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Determination of atovaquone in tablets by Differential Pulse Polarography

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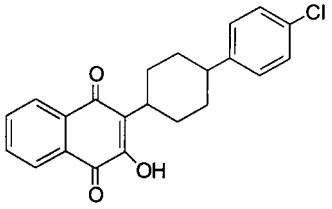
A differential pulse polarographic method is presented for the determination of atovaquone in its pharmaceutical formulations. The polarographic behaviour of atovaquone was examined in various buffer systems over the pH range 3.0–10.0. In Britton Robinson buffer/methanol solution (1 : 1; v/v; pH 8.5) the differential pulse polarograms exhibited reproducible peaks at Ep – 0.43 V vs. silver/silver chloride/potassium chloride (3M). Under these conditions, strict linearity between atovaquone concentration and peak height was observed in the 2.7×10^{-6}–3.5×10^{-5} M concentration range. The detection limit was calculated to be 0.41 μg/ml. The polarographic method was applied to the determination of the content of atovaquone in various pharmaceutical preparations. For the polarographic analysis of atovaquone in Wellvone® and Malarone® tablets no separation step was necessary. In order to evaluate the concentration of atovaquone, the standard addition method was applied. The analysis of Wellvone® tablets led to a mean value of 250.4 mg with a relative standard deviation (S_{rel}) of ±2.1% and of Malarone® tablets 252.3 mg with a S_{rel} of ±2.4%.

1. Introduction

Atovaquone (*trans*-2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphtho-quinone) displays microbicidal activity against pathogenic protozoa [1–3].



Atovaquone is an inhibitor of mitochondrial electron transport in the respiratory chain. It binds to the cytochrome bc_1 (complex III), probably because of its structural similarity to ubiquinone. This subsequently interferes with the dihydroorotate-dehydrogenase, the key enzyme of pyrimidine synthesis. This mechanism also explains the selective effect on protozoa. Unlike protozoa, mamma cells have another way of synthesizing pyrimidines. This mechanism of action seems to be effective against toxoplasmas, Plasmodia and *Pneumocystis carinii*. With regard to *Pneumocystis carinii*, it also appears to reduce ATP synthesis as the respiratory chain is linked to oxidative phosphorylation [4, 5].

Atovaquone is therefore used against *Pneumocystis carinii* infections. *Pneumocystis carinii* pneumonia (PCP) is an opportunistic infection that occurs almost exclusively in immunosuppressed patients. Atovaquone (Wellvone®) is recommended by the US Food and Drug Administration for treatment of PCP, if Cotrimoxazol as the drug of choice is not tolerated [6].

In combination with the active substance proguanil, atovaquone displays a synergistic effect against *Plasmodium falciparum*, the causative organism of tropical malaria [7]. Malarone® (Glaxo Wellcome; UK) is a combination of atovaquone and proguanil-HCl and is used in prevention and treatment of tropical malaria. It is also effective against Plasmodia that are already resistant to conventional malaria drugs.

Suggested methods of analysing the quantity of atovaquone, above all in the biological matrix, are complex chromatographic methods (GC, HPLC) and CZE (capillary zone electrophoresis) [8–10].

This paper is the first report on a differential pulse polarographic (DPP) method for determining atovaquone in tablets (Wellvone® 250 mg and Malarone® 250 mg). The big advantage of this DPP method is that the analysis requires neither separation of the tablet matrix nor extraction of the active substance. Because this analysis only requires little time, it is also suitable for routine analyses.

2. Investigations, results and discussion

2.1. Polarographic behaviour of atovaquone

The DPP analyses showed that atovaquone is reduced at the dropping mercury electrode with a well-defined peak. This peak occurred in dependence on the pH value (3.0–10.0) in a potential range of −0.08 V to −0.53 V. The linear correlation between peak potential (Ep in mV) and pH has the following function:

$$\mathrm{Ep(mV)} = -63.7 \times \mathrm{pH} + 113.6$$

$$(\mathrm{R} = 0.9985) \qquad \mathrm{pH}\ 3.0\text{–}10.0$$

A negative slope of about 64 mV/pH is shown regardless of the buffer system used and confirms that 2 protons and 2 electrons participate in the electrode reaction. This reaction is typical of a quinone-hydroquinone transition [11–13]. The cyclovoltammograms show that this is a reversible electrode process (Fig. 1).

The intensity of the peak current (ip) of atovaquone was influenced both by the type of buffer system (i.e. acetate buffer, Sörensen buffer, BR buffer) and the pH value. The optimum system for analysing atovaquone proved to be the Britton-Robinson buffer (pH 7.5). It was necessary to add methanol to the buffer solution as a solubilizer.

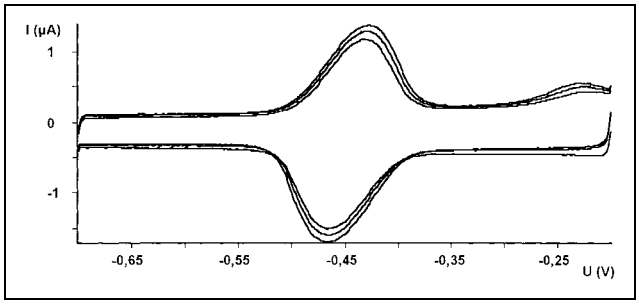


Fig.1: Cyclic voltammogram of $1{,}4 \times 10^{-5}$ M atovaquone solution in BR-buffer/methanol (1 : 1; v/v) pH 8.5; scan rate: 5 Vs^{-1}.

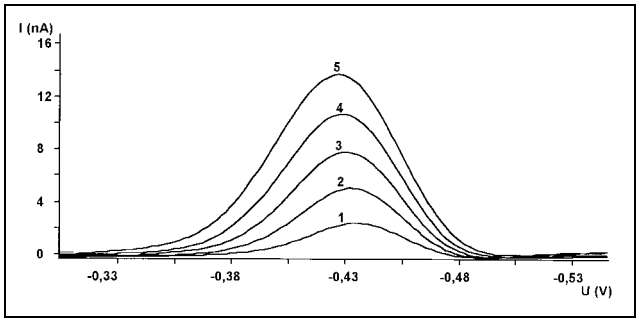


Fig. 2: Differential pulse polarograms of atovaquone in BR-buffer/methanol solution (1 : 1, v/v, pH 8.5). The concentration of atovaquone employed were: (1) 0.34, (2) 0.69, (3) 1.04, (4) 1.39, (5) 1.75×10^{-5} M.

Under optimised conditions strict linearity between peak height and concentration of atovaquone in a range of 1.0–13.0 μg/ml (2.7×10^{-6}–3.5×10^{-5} M) was observed (Fig. 2). Intraday determination of the calibration line (measurement of 4 calibration curves on one day; n = 5 measuring points per curve) produced the following linear equation: ip (nA) = 2.15 × C (μg/ml) − 0.175 (R = 0.9995). The linear equation resulting from the interday analysis (measurement of 3 curves on three days; n = 5 measuring points) led to the following function: ip (nA) = 2.15 × C (μg/ml) − 0.148 (R = 0.9995).

The accuracy of the polarographic method during one day was tested on a solution of atovaquone with a set concentration (3.69 μg/ml; n = 6; corresponding to the concentration in the tablets to be analysed) by means of the standard addition method. This analysis revealed a mean of 3.68 ± 0.07 μg/ml, corresponding to a relative standard deviation of ±1.9%. A recovery of 99.7% was obtained. In addition, interday accuracy was analysed by measuring 3 solutions of atovaquone (3.69 μg/ml) on three different days. The analysis revealed a mean of 3.72 ± 0.08 μg/ml, corresponding to a relative standard deviation of ±2.2%. A recovery of 100.8% was obtained.

According to the Analytical Methods Committee [7], the detection limit (LOD) is defined as the mean value of the intercepts (blank mean y_B) of the calibration curves plus three times of the standard deviation of the intercepts (blank s_B). This led to a LOD of 0.41 μg/ml.

The limit of quantitation (LOQ) is estimated similarly to the LOD value, but: $y_B + 10\ s_B$. This led to a LOQ of 1.03 μg/ml.

2.2. Atovaquone content in Wellvone® tablets (mono-preparation) and in Malarone® tablets (compound preparation)

In order to eliminate inconstancy of weight, 10 Wellvone® tablets were finely pulverized. The average weight per tablet was 343.5 mg. To evaluate the content of atovaquone in tablets, the sample preparation had to be optimised. Several solvents as well as different extraction volumes were examined. Best results were obtained by dissolving an aliquot of 35 mg in methanol at room temperature. Following the analysis of the tablets as described in the working procedure a mean value of 3.77 ± 0.08 μg/ml (n = 10) was obtained. In relation to one Wellvone® tablet this corresponds to a content of 250.4 mg with a relative standard deviation of ±2.1% and a recovery of 100.2%. A typical polarogram from the analysis of atovaquone in tablets is shown in Fig. 3.

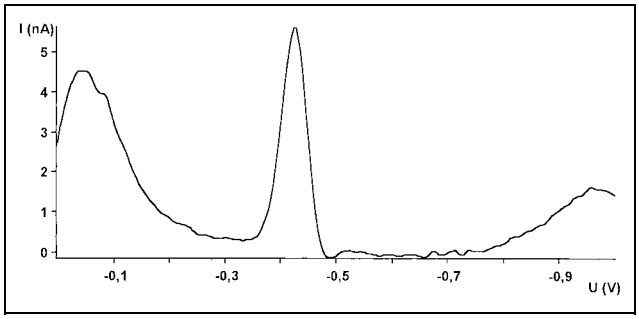


Fig. 3: Typical differential pulse polarogram of a Wellvone® tablet solution (1.03×10^{-5} M atovaquone) in BR-buffer/methanol pH 8.5.

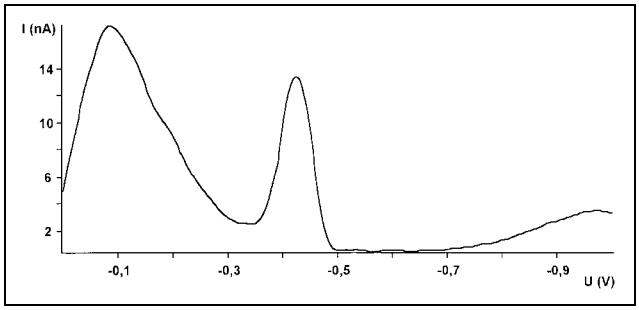


Fig. 4: Typical differential pulse polarogram of a Malarone® tablet solution (1.03×10^{-5} M atovaquone) in BR-buffer/methanol pH 8.5.

Similar investigations were carried out with Malarone® tablets (average weight per tablet was 489.4 mg). A mean value of 3.81 ± 0.09 μg/ml (n = 10) was obtained. In relation to one Malarone® tablet this corresponds to a content of 252.3 mg with a relative standard deviation of ±2.4% and a recovery of 100.9%. A typical polarogram from the analysis of atovaquone in Malarone® tablets is shown in Fig. 4.

The results presented here with a reproducibility of ± 2.1% for Wellvone® tablets and ± 2.4% for Malarone® tablets and an excellent recovery of 100.2 and 100.9%, respectively prove that polarography (DPP) is a robust, precise method of determining contents of atovaquone in commercially available preparations. A major advantage is also the fact that determination of atovaquone can be performed smoothly without separating tablet matrix first. An analysis of atovaquone in combination with proguanil-hydrochloride in the compound preparation Malarone® also shows the high selectivity of the polarographic method described above. Since the analysis is not time-consuming – determining one tablet takes approximately 15 min – this method can also be used for routine tests. Another advantage is the fact that the method can be used in all climate zones of the earth, thus making it suitable for quality assurance of malaria drugs in the known risk areas.

3. Experimental

3.1. Apparatus and polarographic conditions

Polarographic measurements (DPP) were carried out by using a 693 VA Processor (Metrohm AG, Herisau, Switzerland) in combination with a VA stand 694 (Metrohm AG, Herisau, Switzerland). This stand consists of a multimode mercury electrode [static mercury drop (SMDE)] as the working electrode, a silver/silver chloride/potassium chloride (3M) reference electrode and a platinum wire as an auxiliary electrode. For calibration curve and analysis of the tablets the analyser was operated under the following parameters: mode, DPP; drop size, 9; drop time, 1.5 s; potential ramp, −0.2 to −0.6 V; potential step, 4 mV/s; pulse amplitude, 20 mV. Evaluation was performed computer-assisted (Metrohm VA Database 2.0)

using the tangents method. The following apparatus parameters were set for cyclovoltammetric analyses: mode, CV; drop size, 9; potential ramp, 0.0 V to −0.8 V; potential step, 0.1 V−5.0 V/s.
The pH of the solutions was adjusted employing a Metrohm pH meter Model 632 and a glass electrode model 6.0202.000 (Metrohm AG). All measurements were carried out at room temperature.

3.2. Compounds and reagents

Atovaquone (pure substance), Wellvone® 250 mg tablets and Malarone® 250 mg tablets were provided by Glaxo Wellcome (UK). All reagents were of Suprapur® and/or ProAnalysis® grade (E. Merck, Darmstadt, BRD). Distilled water was purified with a Milli-Q Nanopure®(Millipore, Bedford, MA, USA) system and was stored in Nalgene® containers. The nitrogen used was 99.9995% pure, whilst the mercury was 99.999% pure (Ögussa, Graz, Austria). Britton Robinson buffer solutions (O.1M) pH 2.0–10.0, Sörensen buffer (0.1 M) pH 6.88 and acetate buffer (0.1M) pH 4.66 were used as supporting electrolytes for base polarographic tests. The calibration curve and tablet analysis were performed in a BR buffer pH 7.5 mixed with methanol (1 : 1; v/v). This methanolic buffer solution has a pH of 8.5 and is made up fresh once a week. Stock solution of atovaquone was prepared by transferring 25.0 mg atovaquone to a 100 ml volumetric flask, dissolving in methanol p.A. and bringing to volume. This solution contains 0.25 μg/μl and the content remains the same throughout the week. Further standard atovaquone solutions were prepared by diluting the stock solution and making up fresh solution as required.

3.3. Calibration graph of atovaquone (DPP)

BR buffer/methanol mix (10.0 ml), as described above, was transferred to a polarographic cell and purged with nitrogen for 8 min for deoxygenation. After determining the blank value, 5 aliquots (each of 100 μl) of atovaquone stock solution (0.25 μg/μl) were added successively to the cell, and the cell was purged after each addition with nitrogen for another 30 s. The polarogram was recorded with the above described instrumental parameters. The data were evaluated by applying the tangents method, correcting for the increase in volume. Using suitable standard solutions, it is possible to determine atovaquone contents of 1.0–13.0 μg/ml.

3.4. Working procedure for the determination of atovaquone in tablets

3.4.1. Wellvone® 250 mg tablets (monopreparation)

10 Wellvone® 250 mg tablets were weighed and finely pulverized in an analysis mill. An aliquot of this homogenized powder (35.0 mg) was placed in a 100.0 ml volumetric flask, taken to volume with MeOH p.a and allowed to extract for 3 min in an ultrasonic bath. After determination of the blank value (10 ml BR buffer/methanol solution 1 : 1; v/v; pH 8.5), 150 μl of the tablet extract (mean linearity range) was added and the cell was purged with N_2 for another 30 s. The polarogram was then recorded using the instrumental parameters described above. The content was determined applying the standard addition method and the tangents method by adding 150 μl stock solution once (0.25 μg/μl).

3.4.2. Malarone® 250 mg tablets (compound preparation)

10 Malarone® tablets (atovaquone content per tablet: 250 mg; proguanilhydrochloride per tablet: 100 mg) were finely ground in an analysis mill. 50.0 mg of the homogenized powder was placed in a 100 ml volumetric flask, taken to volume with methanol p.a. and allowed to extract for 3 min in an ultrasonic bath. The polarographic analysis was subsequently carried out following the procedure described above.

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