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Journal of Chromatography B, 708 (1998) 316–320

JOURNAL OF CHROMATOGRAPHY B

Short communication

Chromatographic studies on the isolation of peroxydisulphate oxidation products of primaquine

Virendra K. Dua[a,*], Sukesh N. Sinha[a], V.P. Sharma[b]

[a]*Malaria Research Centre, Field Station, Sect. III, BHEL, Ranipur, Hardwar 249403, India*
[b]*Malaria Research Centre, 22 Sham Nath Marg, Delhi 110054, India*

Received 25 June 1997; received in revised form 27 November 1997; accepted 11 December 1997

Abstract

Eight compounds from peroxydisulphate oxidation of primaquine were fractionated on Bio-Gel P-2 column using water as an eluent. A HPLC method employing acetonitrile–methanol–1 *M* perchloric acid–water (30:7:1:95, v/v) as a mobile phase at 1.0 ml/min on μBondapak reversed-phase column and UV detection at 254 nm was developed for the separation and identification of different oxidation products of primaquine. A combination of Bio-Gel chromatography with reversed-phase HPLC was found to be the most suitable analytical technique for the semipreparative isolation of various products formed from the oxidation. Two oxidation products that were isolated had three or four times higher gametocytocidal activity as compared to primaquine. © 1998 Elsevier Science B.V.

Keywords: Primaquine

1. Introduction

Primaquine, a potent tissue schizontocide, is the clinical drug of choice for the radical cure of relapsing malaria. However, the use of primaquine has been restricted by its toxic side effects especially in those patients deficient in glucose-6-phosphate dehydrogenase [1]. Therefore there is always a recognized need for investigations of new less toxic, tissue schizontocide. Several analogs of primaquine have been isolated using chemical [2] and enzymatic methods [3] and tested for the antimalarial activity but none were found better than primaquine. Peroxydisulphate ion is used for the oxidation of aminophenols [4] and aromatic amines [5] in neutral medium. We have carried out oxidation of primaquine by peroxydisulphate ion in aqueous medium with the aim to isolate an oxidative analog of primaquine that had antimalarial property. This paper describes a suitable chromatographic method for the separation and isolation of peroxydisulphate oxidation products of primaquine.

2. Experimental

Primaquine diphosphate (99%) pure was purchased from sigma (St. Louis, MO, USA) while Bio-Gel P-2 was obtained from Bio-Rad Calf (USA). HPLC-grade acetonitrile and methanol were purchased from Spectrochem Bombay while potassium peroxydisulphate and perchloric acid were of analytical grade and were obtained from E. Merck

*Corresponding author.

0378-4347/98/$19.00
PII S0378-4347(97)00639-7

(Bombay, India). All these chemicals were used without further purification.

Stock solution of primaquine (0.25 mg/ml) and potassium peroxydisulphate (0.24 mg/ml) were prepared in distilled water for UV and HPLC study while 2.5 mg/ml of primaquine and 2.4 mg/ml of potassium peroxydisulphate solution were prepared for semipreparative isolation of various oxidation products on Bio-Gel column chromatography. All solutions were kept in dark bottles and stored in the refrigerator.

An ultraviolet spectrophotometer UV 250 (Shimadzu, Japan) was used to detect the formation of different compounds while column chromatography was performed on a Bio-Gel P-2 filled column (100×2 cm) using water for elution. The eluted fractions were tested by the HPLC method.

The HPLC apparatus consisted of a Waters 510 pump, a Rheodyne 7125 injector, a variable wavelength UV 486 detector operated at 254 nm and an integrator. The mobile phase acetonitrile–methanol–(1 *M*) perchloric acid–water (30:7:1.95, v/v) was pumped at a flow-rate of 1.0 ml/min through a μBondapak C_{18} reversed-phase column (300×3.9 mm ID. 5 μm particle size). The mobile phase was filtered on 5-μm filter and ultrasonicated (FS 100; Decon Hove, UK) before use. Chromatography was performed at ambient temperature.

3. Results

Primaquine, on oxidation with peroxydisulphate ion in neutral medium, gave pale yellow, to orange, brown, violet and then yellow colour within 1 h of initiation of reaction. The solution became colourless in two days. The UV spectra recorded at different times of the reaction at various wavelength are given in Fig. 1 which gave different λ_{max} indicating formation of different compounds during the course of the reaction.

Attempts to extract the various reaction products using different polar and nonpolar solvents like methanol, ethylacetate, chloroform, *n*-hexane, petroleum ether and their various combination failed. It may be noted that peroxydisulphate ion behaved entirely different in basic medium and the compounds became labile and degrated during the neutral

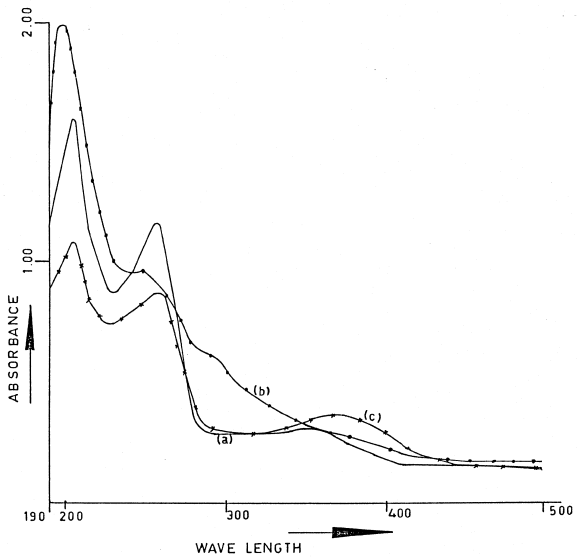


Fig. 1. UV spectra of the reaction of primaquine (0.25 mg/ml) with potassium peroxydisulphate (0.24 mg/ml) recorded at (a) 5 min, (b) 15 min and (c) 30 min after initiation of the reaction.

to basic phase [6]. Therefore the extraction of oxidation products in basic pH was not performed.

Column chromatography using silica gel and alumina as adsorbents also failed for the separation of the reaction products because all compounds were retained on the top surface of the adsorbent and could not be eluted with common solvents. Bio-Gel P-2, previously reported for the fractionation of oligosaccharides [7], was found suitable for the separation of all oxidation products of primaquine using water as an eluent. Different colour bands were obtained on Bio-Gel P-2 column and fractions of different colours were collected manually. Each fraction was further tested for their purity using high-performance liquid chromatography.

Various proportions of acetonitrile, methanol, perchloric acid and water as mobile phase were used for the separation of oxidation products of primaquine. It is found that the separation was best achieved using acetonitrile–methanol–(1 *M*) perchloric acid–water (30:7:1:95, v/v) as mobile phase on μBondapak C_{18} reversed-phase column with a flow of 1.0 ml/min. Fig. 2a shows the HPLC chromatogram of the reaction mixture of 100 μl primaquine (0.25 mg/ml) with 200 μl potassium peroxydisulphate (0.24 mg/

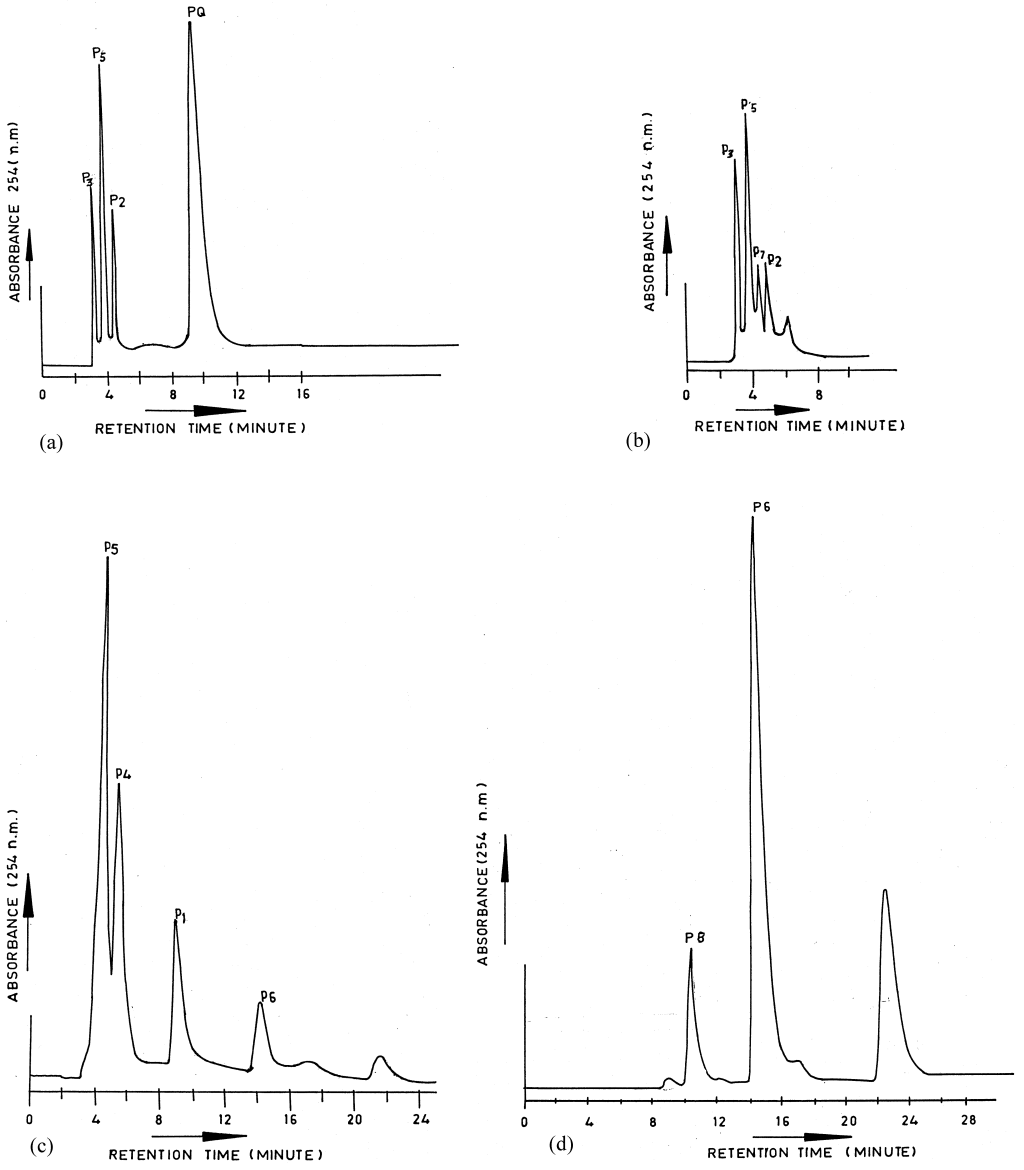
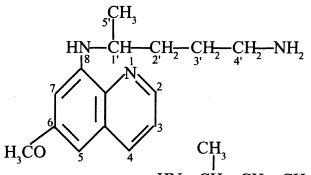
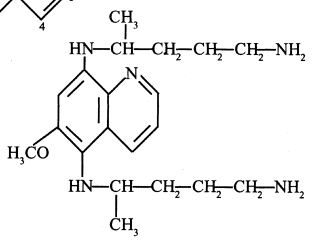
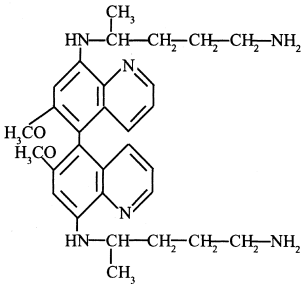
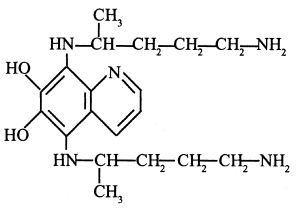
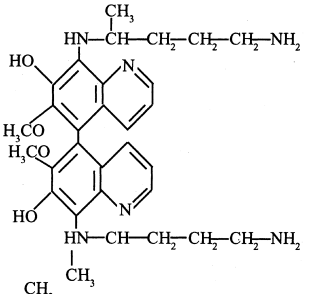
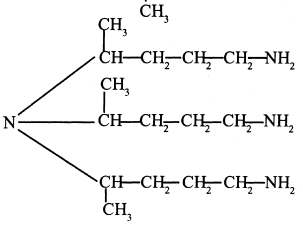


Fig. 2. HPLC chromatogram of reaction mixture of primaquine (0.25 mg/ml) and potassium peroxydisulphate (0.24 mg/ml) injected (5 μl) at (a) 5 min, (b) 15 min, (c) 30 min and (d) 40 min after initiation of reaction, eluted with acetonitrile–methanol–perchloric acid and water and μBondapak C_{18} column at a flow-rate of 1 ml/min. (Refer to Table 1 for the structral representation of the various peaks that appear in the chromatogram).

ml) injected 5 min after the initiation of the reaction. Similarly Fig. 2b–d show HPLC chromatograms of the reaction mixture injected 15 min, 30 min and 40 min after starting the reaction respectively. Retention time of various oxidation products formed from the oxidation of primaquine are given in Table 1.

It may be noted that this HPLC method could not be used for semi preparative isolation of various oxidation products because the mobile phase contained perchloric acid whose removal will be difficult from an isolated sample. Therefore, Bio-Gel column chromatography was used to isolate different prod-

Table 1
Structure, colour, wavelength and retention time of compounds formed from the oxidation of primaquine by peroxidisulphate ion

Compound Code	Chemical Name	Retention Time	Structure
Primaquine (PQ)	6-methoxy-8(4'-amino-1'-methylbutyl amino)Quinoline	10.0	
P_1 (Dark Brown, λ_1=372 λ_2=254, λ_3=204)	6-methoxy-5,8 di-(4'-amino-1'-methylbutyl amino)Quinoline	9.0	
P_2(Orange, λ_1=384 λ_2=264, λ_3=204)	5,5 di-[6 methoxy, 8(4'-amino-1'-methylbutyl amino) Quinoline]	4.6	
P_3 (Pale Yellow, λ_1=280 λ_2=200)	5,8 di-[4'-amino-1'-methylbutylamino] 6,7 dihydroxy Quinoline	3.0	
P_4(Red, λ_1=378 λ_2=258,λ_3=206)	5,5 di-[7-hydroxy, 6-methoxy 8(4'-amino -1'-methylbutyl amino)Quinoline]	5.4	
P_5(Orange,λ_1=258 λ_2=202)	N, N tri-(4-amino-1-methylbutylamine)	4.0	

ucts. Each eluted Bio-Gel fraction was tested on HPLC and the fractions containing single similar peaks were pooled and freeze dried. Mixed fractions were pooled and rechromatographed on Bio-Gel column and tested on HPLC until pure compounds were obtained. Thus eight compounds were isolated above 90% purity from the oxidation of primaquine. The structures of 5 major compounds were determined using 1R, MS and NMR studies and are given in Table 1 along with their colour and λ_{max}.

4. Discussion

Oxidation of primaquine by peroxydisulphate ion in neutral medium gave different oxidation products and UV spectra of reaction mixture also showed different λ max at different times. Solvent extraction methods failed to isolate various compounds and the same was true with silica or alumina column chromatography. All compounds were fractioned on Bio-Gel P-2 column. Since the molecular weight of the isolated compounds were not related to the elution pattern, it clearly implied that other factors played some role in separation besides size exclusion.

A reversed-phase HPLC method was developed for the separation and identification of various oxidation products of primaquine. Slight modification was made of the method reported by Dua et al. [8] for the determination of primaquine. It may be noted that with the slight change in mobile phase composition, base line separation between some oxidation products was obtained. The HPLC separation followed reversed-phase and ion pair mechanism as stated earlier [9].

In vitro study on the five structurally confirmed compounds (Table 1) showed that compounds P_1 and P_2 had 3–4 times higher gametocytocidal activity as compared to primaquine.

Our study clearly showed that the combination of Bio-Gel column chromatography with reversed-phase HPLC was the most suitable analytical technique for the isolation of various products in pure and semipreparative scale, formed from the oxidation of primaquine by peroxydisulphate ion.

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