluid (Phillips et al., 1989). The results obtained showed that the differential dissolution profiles produced using the 'open-loop' configuration were more discriminating in describing the release characteristics according to the relative release rates than the corresponding 'closed-loop' configuration release profiles.

The work described below details the development of a suitable dissolution test for atovaquone in Malarone® tablets using an 'open-loop' system.

2. Materials and methods

2.1. Apparatus

The flow-through dissolution system is shown schematically in Fig. 1. All work was carried out on the following Sotax equipment: a CE6 Dis-

sotest, a CY7 piston pump, an MS36 media switcher controlled by a PD29 Technical Interface. In addition, an Ismatec IPS peristaltic pump and a Perkin–Elmer Lambda 7 double-beam UV/Vis spectrophotometer equipped with a six-position cell holder were also used.

2.2. Chemicals

Sodium hydroxide solution (0.1 M) 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 in order to create laminar media flow. For this test, 22.6 mm diameter flow cells were used. The tablets (adult strength) 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. Undissolved solid material was retained in the cells by a filter system[2] housed in the filter head positioned on the top of the cell. The effluent from the cell was split using a glass T-piece and a representative fraction passed through the spectrophotometer for measurement of UV absorbance of atovaquone in 1 mm cuvettes at 487 nm. Solvent debubblers placed between the peristaltic pump and the spectrophotometer were used to remove air-bubbles from the system. Analysis was carried out against a 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] The filter system was made up of Whatman filters placed in the filter head in the following order: GF/F, GF/B, GF/C, GF/A and GF/D. A glass wool plug was also added to prevent blockage of the filters by undissolved material and maintain a flow rate of 16 ml/min for the duration of the test.

3. Results and discussion

The initial development of a dissolution test for atovaquone/proguanil tablets (Malarone® tablets) was carried out using the EP/USP paddle apparatus. Sodium hydroxide was chosen because the atovaquone molecule contains a weakly acidic -OH group which ionises in alkaline solution. In this media, the saturated solubility is 4.6 mg/ml at 25°C whereas the solubility in water and 0.1 M hydrochloric acid is < 0.0002 mg/ml indicating that these latter two solvents are unsuitable for a dissolution test under sink conditions. Testing was carried out in 0.1 M sodium hydroxide with a paddle speed of 50 rpm. A two stage dissolution test was employed because of the hypromellose film-coating material used to film-coat the tablets which is insoluble in alkaline conditions. The initial dissolution fluid was 850 ml of water which allowed for the dispersion of the film-coat and the tablet to disintegrate prior to the addition of alkali. After 5 min, 50 ml of 1.8 M sodium hydroxide was added to give a final dissolution fluid of 900 ml of 0.1 M sodium hydroxide. During the first stage of the test prior to the addition of alkali a significant amount of proguanil hydrochloride was found to dissolve; the solubility of proguanil hydrochloride is 1 in 110 parts of water at 20°C.

The results obtained showed that the amount of atovaquone released after 45 min was less than 40% label strength. The results obtained were surprising because under the conditions used sink conditions should have been achievable for the full strength product. Furthermore little improvement was found in the amount of drug released when the concentration of sodium hydroxide was increased to 0.2 or 0.5 M.

In an attempt to explain these low results it was hypothesised that the solubility of atovaquone was reduced in the presence of proguanil hydrochloride. This was subsequently proved by solubility experiments which showed that the solubility of atovaquone decreased with increasing proguanil hydrochloride concentration, the results from this experiment are shown in Table 1 and graphically in Fig. 2. The results confirmed that sink conditions for atovaquone could not be ob-

Table 1
Solubility of atovaquone in 0.1 M sodium hydroxide at 20°C in the presence of proguanil hydrochloride

Concentration of proguanil hydrochloride mg/100 ml	Resulting atovaquone concentration mg/100 ml
0.0	67.9
5.5	18.6
8.0	17.7
11.0	17.4

tained under the experimental conditions used. As a consequence the EP flow-through apparatus (USP Apparatus 4) was investigated.

Initial experiments using the EP flow-through apparatus were carried out in 0.1 M sodium hydroxide using a flow rate of 16 ml/min. The results obtained were found to be unsatisfactory with less than 4% of drug released after 120 min. These results were attributed to the poor dispersion of the film-coat preventing disintegration of the tablet and the presence of proguanil hydrochloride preventing the dissolution of atovaquone. In order to ensure dispersion of the film-coat, and dissolution of proguanil hydrochloride the procedure was modified by passing water through the flow cell for 1 h. This approach removed the film-coat and was also effective in removing the water soluble proguanil hydrochloride from the system allowing atovaquone to dissolve when the dissolution medium was switched to 0.1 M sodium hydroxide. This 1 h water wash stage was included in all subsequent experiments. The results obtained are given in Table 2 showing the percent released at four time points during the experiment and shown graphically in Fig. 3.

Table 2
Dissolution results for atovaquone in 0.1 M sodium hydroxide using a flow rate of 16 ml/min

Time (min)	Mean result (%)	RSD (%)
75	59	6.0
90	87	4.4
105	93	5.2
120	96	5.2

Readings were taken every 30 s after the initial hour in water. The above time intervals were chosen to allow simple evaluation of drug release in a QA enviroment.

The influence of both flow rate and sodium hydroxide concentration on the dissolution rate were investigated. In one experiment the sodium hydroxide concentration was varied to 0.05 and 0.15 M. In a second experiment the flow rate was adjusted to 8 ml/min and a sodium hydroxide concentration of 0.1 M used. The results obtained for both are shown in Tables 3 and 4, and graphically in Figs. 4 and 5 respectively. The data in

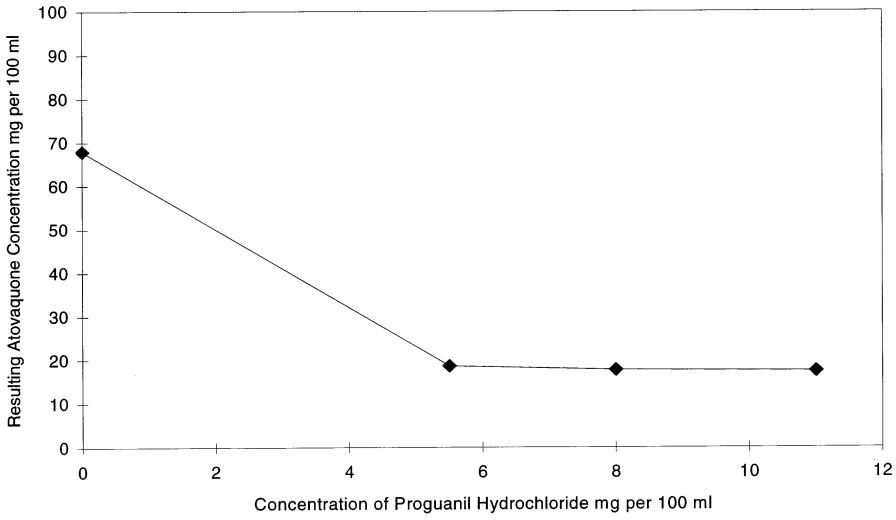


Fig. 2. Solubility of atovaquone in 0.1 M sodium hydroxide at 20°C in the presence of proguanil hydrochloride.

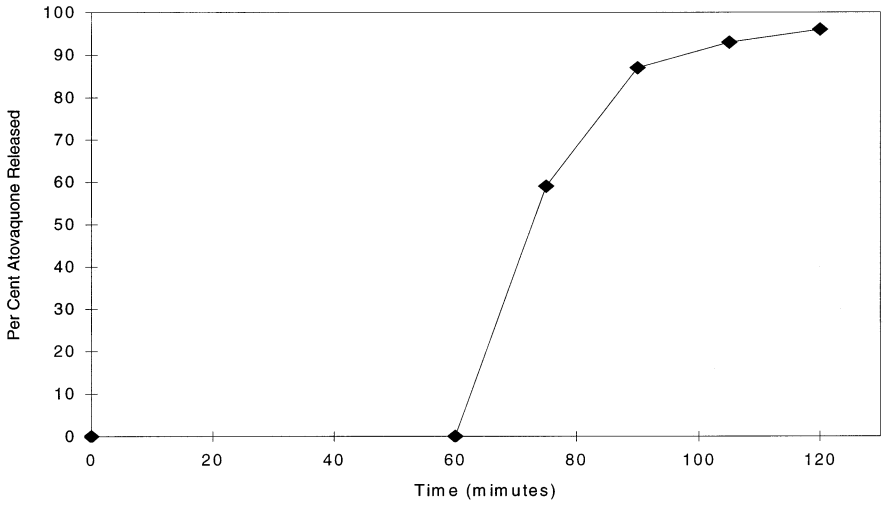


Fig. 3. Atovaquone dissolution profile in 0.1 M sodium hydroxide at 16 ml/min.

Table 3
Dissolution results for atovaquone in 0.05 and 0.15 M sodium hydroxide with a flow rate of 16 ml/min

Time (min)	Mean result in 0.05 M*	RSD (%)	Mean result in 0.15 M**	RSD (%)
75	31	5.9	74	13.5
90	62	5.4	90	9.4
105	77	5.7	94	7.0
120	84	5.9	95	5.2

* $n = 4$,
** $n = 6$.

Table 3 show that the dissolution rate of atovaquone in 0.05 M sodium hydroxide was found to be slower than that in 0.1 M sodium hydroxide, with 84% released after 120 min compared to 96% released at the same time point as shown in Table 2. In 0.15 M sodium hydroxide the dissolution rate in the early stages of the test was found to be faster than that in 0.1 M sodium hydroxide with 74% released after 75 min compared to 59% at the same time point (Table 2). On the basis of the results obtained it was felt that the dissolution rate in 0.05 M sodium hydroxide to be too slow, and that in 0.15 M sodium hydroxide too fast particularly in the early stages of the test to provide a discriminating test for atovaquone. It was decided therefore that 0.1 M was the optimum medium concentration.

Table 4
Dissolution results for atovaquone in 0.1 M sodium hydroxide using a flow rate of 8 ml/min

Time (min)	Mean result (%)	RSD (%)
75	26	5.6
90	50	6.0
105	59	5.9
120	64	6.3
180	72	5.9
240	76	5.3

The results from the second experiment using a flow rate of 8 ml/min and sodium hydroxide concentration of 0.1 M showed that the dissolution rate was significantly slower. The results obtained showed that only 64% was released after 120 min compared to 96% released at the same time point using a flow rate of 16 ml/min in the same medium concentration; after 240 min the amount of atovaquone released was found to be

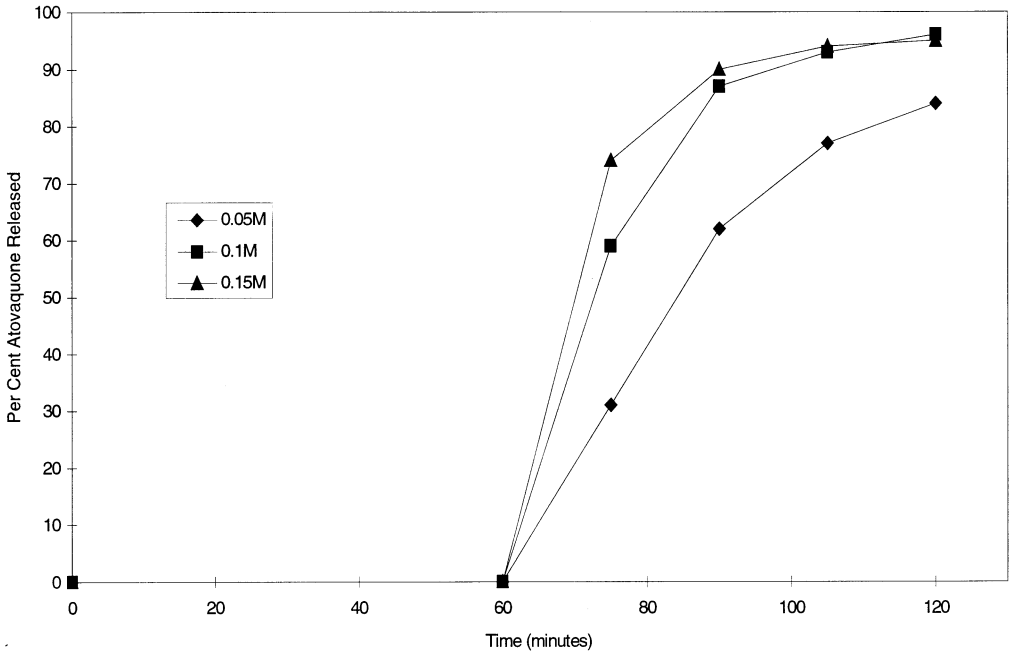


Fig. 4. Atovaquone dissolution profiles in 0.05, 0.1 and 0.15 M sodium hydroxide.

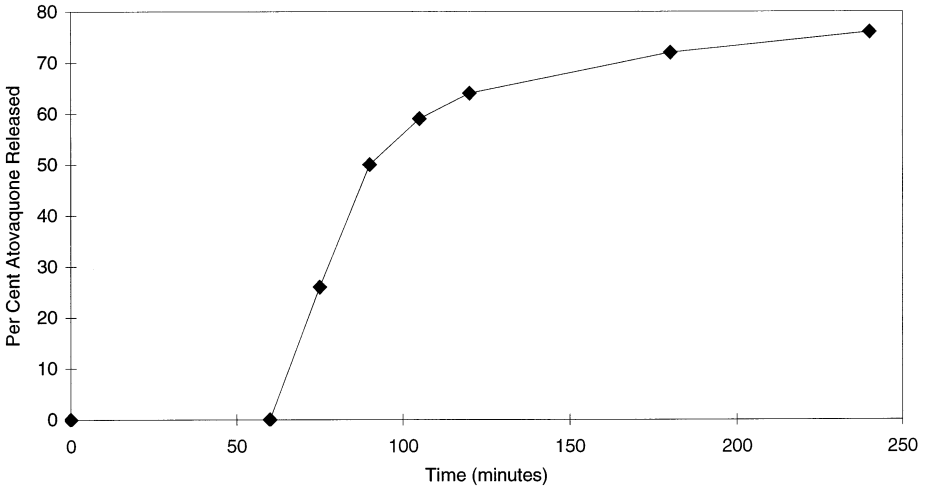


Fig. 5. Atovaquone dissolution profile in 0.1 M sodium hydroxide at 8 ml/min.

only 76%. The results obtained from this experiment therefore indicated that 16 ml/min was the preferred flow rate. The data obtained from both experiments suggested that the preferred conditions of medium concentration and flow rate was 0.1 M and 16 ml/min. The test was therefore validated using these conditions.

The use of the above procedure to monitor proguanil hydrochloride release during the initial 1 h 'water wash' period was considered. Experiments were carried out in water using a flow rate of 16 ml/min. Measurement of the dissolution rate was carried out by on-line UV analysis at 253 nm. The results from these experiments were found to

be variable and the dissolution profiles obtained showed that the dissolution rate under these conditions was too rapid to be monitored accurately, with most of the drug released in under 10 min. Attempts to slow the release rate by reducing the flow rate to 8 ml/min provided no improvement in release profile. As a consequence, a separate dissolution test in water using the EP/USP paddle apparatus is used for proguanil hydrochloride.

4. Validation

4.1. Linearity of the method

The linearity of the method was determined by measuring the standard response of atovaquone in 0.1 M sodium hydroxide solution over the range 0–250% of the standard level. This equates to approximately 2 absorbance units which covers the expected maximum absorbance of a typical test sample (around 1.0 absorbance unit). Linear response of absorbance versus concentration (as a percentage of the standard level) was obtained with the relationship: $y = 0.00798x - 0.00077$, $r = 0.99996$.

4.2. Specificity

An inert formulation with respect to atovaquone was subjected to the dissolution test. A response of 0.45% w/w of the expected atovaquone response at the maximum dissolution rate was obtained. The method was considered specific for the dissolution of atovaquone in the tablet formulation.

Table 5
Recovery of atovaquone in the dissolution test

Recovery level (%)	Recovered (%)
50	98.2, 97.8
80	99.6, 100.1
100	98.5, 101.8
120	100.5, 101.3

4.3. Accuracy

The accuracy of the method was determined by recovery experiments. A series of recovery solutions equivalent to 50, 80, 100 and 120% of the nominal content were prepared by dissolving atovaquone in 0.1 M sodium hydroxide solution. The formulation materials, excluding atovaquone, were added to each flow cell. Water was passed through the cells for 1 h according to the first stage of the dissolution test. The medium was then switched to the atovaquone recovery solution and the filtrate from each cell collected and assayed to determine the amount of atovaquone recovered. This was repeated for each recovery solution, the results obtained are given in Table 5. These results were considered satisfactory.

4.4. Precision

The repeatability of the assay was assessed by carrying out ten absorbance measurements of a standard solution on a single occasion. An RSD of 0.06% was obtained indicating that the method is sufficiently precise for the assay of dissolution samples. The reproducibility of the method was determined by examination of twelve tablets from a single batch on three separate occasions. The mean result obtained was 89.8% with an RSD of 5.2%.

5. Conclusions

A flow-through dissolution test has been developed for a two component anti-malarial tablet formulation as an alternative to a test using the EP/USP paddle apparatus which has been shown to provide unsatisfactory release data for the low solubility component in the formulation. During method development, variations in test parameters were investigated, and the results from the experiments performed led to the conclusion that a suitable test for atovaquone can be carried out in 0.1 M sodium hydroxide using a flow rate of 16 ml/min. Validation of the method for linearity and precision of the method for atovaquone was found to be satisfactory. The method has been

successfully transferred to other laboratories and is used for the testing of commercial batches and stability testing. This approach can be applied to similar formulations containing drugs which have markedly differing solubility properties and where conventional dissolution methods are shown to be inappropriate.

Acknowledgements

We would like to thank A. Crumpton, G. Knight, Dr P.A. Haywood and Dr J. Wahlich for their advice on writing this paper and to P. Raja for the secretarial support for her help in its production.

References

Aiache, M., Islasse, M., Bessac, E., Aiache, S., Renoux, R., Kantelip, J.-P., 1987. Kinetics of indomethacin release from suppositories. In vitro–in vivo correlation. Int. J. Pharm. 39, 235–242.

Chaudhary, R.S., Gangwal, S.S., Gupta, V.K., Shah, Y.N., Jindal, K.C., Khanna, S., 1994. Dissolution system for nifedipine sustained release Formulations. Drug. Dev. Ind. Pharm. 20 (7), 1267–1274.

Gjellan, K., Graffner, C., Quiding, H., 1994. Influence of amount of hard fat in suppositories on the in vitro release rate and bioavailability of paracetamol and codeine. I. A comparison of three suppository compositions in vivo. Int. J. Pharm. 102, 71–80.

Möller, H., Langenbucher, F., 1982. In vitro and in vivo dissolution of some sustained release tablets of theophylline. Pharm. Ind. 44 (10), 1065–1071.

Nicklasson, M., Orbe, A., Lindberg, J., Borga, B.B., Magnusson, A.B., Nilsson, G., Ahlgren, R., Jacobsen, L., 1991. A collaborative study of the in vitro dissolution of Phenacetin crystals comparing the flow through method with the USP Paddle method. Int. J. Pharm. 69 (3), 255–264.

Phillips, J.G., Chen, Y., Wakeling, I.N., 1989. A flow-through dissolution approach to in vivo/in vitro correlation of adinazolam release from sustained release formulations. Drug. Dev. Ind. Pharm. 15, 2177–2195.

Post, J., Speiser, P.P., 1980. Sink conditions in the flow through cell during dissolution. Int. J. Pharm. 5 (2), 101–107.

Talukdar, M.M., Plaizier-Vercammen, J.A., 1992. Flow-through method is advantageous for dissolution experiment of small dosage forms. Pharmazie 47 (6), 458–459.

Wakerly, Z., Fell, J.T., Attwood, D., Parkins, D., 1994. Pectin/ethylcellulose film coating formulations for colonic drug delivery. Pharma. Res. 13 (8), 1210–1212.

Zhang, G.H., Vadino, W.A., Yang, T.T., Cho, W.P., Chaudry, I.A., 1994. Evaluation of the flow-through cell dissolution apparatus: effects of flow rate, glass beads and tablet position on drug release from different type of tablets. Drug Dev. Ind. Pharm. 20 (13), 2063–2078.