Primaquine Diphosphate: Comprehensive Profile

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1. DESCRIPTION

1.1. Nomenclature

1.1.1. Chemical names

N^4-(6-methoxy-8-quinolinyl)-1,4-pentanediamine diphosphate.
N^4-(6-methoxy-8-quinolinyl)-1,4-diaminopentane diphosphate.
(*RS*)-8-(4-amino-1-methylbutylamino)-6-methoxy quinoline diphosphate.
1,4-Diaminopentane, N^4-(6-methoxy-8-quinolinyl) diphosphate.
1,4-Pentanediamine, N^4-(6-methoxy-8-quinolinyl) diphosphate [1–6].

1.1.2. Nonproprietary names

Base: Primaquine, Primaquin, Primaquinum, Primaquina.
Phosphate: Primaquine phosphate, Primaquine (diphosphate de), Primaquini diphosphas, Primaquinbisdihydrogenphosphat, Primaquine diphosphate, Difosfato de Primaquina, Primachina Fosfato, Primaquinum Phosphoricum SN-13272 [1–6].

1.1.3. Proprietary names

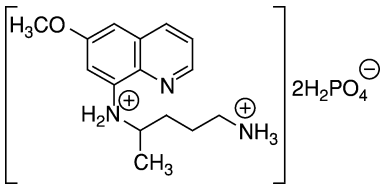
PMQ-Inga, Primachina fosfato, Primaquine [1–6].

1.2. Formulae

1.2.1. Empirical formula, molecular weight, and CAS number

Primaquine	$C_{15}H_{21}N_3O$	259.35	[90–34–6]
Primaquine diphosphate	$C_{15}H_{21}N_3O{\cdot}2H_3PO_4$	455.34	[63–45–6]
Primaquine oxalate	$C_{15}H_{21}N_3O{\cdot}C_2H_2O_4$	349.39	–

1.2.2. Structure



1.3. Elemental composition

Primaquine	C 69.47%;	H 8.16%;	N 16.20%;	O 6.17%.	
Primaquine diphosphate	C 39.56%;	H 5.93%;	N 9.23%;	O 31.65%;	P 13.63%.
Primaquine oxalate	C 58.44%;	H 6.63%;	N 12.03%;	O 22.90%	

1.4. Appearance

Primaquine: A viscous liquid.
Primaquine diphosphate: orange-red or orange crystalline powder, odorless and has bitter taste, solution is acid to litmus.
Primaquine oxalate: Yellow crystals from 80% ethanol.

1.5. Uses and application

Primaquine is an 8-aminoquinoline antimalarial agent, which is effective as tissue schizontocide against intrahepatic forms of all types of malaria parasite and is used to produce radical cure of *vivax* and *ovale* malarias. When primaquine is used for radical cure of *vivax* and *ovale* malarials, a course of treatment with a blood schizontocide must be given first to kill any erythrocytic parasite. Primaquine phosphate is then administered by mouth, usually in a dose equivalent to 15 mg of the base daily for 14 days but higher doses or longer courses may be required to overcome resistance in some strains of *Plasmodium vivax*. World Health Organization has advised that a treatment period of 21 days should be employed to achieve radical cure in most of South-East Asia and Oceania. A suggested dose for children is 250 mg/kg body weight daily for 14 days. Regimens of 30 mg (children, 500–750 μg/kg body weight) once every 7 days for 8 weeks have been suggested to minimize haemolysis in patients with glucose-6-phoshate dehydrogenase deficiency [3].

Primaquine is also gametocytocidal and a single dose of 30–45 mg has been suggested to prevent transmission of *falciparum* malaria particularly in areas where there is a potential for reintroduction of malaria. Primaquine is also used in the treatment of *Pneumocystis carinii* pneumonia in AIDS patients in combinations with clindamycin [3].

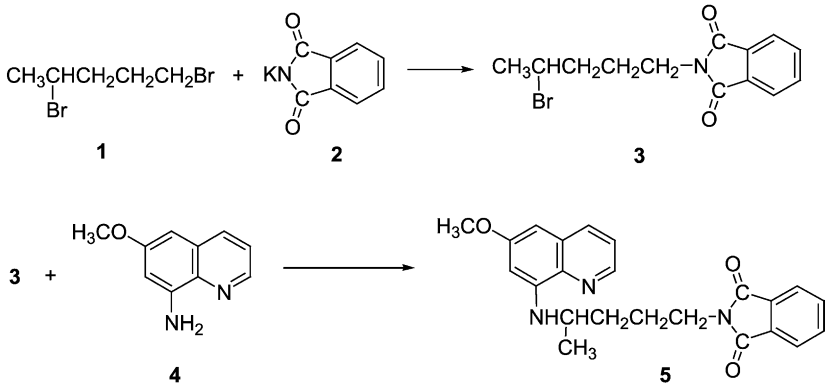
Primaquine should be administered cautiously to actually ill patients with any serious systemic disease characterized by a tendency to granulocytopenia such as rheumatoid arthritis or lupus erythematosus. The drug should be used with care in patients with glucose-6-phosphate dehydrogenase deficiency. Primaquine should be withdrawn if signs of haemolysis or methaemoglobinaemia occur and the blood

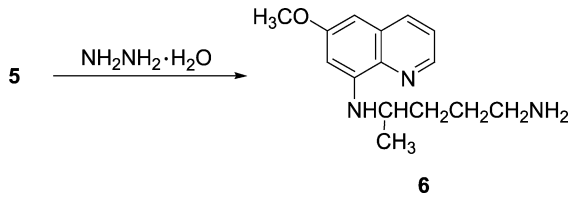
count should be monitored periodically. Primaquine should not be administered concomitantly with drugs liable to induce haemolysis or bone marrow depression. Mepacrine may increase the plasma concentrations of primaquine, resulting in a higher risk of toxicity, and it has been recommended that these drugs should not be used concomitantly [3].

2. METHODS OF PREPARATION

Elderfield *et al.* [7] prepared primaquine diphosphate as follows.

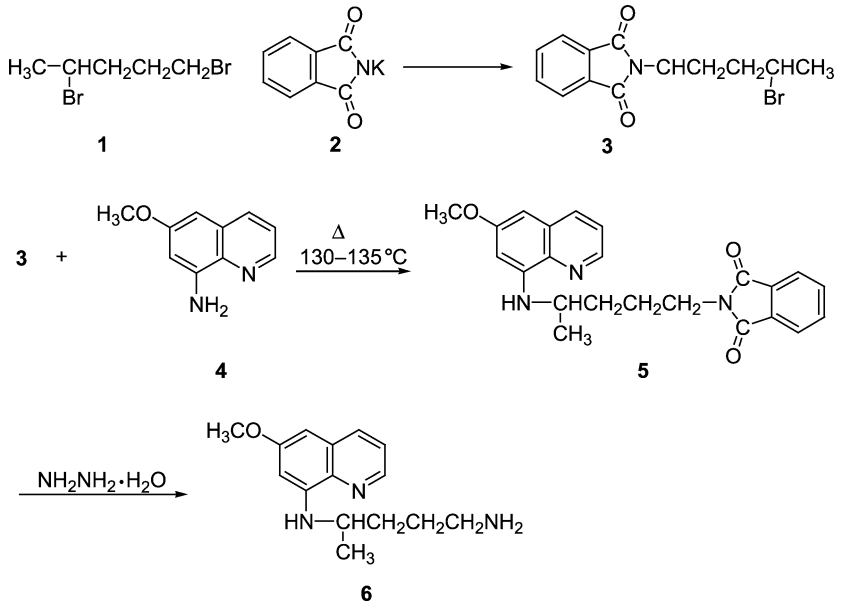
1,4-Dibromopentane **1** (460 g) and potassium phthalimide **2** (278 g) and 1.5 L acetone was refluxed for 24 h with stirring, the potassium bromide was filtered off, and the filtrate fractionated gave 67% 4-bromo-1-phthalimidopentane **3** as pale yellow oil. 6-Methoxy-8-aminoquinoline **4** (348 g) and 4-bromo-1-phthalimidopentane **3** (296 g) in 1 L ethanol was refluxed for 72 h with stirring. The mixture was cooled, diluted with diethyl ether and filtered, the residue of 6-methoxy-8-aminoquinoline hydrobromide was washed with diethyl ether, the combined filtrate and washings was washed with aqueous potassium carbonate and water, dried and evaporated, the dark brown residue was boiled in ethanol with decolorizing carbon and filtered. The filtrate was cooled and the crystal deposit was recrystallized from ethanol and gave the 4-phthalimido-1-methyl butyl amino analog **5** of primaquine. 6-Methoxy-8-aminoquinoline (17.4 g) **4**, 4-bromo-1-phthalimidopentane **3** (29.6 g), ethanol (30 mL) and phosphate buffer, pH 8, (100 mL) was heated for 79 h with stirring at 75–80 °C yielded 49% of crude 4-phthalimido-1-methyl butyl amino analog **5** of 6-methoxy-8-(4-amino-1-methyl butylamino)quinoline (primaquine) **6**. Compound **5** was refluxed for 2 h in ethanol with the equivalent amount of hydrazine hydrate and extracted with ethanol, the extract was treated with swirling with the calculated amount of 85% phosphoric acid, the precipitated orange oil was treated with ethanol, and the crystal material was recrystallized from 90% ethanol yielded 80% of primaquine diphosphate **6**. When primaquine base, in diethyl ether, was treated with 1 mol equivalent of oxalic acid in ethanol and the precipitate recrystallized from 80% ethanol gave primaquine oxalate.





Chu *et al.* reported the synthesis of primaquine diphosphate [8] as follows.

4-Bromo-1-phthalimidopentane **3** was obtained in 72–82 g yield by refluxing 92 g of 1,4-dibromopentane **1**, 55.5 g of potassium phthalimide **2**, and 200 mL dry acetone on a steam bath for 30 h. Compound **3** (30 g) and 42 g 6-methoxy-8-aminoquinoline **4** refluxed at 130−135 °C for 6 h, extracted with benzene to separate insoluble 6-methoxy-8-aminoquinoline hydrobromide, the residue from evaporation of the benzene was refluxed with stirring with 100 mL of an alcoholic solution of 6 g hydrazine hydrate for 4 h, the solution was concentrated, made acidic to congo red with 8 N hydrochloric acid, filtered, and washed with boiling water. The combined filtrate and washings was concentrated, made alkaline, extracted with benzene, and distilled *in vacuo* to give 20.5 g primaquine **6**, which was treated with 19 mL 85% phosphoric acid in absolute ethanol, formed 42.5% primaquine diphosphate.



3. PHYSICAL CHARACTERISTICS

3.1. Solubility characteristics

Primaquine base: Soluble in ether, moderately soluble in water.

Primaquine diphosphate: Soluble 1 in 16 of water, practically insoluble in ethanol, chloroform, and ether.

3.2. X-ray powder diffraction pattern

The X-ray powder diffraction pattern of primaquine diphosphate was performed using a Simons XRD-5000 diffractometer. Table 1 shows the values of the scattering angles (degrees 2θ), the interplanar d-spacings (Å), and the relative intensities (%) for primaquine diphosphate, which were automatically obtained on a digital printer. Figure 1 shows the X-ray powder diffraction pattern of the drug, which was carried out on a pure sample of the drug.

3.3. Thermal methods of analysis

3.3.1. Melting behavior

Primaquine diphosphate melts at about 197–198 °C and primaquine oxalate melts at 182.5–185 °C. Primaquine as a base is viscous liquid that boils at 175–179 °C. Primaquine oxalate m.p. 182.5–185 °C.

3.3.2. Differential scanning calorimetry

The differential scanning calorimetry (DSC) thermogram of primaquine diphosphate was obtained using a DuPont TA-9900 thermal analyzer attached to a DuPont Data unit. The thermogram shown in Fig. 2 was obtained at a heating rate of 10 °C/min, and was run from 50 to 300 °C. The compound was found to melt at 209.8 °C.

3.4. Spectroscopy

3.4.1. UV–Vis spectroscopy

The ultraviolet spectrum of primaquine diphosphate in methanol (0.0016%) shown in Fig. 3 was recorded using a Shimadzu Ultraviolet visible spectrometer 1601 PC. The drug exhibited two maxima at 361 and 266.1 nm. Clarke reported the following: aqueous acid −265 nm (A 1%, 1 cm = 579a), 282 nm (A 1%, 1 cm = 574a), and 334 nm [2].

3.4.2. Vibrational spectroscopy

The infrared (IR) absorption spectrum of primaquine diphosphate was obtained as KBr disc using a Perkin-Elmer infrared spectrometer. The infrared spectrum is shown in Fig. 4 and the principal peaks are at 2946, 1612, 1469, 1430, 1384, 1200, 1050, 956, 815, and 760 cm^{-1}. The assignments of the infrared absorption bands of primaquine diphosphate are shown in Table 2. Clarke reported the following principal peaks at 1611, 1595, 815, 1230, 1572, and 1170 cm^{-1} (KBr disk) [2].

3.4.3. Nuclear magnetic resonance spectrometry

3.4.3.1. ^{1}H NMR spectra

The proton nuclear magnetic resonance (NMR) spectra of primaquine diphosphate was obtained using a Bruker instrument operating at 300, 400, or 500 MHz.

Table 1. Crystallographic data from the X-ray powder diffraction pattern of primaquine diphosphate

Scattering angle (degrees 2θ)	*d*-spacing (Å)	Relative intensity (%)	Scattering angle (degrees 2θ)	*d*-spacing (Å)	Relative intensity (%)
5.698	15.4976	4.90	5.945	14.8540	0.18
6.117	14.4356	0.22	10.320	8.5644	3.35
11.313	7.8149	100.00	12.260	7.2134	1.92
14.467	6.1176	3.97	15.318	5.7794	0.78
16.975	5.2190	45.33	17.765	4.9887	0.64
18.355	4.8295	1.15	18.998	4.6674	3.22
19.471	4.5552	2.60	20.000	4.4358	1.37
20.675	4.2925	14.76	21.611	4.1088	0.82
21.844	4.0655	1.64	22.650	3.9225	27.50
23.533	3.7773	0.75	24.121	3.6866	3.72
24.391	3.6463	2.79	25.083	3.5473	0.83
25.586	3.4787	1.18	25.840	3.4451	0.75
26.458	3.3660	0.72	27.3566	3.2332	3.19
29.295	3.0461	10.47	29.680	3.0075	0.72
30.204	2.9565	0.31	31.188	2.8654	2.21
31.520	2.8360	0.20	32.334	2.7665	0.43
32.818	2.7268	0.27	34.230	2.6174	9.36
34.514	2.5965	3.88	34.880	2.5701	1.17
35.593	2.5202	0.23	36.460	2.4623	0.39
39.040	2.3053	0.48	40.148	2.2442	3.34
40.509	2.2250	4.10	41.990	2.1499	0.33
42.528	2.1240	6.16	43.218	2.0916	0.63
43.625	2.0730	0.90	44.0.46	2.0542	2.17
45.093	2.0089	0.39	45.526	1.9908	1.71
46.162	1.9649	9.46	46.813	1.9390	0.39
47.677	1.9059	0.49	48.834	1.8634	0.35
49.983	1.8232	0.38	50.736	1.7979	0.67
52.353	1.7461	0.60	53.085	1.7238	0.42
53.800	1.7025	0.31	55.431	1.6562	0.15
56.765	1.6204	0.59	58.689	1.5718	0.79
59.018	1.5638	0.86			

Standard Bruker Software was used to execute the recording of DEPT, COSY, and HETCOR spectra. The sample was dissolved in DMSO-d_6 and all resonance bands were referenced to tetramethylsilane (TMS) internal standard. The ^{1}H NMR spectra of primaquine diphosphate are shown in Figs. 5–8. The ^{1}H NMR assignments of primaquine diphosphate are shown in Table 3.

3.4.3.2. ^{13}C NMR spectra

The ^{13}C NMR spectra of primaquine diphosphate were obtained using a Bruker instrument operating at 75, 100, or 125 MHz. The sample was dissolved in DMSO-d_6 and tetramethylsilane (TMS) was added to function as the internal standard. The ^{13}C NMR spectra are shown in Fig. 9. The HSQC and HMBC spectra are shown in Figs. 10 and 11, respectively. The DEPT 90 and DEPT 135 are shown in Figs. 12 and 13, respectively. The assignments for the observed resonance bands associated with the various carbons are provided in Table 4.

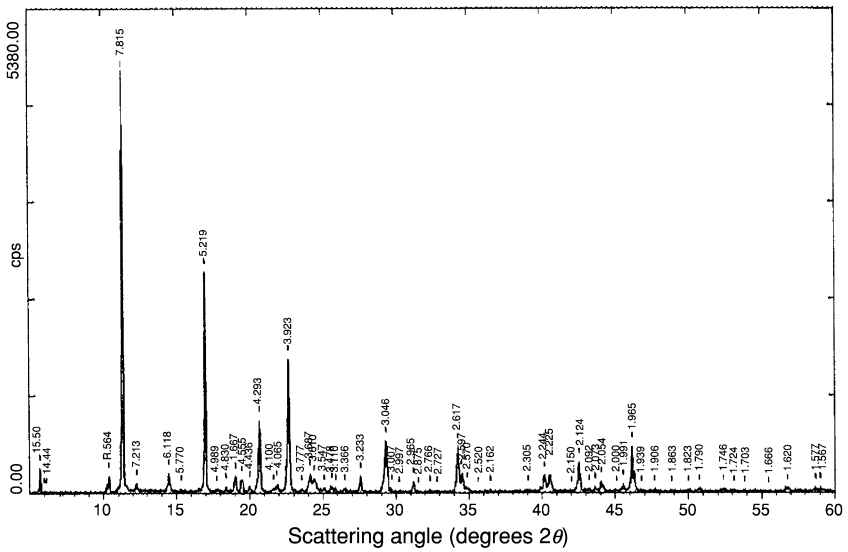


Fig. 1. The X-ray powder diffraction pattern of primaquine diphosphate.

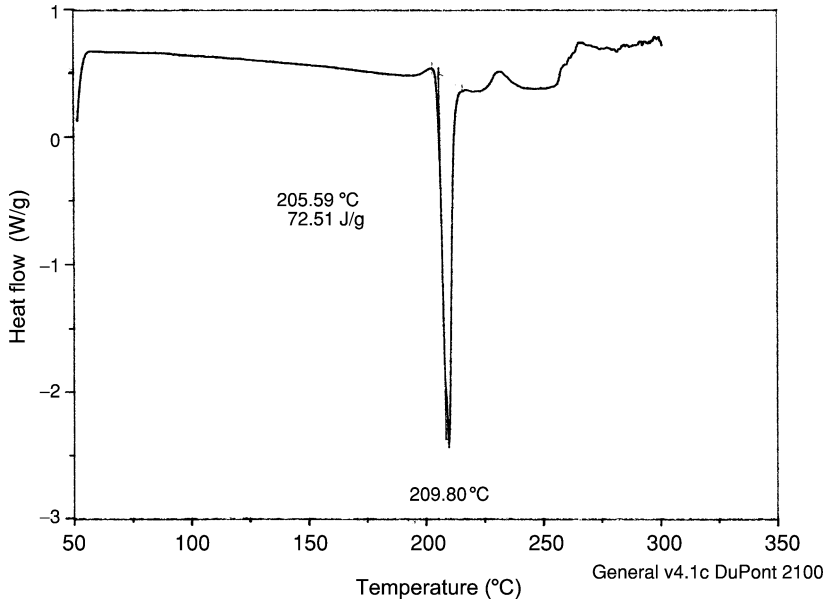


Fig. 2. Differential scanning calorimetry thermogram of primaquine diphosphate.

3.5. Mass spectrometry

The mass spectrum of primaquine diphosphate was obtained using a Shimadzu PQ-5000 mass spectrometer. The parent ion was collided with helium as a carrier gas. Figure 14 shows the detailed mass fragmentation pattern for primaquine diphosphate. Table 5 shows the proposed mass fragmentation pattern of the drugs. Clarke reported the following principal peaks at *m/z* 201, 81, 98, 175, 259, 176, 202, and 242 [2].

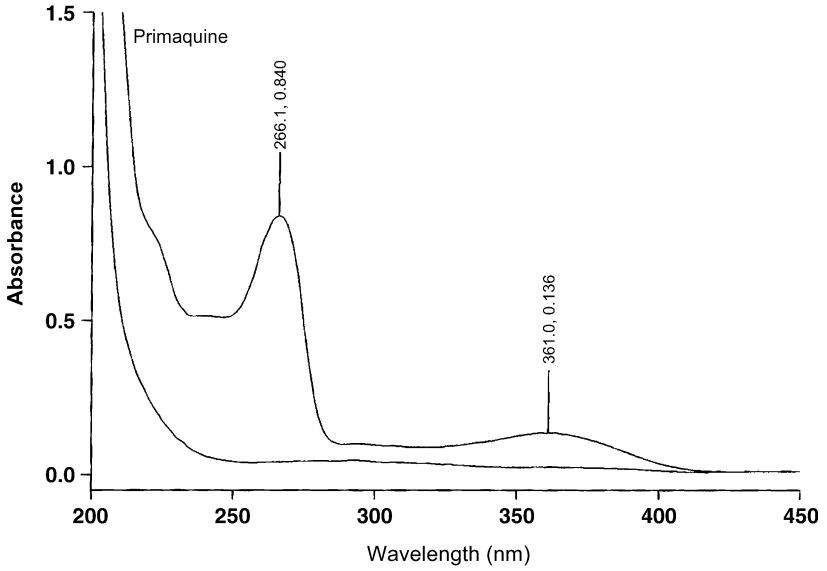


Fig. 3. Ultraviolet absorption spectrum of primaquine diphosphate.

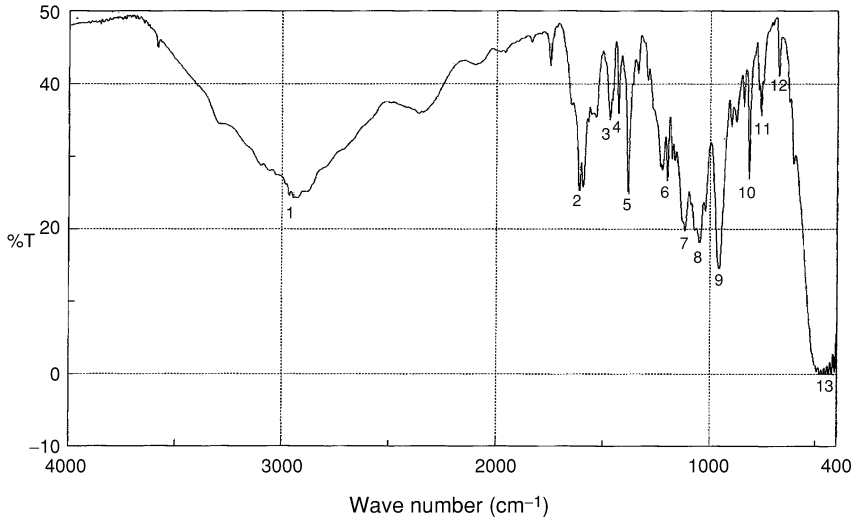


Fig. 4. The infrared absorption spectrum of primaquine diphosphate (KBr pellet).

4. METHODS OF ANALYSIS

4.1. Compendial method of analysis

4.1.1. British pharmacopoeia compendial methods [3]

4.1.1.1. Primaquine phosphate

Primaquine phosphate contains not less than 98.5% and not more than the equivalent of 101.5% of (*RS*)-8-(4-amino-1-methylbutylamino)-6-methoxyquinoline diphosphate, calculated with reference to the dried substance.

Table 2. Vibrational assignments for primaquine diphosphate infrared absorption bands

Frequency (cm^{-1})	Assignments
3300–3150	NH_2 and NH stretch
2946–2900	CH_3 and CH_2 stretch
1612	 stretch
1469, 1430	CH_2 bending
1384, 1200	C–H bending
1050	C–O stretch
956, 815, 760	C–H bending

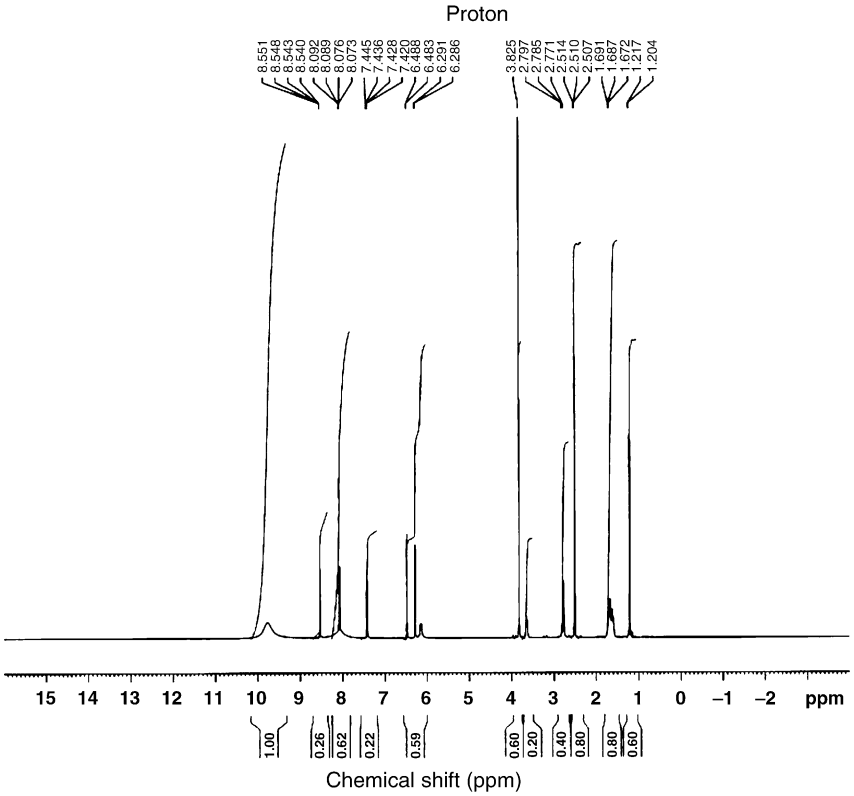


Fig. 5. The ^1H NMR spectrum of primaquine diphosphate in DMSO-d_6.

Identification

Test 1: Dissolve 15 mg of primaquine phosphate in 0.01 M *hydrochloric acid* and dilute to 100 mL with the same acid. Examined between 310 and 450 nm, according to the general procedure (2.2.25), the solution shows two absorption maxima, at 332

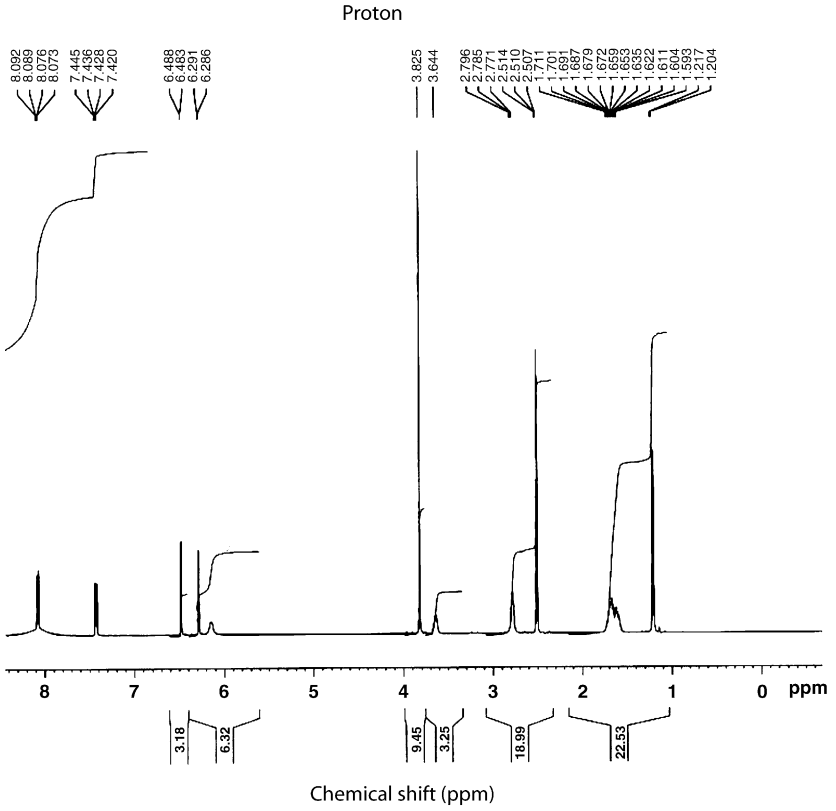


Fig. 6. Expanded ^{1}H NMR spectrum of primaquine diphosphate in DMSO-d_6.

and 415 nm. The specific absorbances at the maxima are 45–52 and 27–35, respectively. Dilute 5 mL of the solution to 50 mL with 0.01 M *hydrochloric acid*. Examined between 215 and 310 nm, the solution shows three absorption maxima, at 285, 265, and 282 nm. The specific absorbances at the maxima are 495–515, 335–350, and 330–345, respectively.

Test 2: Examine by infrared absorption spectrophotometry, according to the general procedure (2.2.24), comparing with spectrum obtained with *primaquine diphosphate* Chemical Reference Substance (*CRS*). Examine the substance as discs prepared as follows: dissolve separately 0.1 g of primaquine diphosphate and the reference substance in 5 mL of *water R*, add 2 mL of *dilute ammonia R* and 5 mL of *chloroform R* and shake; dry the chloroform layer over 0.5 g of *anhydrous sodium sulfate R*; prepare a blank disc using about 0.3 g of *potassium bromide R*, apply dropwise to the disc 0.1 mL of the chloroform layer, allowing the chloroform to evaporate between applications; dry the disc at 50 °C for 2 min.

Test 3: Examine by thin-layer chromatography, as specified in the general procedure (2.2.27), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Carry out all operations as rapidly as possible protected from light. Prepare the test and reference solutions immediately before use.

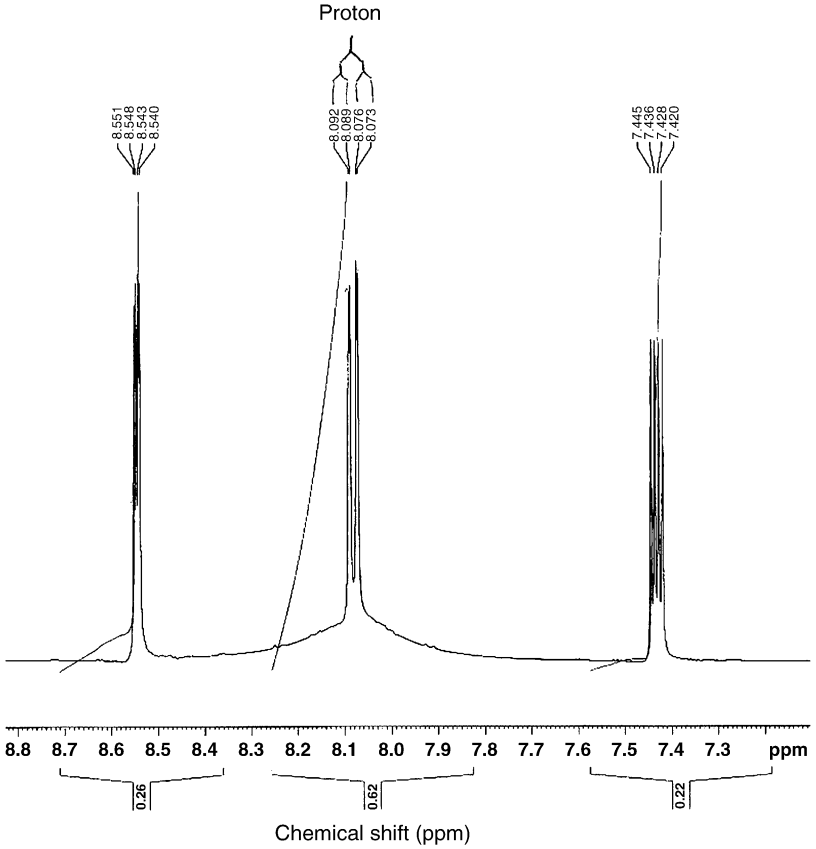


Fig. 7. Expanded ^{1}H NMR spectrum of primaquine diphosphate in DMSO-d_6.

Test solution: Dissolve 0.2 g of primaquine diphosphate in 5 mL of *water R* and dilute to 10 mL with *methanol R*. Dilute 1 mL of the solution to 10 mL with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution: Dissolve 20 mg of primaquine diphosphate CRS in 5 mL of water R and dilute to 10 mL with *methanol* R.

Carry out prewashing of the plate with a mixture of 1 volume of *concentrated ammonia R*, 40 volumes of *methanol R* and 60 volumes of *chloroform R*. Allow the plate to dry in air. Apply separately to the plate 5 μL of each solution. Develop over a bath of 15 cm using the mixture of solvents prescribed for prewashing. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

Test 4: Dissolve 50 mg of primaquine diphosphate in 5 mL of *water R*. Add 2 mL of *dilute sodium hydroxide solution R* and shake with two quantities, each of 5 mL, of *chloroform R*. The aqueous layer, acidified by addition of *nitric acid R*, gives reaction (b) of phosphates, as described in the general procedure (2.3.1).

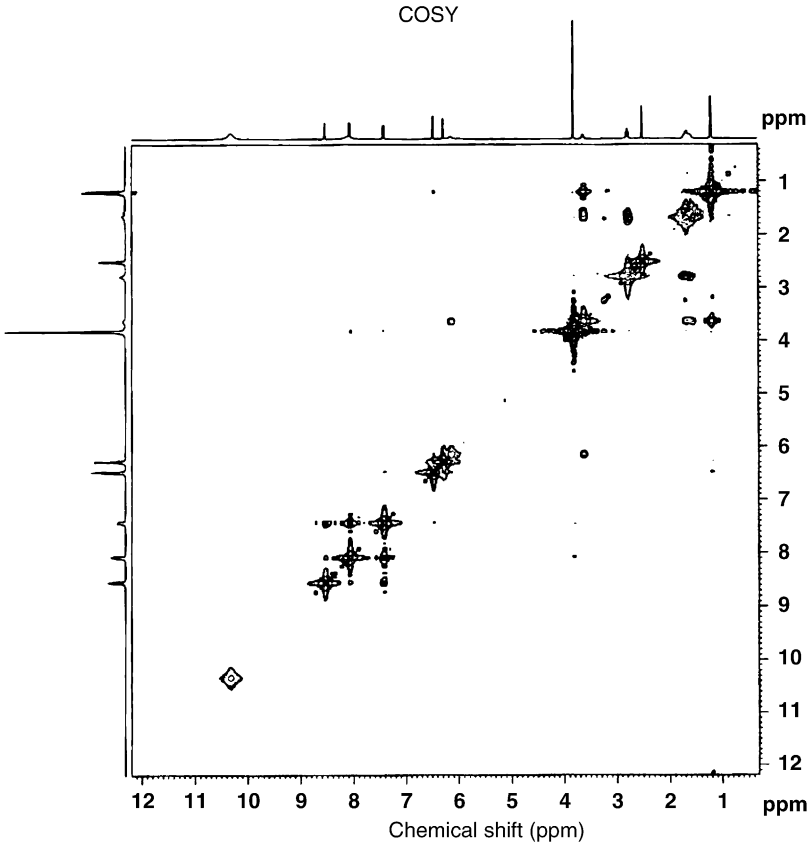


Fig. 8. COSY ^{1}H NMR spectrum of primaquine diphosphate in DMSO-d_6.

Tests

Related substances. Examine by liquid chromatography as described in the general procedure (2.2.29).

Test solution: Dissolve 50 mg of primaquine diphosphate in *water R* and dilute to 5 mL with the same solvent. To 1 mL of this solution add 0.2 mL of *concentrated ammonia R* and shake with 10 mL of the mobile phase. Use the clear lower layer.

Reference solution (a): Dissolve 50 mg of *primaquine diphosphate CRS* in *water R* and dilute to 5 mL with the same solvent. To 1 mL of this solution, add 0.2 mL of *concentrated ammonia R* and shake with 10 mL of the mobile phase. Use the clear lower layer.

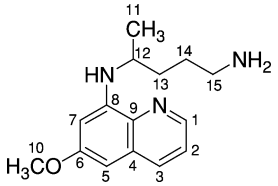
Reference solution (b): Dilute 3 mL of the test solution to 100 mL with the mobile phase.

Reference solution (c): Dilute 1 mL of the test solution to 10 mL with the mobile phase. Dilute 1 mL of this solution to 50 mL with the mobile phase.

The chromatographic procedure may be carried out using:

– a column 0.2 m long and 4.6 mm in internal diameter packed with *silica gel* for *chromatography R* (10 μm).

Table 3. Assignments of the resonance bands in the ^{1}H NMR spectrum of primaquine diphosphate



Chemical shift (ppm, relative to TMS)	Number of protons	Multiplicity*	Assignment (proton at carbon number)
9.6–9.90	2	bs	NH_2
8.54–8.55	1	m	1
8.07–8.09	2	dd	3 and NH
7.42–7.44	1	q	2
6.48	1	s	7
6.29	1	s	5
3.82	3	s	10
3.64	1	bs	12
2.77–2.79	2	t	15
1.59–1.71	2	m	13 and 14
1.20–122	3	t	11

*s, singlet; dd, double doublet; m, multiplet; bs, broad singlet; q, quartet.

– a mixture of 0.1 volume of *concentrated ammonia R*, 10 volumes of *methanol R*, 45 volumes of *chloroform R*, and 45 volumes of *hexane R* as the mobile phase at a flow rate of 3 mL/min.
– as detector a spectrophotometer set at 261 nm.
– a loop injector.

Inject 20 µL of each solution and continue the chromatography for at least twice the retention time of primaquine. In the chromatogram obtained with the test solution, the sum of the areas of any peaks, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (3%). Disregard peak due to solvent and any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (c). The test is not valid unless in the chromatogram obtained with reference solution (a), just before the principal peak there is a peak whose area is about 6% of that of the principal peak and the resolution between these peaks is not less than 2; in the chromatogram obtained with reference solution (c) the signal-to-noise ratio of the principal peak is not less than five.

Loss on drying. When the experiment is carried out according to the general procedure (2.2.32), not more than 0.5% is determined on 1 g by drying in an oven at 100–105 °C.

Assay

Dissolve 0.2 g of primaquine phosphate in 40 mL of *anhydrous acetic acid R*, heating gently. Titrate with 0.1 M *perchloric acid* determining the endpoint

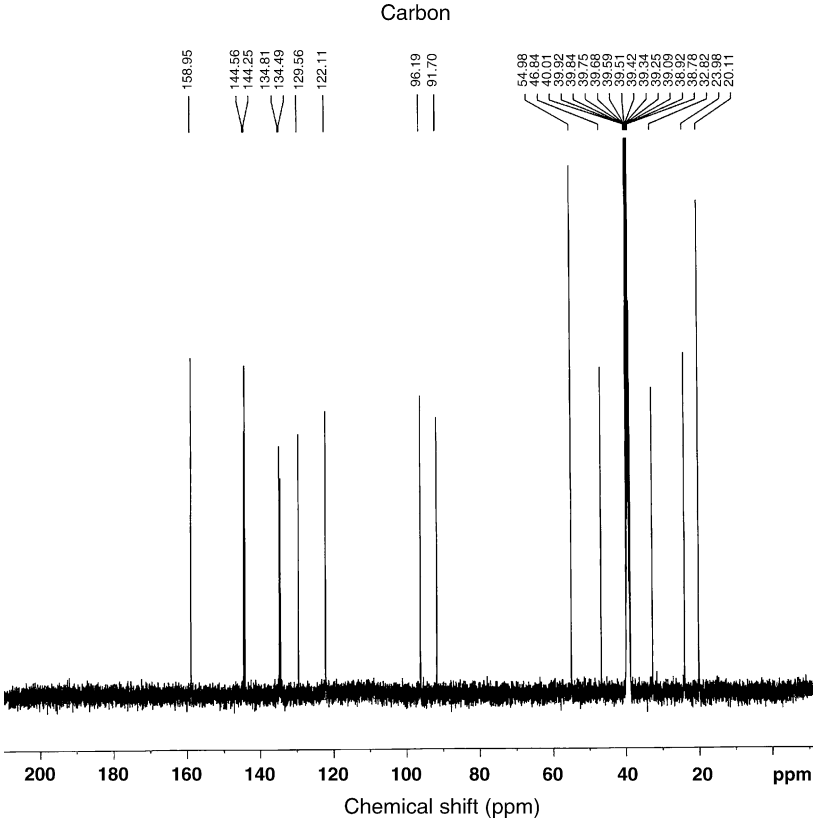


Fig. 9. ^{13}C NMR spectrum of primaquine diphosphate in DMSO-d_6.

potentiometrically as described in the general procedure (2.2.20). One milliliter of 0.1 M *perchloric acid* is equivalent to 22.77 mg of $C_{15}H_{27}N_3O_9P_2$.

Storage

Store protected from light.

4.1.2. United States pharmacopoeia compendial methods [4]

4.1.2.1. Primaquine phosphate

Primaquine phosphate contains not less than 98% and not more than 102% of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$ calculated on the dried basis.

Identification

Test 1: Infrared absorption. This test must be carried out according to the general procedure <197K>. The spectrum obtained from the sample of primaquine phosphate should be identical with that spectrum obtained from *USP Primaquine Phosphate RS*.

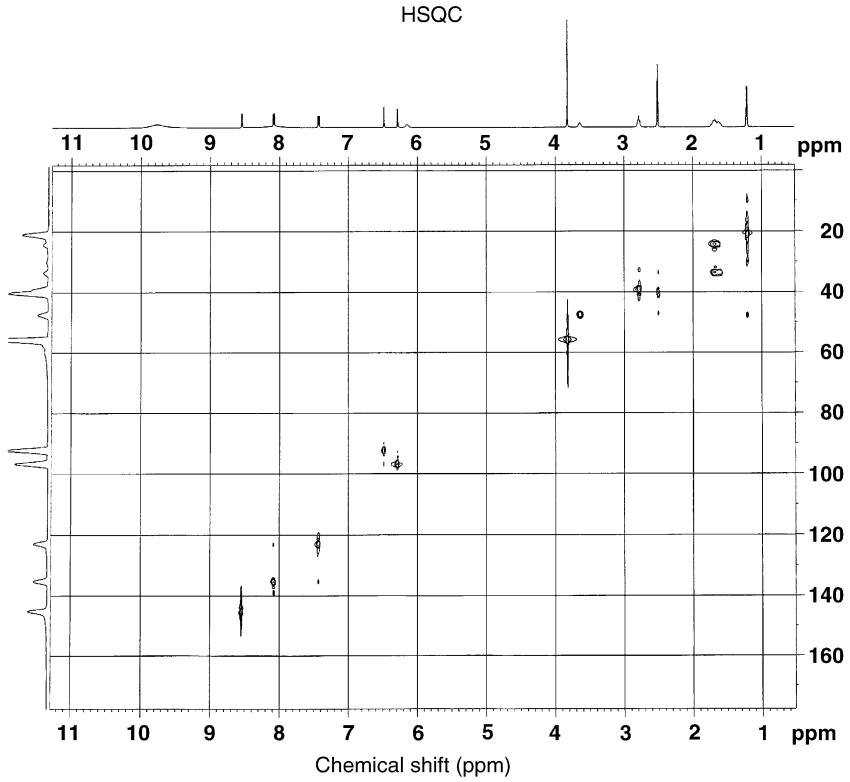


Fig. 10. The HSQC NMR spectrum of primaquine diphosphate in DMSO-d_6.

Test 2: The residue obtained by ignition of primaquine phosphate responds to the test for pyrophosphate as described under phosphate, the test must be carried out as described in the general procedure <191>.

Loss on drying. By following the general procedure <731>, dry primaquine phosphate at 105 °C for 2 h. It loses not more than 1% of its weight.

Organic volatile impurities. By following method 1 in the general procedure <467>: meets the requirements.

Assay

Dissolve about 700 mg of primaquine phosphate, accurately weighed, in about 75 mL of water in a beaker, add 10 mL of hydrochloric acid, and proceed as directed under Nitrite Titration in the general procedure <451>, beginning with "cool to about 15 °C". Each milliliter of 0.1 M sodium nitrite is equivalent to 45.53 mg of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$.

4.1.2.2. Primaquine phosphate tablets

Primaquine phosphate tablets contain not less than 93% and not more than 107% of the labeled amount of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$.

Packaging and storage. Preserve in well-closed, light-resistant container.

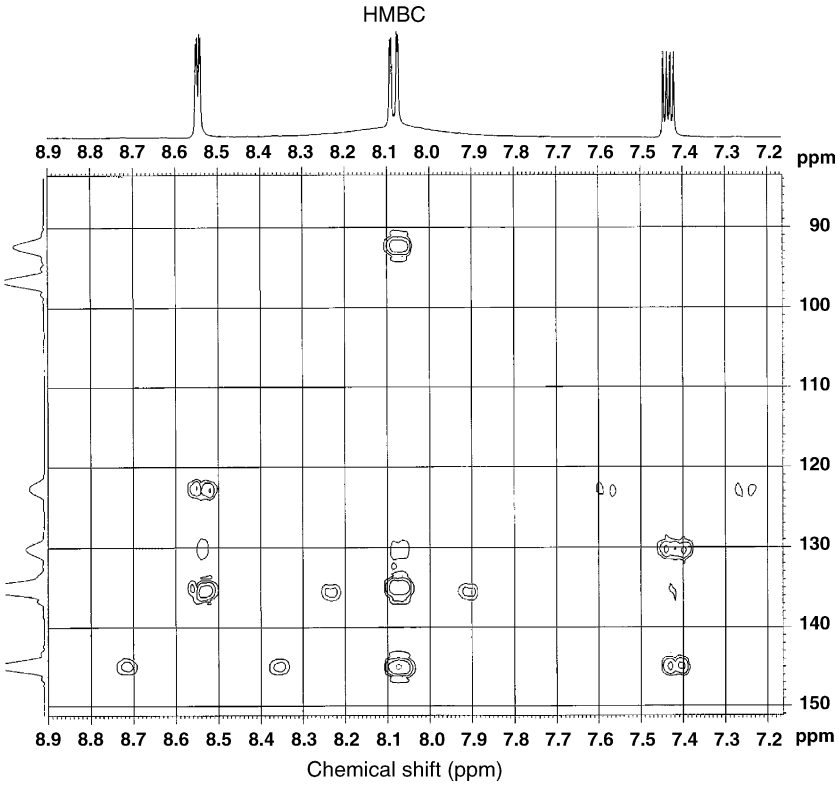


Fig. 11. The HMBC NMR spectrum of primaquine diphosphate in DMSO-d_6.

USP Reference Standards <11>. *USP Primaquine Phosphate RS.*

Identification

Digest a quantity of finely powdered tablets, equivalent to about 25 mg of primaquine phosphate, with 10 mL of water for 15 min, and filter.

Test 1: Dilute 0.1 mL of the filtrate with 1 mL of water, and add one drop of gold chloride TS: a violet blue color is produced at once.

Test 2: To the remainder of the filtrate add 5 mL of trinitrophenol TS: a yellow precipitate is formed. Wash the precipitate with cold water, and dry at 105 °C for 2 h: the picrate melts between 208 °C and 215 °C. [*Caution: Picrate may explode.*]

Dissolution. To be carried out according to the general procedure <711>:

Medium: 0.01 N Hydrochloric acid; 900 mL.
Apparatus 2: 50 rpm.
Time: 60 min.

Determine the amount of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$ dissolved by employing the following method:

1-Pentanesulfonate sodium solution: Add about 961 mg of sodium-1-pentanesulfonate and 1 mL of glacial acetic acid to 400 mL of water, and mix.

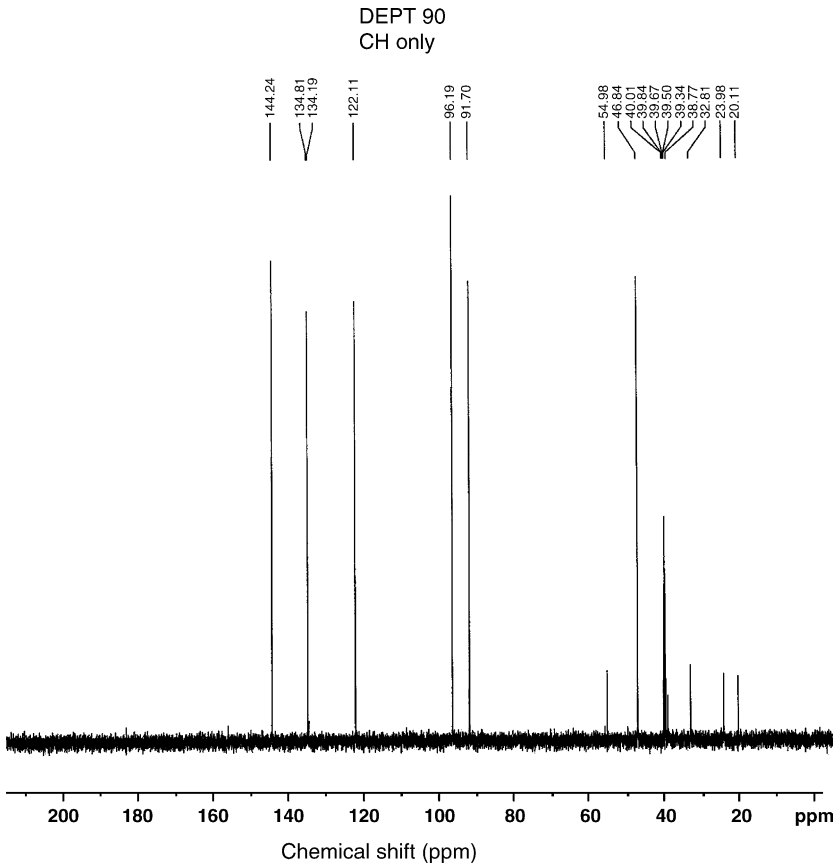


Fig. 12. The DEPT 90 ^{13}C NMR spectrum of primaquine diphosphate in DMSO-d_6.

Mobile phase: Prepare a filtered and degassed mixture of methanol and *1-pentanesulfonate sodium solution* (60:40). Make adjustments if necessary (see *system suitability under Chromatography* <621>).

Chromatographic system: See Chromatography <621>.

The liquid chromatograph is equipped with a 254 nm detector and a 3.9 mm × 30 cm column that contains packing L1. The flow rate is about 2 mL/min. Chromatograph replicate injections of the Standard solution and record the peak responses as directed for *procedure*: the relative standard deviation is not more than 3%.

Procedure: Separately inject into the chromatograph equal volumes (about 20 μL) of the solution under the test and a Standard solution having a known concentration of *USP primaquine phosphate RS* in the same *Medium* and record the chromtograms. Measure the responses for the major peak, and calculate the amount of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$ dissolved.

Tolerances: Not less than 80% (*Q*) of the labeled amount of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$ is dissolved in 60 min.

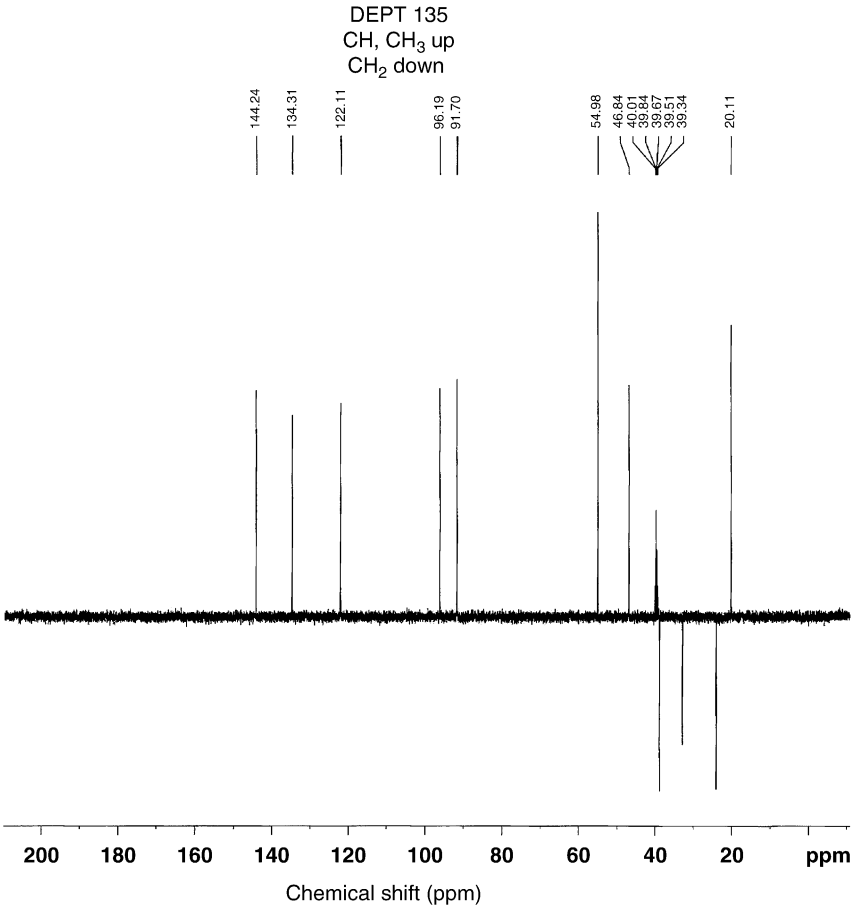


Fig. 13. The DEPT 135 ^{13}C NMR spectrum of primaquine diphosphate in DMSO-d_6.

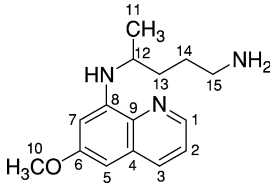
Uniformity of dosage units. When the test is carried out as directed in the general procedure <905>: meet the requirements.

Procedure for content uniformity: Transfer 1 tablet, previously crushed or finely powdered, to a beaker, add 5 mL of hydrochloric acid and about 25 g of crushed ice, then add water to bring the total volume to about 50 mL. Proceed as directed under *Nitrite Titration* under the general procedure <451>, beginning with "slowly titrate", and using as the titrant 0.01 M sodium nitrite VS, freshly prepared from 0.1 M sodium nitrite. Concomitantly perform a blank titration, and make any necessary correction. Each milliliter of 0.01 M sodium nitrite is equivalent to 4.553 mg of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$.

Assay

Weigh and finely powder not less than 30 tablets. Weigh accurately a portion of the powder, equivalent to about 700 mg of primaquine phosphate, and transfer to a

Table 4. Assignments for the resonance bands in the ^{13}C NMR spectrum of primaquine diphosphate



Chemical shift (ppm relative to TMS)	Assignment (carbon number)	Chemical shift (ppm relative to TMS)	Assignment (carbon number)
158.95	6	91.70	7
144.56	8	54.98	10
144.25	1	46.84	12
134.81	9	38.77	15
134.49	3	32.82	14
129.56	4	23.98	13
122.11	2	20.11	11
96.19	5		

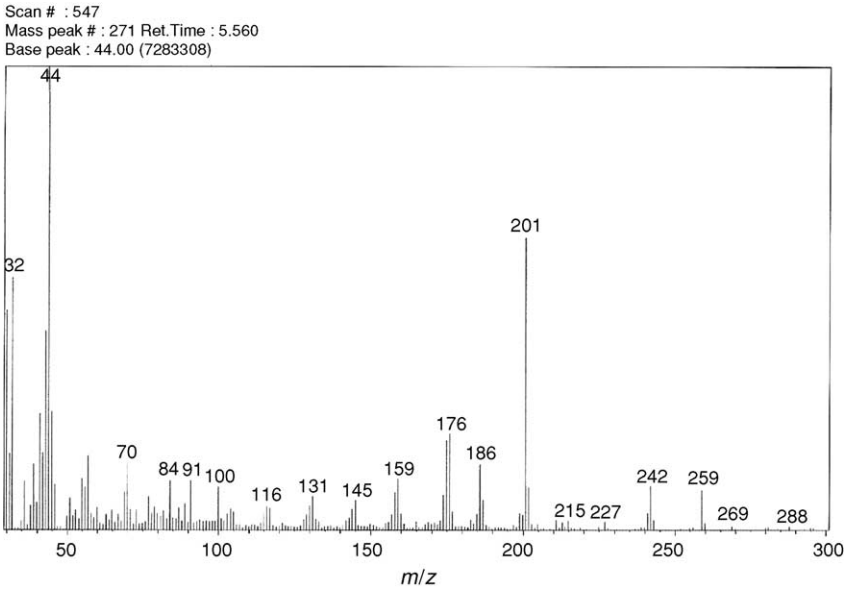
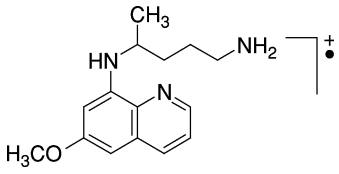
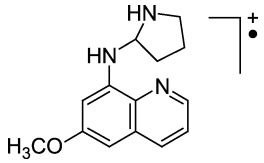
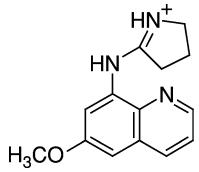
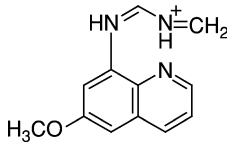
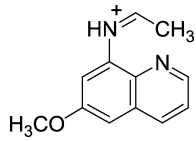
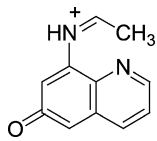
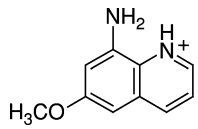
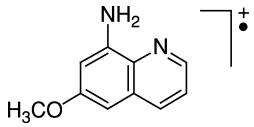


Fig. 14. Mass spectrum of primaquine diphosphate.

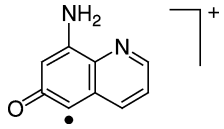
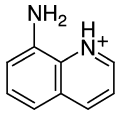
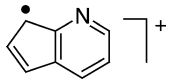
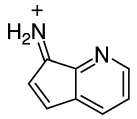
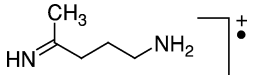
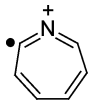
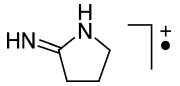
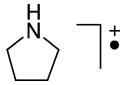
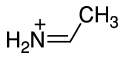
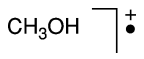
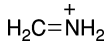
beaker. Add 50 mL of water and sufficient hydrochloric acid to provide about 5 mL in excess, and proceed as directed under *Nitrite Titration* as described in the general procedure <451>, beginning with "cool to about 15 °C". Each milliliter of 0.1 M sodium nitrite is equivalent to 45.53 mg of $C_{15}H_{21}N_3O{\cdot}2H_3PO_4$.

Table 5. Mass spectral fragmentation pattern of primaquine diphosphate

m/z	Relative intensity (%)	Fragment	
		Formula	Structure
259	9	$C_{15}H_{21}N_3O$	
243	2	$C_{14}H_{17}N_3O$	
242	10	$C_{14}H_{16}N_3O$	
215	2	$C_{12}H_{13}N_3O$	
201	62	$C_{12}H_{13}N_2O$	
186	14	$C_{11}H_{10}N_2O$	
175	19	$C_{10}H_{11}N_2O$	
174	7	$C_{10}H_{10}N_2O$	

(*Continues*)

Table 5. (*Continued*)

m/z	Relative intensity (%)	Fragment	
		Formula	Structure
159	11	$C_9H_7N_2O$	
145	7	$C_9H_9N_2$	
116	4	C_8H_6N	
131	8	$C_8H_7N_2$	
100	5	$C_5H_{12}N_2$	
91	10	C_6H_5N	
84	9	$C_4H_8N_2$	
70	14	C_4H_8N	
44	100	C_2H_6N	
32	53	CH_4O	
30	53	CH_4N	

4.2. Reported methods of analysis

4.2.1. Identification

Clarke recommended the following three identification tests [2]:

Test 1: Dissolve 10 mg of primaquine diphosphate in 5 mL of water and add 1 mL of a 5% solution of ceric ammonium sulfate in dilute nitric acid, a violet color is produced (distinction from chloroquine) [2].

Test 2: Mandelin's Test: Dissolve 0.5 g of ammonium vanadate in 1.5 mL of water and dilute to 100 mL with *sulfuric acid*. Filter the solution through glass wool. Add a drop of the reagent to the primaquine phosphate sample on a white tile. A orange → violet color is produced [2].

Test 3: Marquis Test: Mix 1 volume of *formaldehyde solution* with 9 volumes of *sulfuric acid*. Add a drop of the reagent to the primaquine phosphate sample on a white tile; an orange color is produced [2].

4.2.2. Titrimetric methods

Nickel used nitromethane and acetonitrile for the titration of primaquine and other basic pharmaceuticals in various solvents with 0.05 N perchloric acid in dioxane [9]. Half-neutralizaton potentials were determined in acetonitrile, methyl nitrite, and acetic acid anhydride with respect to pyramidon, arbitrarily taken as 500 mV and were shown in a table.

Walash *et al.* [10] determined primaquine and other quinoline drugs in bulk and in pharmaceuticals by a titrimetric method. The method is based on reaction with 1,3-dibromo-5,5-dimethylhydantoin or *N*-bromosuccinimide as the titrant. Primaquine was determined either by usual titration or by potentiometric titration with the brominating agents. The recovery was approximately 100%. The method was simple, precise, and accurate.

Ahmad and Shukla [11] determined primaquine and other antimalarial aminoquinolines by vanadium titration. The drugs were determined by oxidation with aqueous ammonium vanadate solution and back titration of the unconsumed reagent with aqueous acidic ammonium ferrous sulfate with *N*-phenyl anthranilic acid indicator.

Yang *et al.* [12] determined the ionization constants of primaquine by a titrimetric method and studied its coordination ratio with vitamin C. The ionization constants of primaquine in 50% (v/v) ethanol in water determined at 25 °C in the ionic strength range of 5×10^{-3} to 5×10^{-2} mol/L are given. The coordination ratio of primaquine to vitamin C is determined by continuous variation and mole ratio methods based on pH and conductance measurements to be 1:1, indicating that the coordination compound formed in the solution is mainly a 1:1 compound.

Hufford *et al.* [13] determined the dissociation constants of primaquine by titration with 0.1 N hydrochloric acid in acetonitrile–water mixture and values were extrapolated to water by using linear regression analysis.

4.2.3. Conductometric methods

Papovic *et al.* [14] used a conductometric method for the determination of primaquine phosphate. This method is recommended with perchloric acid, silicotungstic acid, and sodium hydroxide as titrants. Conductometric curves obtained with

various titrants differ in their shapes, depending on the changes occurring in conductivity of the solution before and after the titration endpoint, and their character is such that the titration endpoint can be accurately and reproducibly determined. In titration with perchloric acid, no individual protonations were observed on conductometric curves and only one inflexion point appeared although both the nitrogen atoms were protonated. Moreover, in the titration of primaquine phosphate with silicotungstic acid one inflexion point was obtained. With sodium hydroxide, deprotonation was effected only on the primary amine of the drug.

Amin and Issa [15] described a conductometric and indirect atomic absorption spectrometric methods for the determination of primaquine phosphate and other antimalarial agents. The method is based on the formation of their ion-associates with [Cd^{2+}, Co^{2+}, Mn^{2+}, and Zn^{2+}] thiocyanate, ammonium reineckate, and/or sodium cobaltinitrate. The molar combining ratio reveal that (1:1) (drug:reagent) ion-associates are formed for all reagents except for ammonium reineckate which forms (1:2) ion-associates with all studied drugs. The optimum conditions for the ion-association have been studied. Conductometric method was applied for the drug determination of the suggested drugs in bulk powders, whereas indirect atomic absorption spectrometric method, depending on the measurement of the excess metal ion present in supernatant solutions after precipitation of the ion associates is used to calculate the drug concentration. Optimum concentrations ranges for the determination of primaquine and the drugs under consideration were 0.46–12.9 and 0.155–3.87 mg using conductometric and indirect atomic absorption spectral methods, respectively. These procedures have been applied successfully to the analysis of these drugs in formulations and the results are favorably comparable to the official methods.

4.2.4. Spectrophotometric methods

Abou-Ouf *et al.* [16] described a spectrophotometric method for the determination of primaquine phosphate in pharmaceutical preparation. Two color reactions for the analysis of primaquine phosphate dosage form, which are based on 2,6-dichloroquinone chlorimide and 1,2-naphthoquinone-4-sulfonate, were described. The reactions depend on the presence of active centers in the primaquine molecule. These are the hydrogen atoms at position 5 of the quinoline nucleus and the primary amino group of the side chain. The method was applied to tablets of primaquine phosphate and a combination of primaquine phosphate and amodiaquine hydrochloride.

Prasad *et al.* [17] recommended a spectrophotometric assay method for the determination of primaquine in plasma.

Hassan *et al.* [18] used a spectrophotometric method for the simultaneous determination of primaquine with amodiaquine mixtures in dosage forms. Crushed tablets containing primaquine phosphate and amodiaquine hydrochloride were extracted with 0.1 M hydrochloric acid, and the mixture was filtered. A portion of the filtrate was diluted with 0.1 M hydrochloric acid, and the absorbance of this solution was measured at 282 and 342 nm against hydrochloric acid.

Sulaiman and Amin [19] used a spectrophotometric method for the determination of primaquine and chloroquine with chloranil. Treat the sample solution with 5 mL

each of 0.05 M borate buffer (pH 9) and 1 mM chloranil and dilute to 25 mL with water. Heat the stoppered flask at 65 °C for 20 min, cool to room temperature and measure the absorbance at 305 nm versus a reagent blank. The epsilon value for primaquine phosphate is 20.000.

Chatterjee *et al.* [20] quantitatively separated primaquine from amodiquine by a selective precipitation method. A powdered sample containing primaquine and amodiaquine was dissolved in 0.01 N hydrochloric acid 4 N ammonia was added to precipitate amodiaquine. The mixture was filtered and the combined filtrate and washings containing primaquine was diluted with water and 0.1 N hydrochloric acid. The absorbance of this solution was measured at 282 nm versus a solvent blank.

Abdel-Salam *et al.* [21] described a sensitive and simple spectrophotometric method for the determination of primaquine and other antimalarial drugs. The method is based on the formation of complexes between iodine (as an acceptor) and the basic drug in chloroform solution. Optimum conditions were established for the determination of primaquine, in pure form or in pharmaceutical preparation. Results were accurate and precise.

Vishwavidyalaya *et al.* [22] used a difference-spectrophotometric method for the estimation of primaquine phosphate in tablets. One portion of powdered tablets, equivalent to 7.5 mg of primaquine phosphate, was extracted with hydrochloric acid–potassium chloride buffer (pH 2) and a second portion was extracted with phosphate buffer (pH 10). Primaquine phosphate was determined from the difference in absorbance of the acid and alkaline extracts at 254.2 nm. The calibration graph was rectilinear from 2 to 14 μg/mL of primaquine phosphate. Recovery was 98.6% and no interference was observed from excipients. Results compared with those by the British Pharmacopoeial method.

Min *et al.* [23] determined primaquine phosphate, in tablets, by an ultraviolet spectrophotometric method. Sample was treated with 0.01 M hydrochloric acid and the resulting solution (500 μg/mL) was diluted to 50 mL with 0.01 M hydrochloric acid. The absorbance of the solution was measured at 265 nm versus a reagent blank. Beer's law was obeyed from 8 to 20 μg/mL of primaquine phosphate. Recovery was 100.2% ($n = 5$) and the coefficient of variation was 0.5%. Results were consistent with those obtained by a pharmacopoeial method.

Refaat *et al.* [24] used a spectrophotometric method for the determination of primaquine, and 16 other tertiary amine drugs, in bulk or in pharmaceuticals. The method involved the condensation of malonic acid with acetic anhydride in the presence of a tertiary amine in an aliphatic or a heterocyclic system. The condensation product is highly fluorescent and allows the spectrofluorimetric determination of the drug in the ng/mL ranges ($\lambda_{ex} = 415$ nm and $\lambda_{em} = 455$ nm). The product is also colored and allows the spectrophotometric determination of the drug and the other tertiary amine drugs ($\lambda_{max} = 333$ nm). Results of analysis of pure drugs and their dosage forms by these methods are in good agreement with those of the official British Pharmacopoeia and United States Pharmacopoeial procedures.

Artemchenko *et al.* [25] used a spectrophotometric method with *p*-quinone chlorimide in isopropanol in a pH 8 universal buffer for the determination of primaquine.

Talwar *et al.* [26] described a difference spectrophotometric method for the estimation of primaquine phosphate in tablets. The method is based on the

measurement of the absorbances at 265.5 and 281.3 nm (acidic solution) and 259 nm (alkaline solution). The λ_{max} of difference spectra was 254.2 nm. Beer's law was obeyed in the concentration range 2−14 μg/mL. The recovery was 98.62−99.19%.

4.2.5. Spectrofluorimetric methods

Refaat *et al.* [24] used a spectrophotometric method for the determination of primaquine, and 16 other tertiary amine drugs, in bulk or in pharmaceuticals. The method involved the condensation of malonic acid with acetic anhydride in the presence of a tertiary amine in an aliphatic or a heterocyclic system. The condensation product is highly fluorescent and allows the spectrofluorimetric determination of the drug in the ng/mL ranges ($\lambda_{ex} = 415$ nm and $\lambda_{em} = 455$ nm).

Gu *et al.* [27] used a spectrofluorimetric method for the determination of the long-acting antimalarial primaquine octanoate.

Aaron *et al.* [28] described a photochemical fluorimetric method for the determination of primaquine absorbed on silica gel chromatoplates.

Tsuchiya *et al.* [29] used a photochemical fluorimetric method for the determination of primaquine in a flowing solvent with application to blood serum. The standard or sample solution, as a 1:1 mixture of phosphoric acid solution (containing primaquine) and dioxan, was placed in a separating funnel and allowed to flow through a photochemical reactor and fluorescence cell at 0.3 mL/min. Serum (1 mL) was centrifuged after adding 1 mL of trichloroacetic acid and the supernatant solution (1 mL) was diluted to 50 mL with 0.7 M phosphoric acid–dioxan (1:1). The fluorescence background of serum was very low, allowing detection limit of $<$0.1 μg/mL.

Ibrahim *et al.* [30] described a fluorimetric method for the determination primaquine and two other aminoquinoline antimalarial drugs using eosin. Powdered tablets or ampule contents containing the equivalent of 50 mg of the drug was extracted with or dissolved in water (100 mL). A 10 mL aliquot was mixed with 10 mL of aqueous ammonia, 1 mL of 0.001% eosin (C.I. acid red 87) in dichloroethane, and dichloroethane was added to volume. Primaquine was determined fluorimetrically at 450 nm (excitation at 368 nm). Calibration graphs were rectilinear for 0.1–5 μg/mL of primaquine. Recoveries were quantitative. The method could be readily adapted for determination of the drug in biological fluids.

Cheng *et al.* reported the use of a synchronous fluorimetric method for the determination of primaquine in two-component antimalarial tablets [31]. Ground tablets were dissolved in water and the mixture was filtered. The fluorescence intensities of chloroquine phosphate and primaquine phosphate, in the filtrate, were measured at 380 nm (excitation at 355 nm) and 505 nm (excitation at 480 nm), respectively. The calibration graphs were linear from 1 to 8 μg/mL of chloroquine phosphate and 10 to 110 μg/mL of primaquine phosphate. The mean recoveries were 98.2–101.49% and the relative standard deviations were 2.23%.

4.2.6. Colorimetric methods

Fuhrmann and Werrbach [32] used a field test for the quantitative detection of primaquine in urine. Filter urine and place 10 mL in a 50-mL graduated cylinder. Add 1 mL of 25% ammonium hydroxide and 20 mL ammonium acetate and shake

immediately and thoroughly for 3 s. Allow to stand for 10–20 min, and transfer 15 mL of the ammonium acetate to a 25-mL measuring cylinder. Add 10 mL of 0.2 N sulfuric acid, shake, and after layer separation, withdraw 3 mL of the sulfuric acid without adherence of the ammonium acetate. Put the acid extract in a test tube; add 0.25 mL of diazo reagent (10 mL of a solution containing 4 g sulfanilic acid, 12 mL hydrochloric acid and water to 800 and 0.25 mL of 0.5% sodium nitrite). After 10 min, add 0.5 mL of Fehling reagent (40 g sodium hydroxide and 140 g sodium potassium tartarate per liter), mix, and determine the orange-red color colorimetrically or photometrically (Hg 436 filter). Determine the concentration by reference to a standard curve, which is linear in the range 0.3–2.1 mg% primaquine.

Rao and Rao [33] used a rapid, sensitive, and simple colorimetric method for the estimation of primaquine phosphate. The method is based on its reaction with sodium vandate to give a pink color, which has an absorbance maximum at 550 nm. Beer's law is obeyed for 2–30 μg/mL.

Issa *et al.* [34] used 2,3-dichloro-5,6-dicyano-*p*-benzoquinone for the spectrophotometric determination of primaquine and other antimalarials. The drugs were determined in tablets by a spectrophotometric method based on the reaction with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone and measurement of the absorbance at 460 nm. The reaction occurred fastest in methanol and acetonitrile to yield a radical anion, which was detected by electron spin resonance. The color attained its maximum intensity after 5 min and remained stable for at least 1 h. The absorbance versus concentration curve obeyed Beer's law in the concentration range 1–4 mg per 100 mL. The recovery was 99.9–102.6%.

De Faria and Santos [35] studied primaquine diphosphate and its oxidation product by visible, ultraviolet, Raman and electron paramagnetic resonance spectroscopy as well as cyclic voltammetry. The doubly protonated form of the drug is oxidized to a radical that absorbs strongly in the visible region at 552 nm. Such a species is highly reactive, and its time-decay is mainly due to its interaction with the nonoxidized drug. Another radical can be generated at higher pH, possibly originating from the singly protonated form of the drug.

El-Ashry *et al.* [36] studied the complex formation between the bromophenol blue, primaquine, and other important aminoquinoline antimalarials. The colorimetric method used was described as simple and rapid and is based on the interaction of the drug base with bromophenol blue to give a stable ion-pair complex. The spectra of the complex show maxima at 415–420 nm with high apparent molar absorptivities. Beer's law was obeyed in the concentration range 1–8, 2–10, and 2–12 μg/mL for amodiaquine hydrochloride, primaquine phosphate, and chloroquine phosphate, respectively. The method was applied to the determination of these drugs in certain formulations and the results were favorably comparable to the official methods.

John *et al.* [37] described a colorimetric method for the estimation of primaquine phosphate. Sample solutions of different dilutions (0.15–0.6 mL) of the drug (6–24 μg/mL) were treated with 5 mL of 1% cerric ammonium sulfate in dilute nitric acid and made up to 25 mL with water. The absorbance of the resulting light purple solution was measured at 480 nm after similar 30 min. Beer's law was obeyed from 5–30 μg/mL of primaquine phosphate. The method is applicable to bulk formulations in addition to tablets and capsule formulation.

Amin and Issa [38] described a simple, rapid, accurate, and sensitive spectrophotometric method for the microdetermination of primaquine phosphate and other aminoquinoline antimalarials. The method is based on the interaction of these drugs with calmagite indicator to give a highly colored ion-pair complex that exhibit maximum absorption at 666 nm for primaquine phosphate. Beer's law is obeyed in the concentration range 1–33 μg/mL for primaquine phosphate. For more accurate analysis, the Ringbom optimum concentration range is 3–30 μg/mL for primaquine phosphate. The method was applied to the determination of primaquine and the other two antimalarial agents in certain formulations, with results that compared favorably with those obtained by the official methods.

Hassan *et al.* [39] used a sensitive color reaction method for the determination of primaquine in pharmaceutical preparation. Primaquine was treated with diazo-*p*-nitroaniline in acidic medium to give an orange-yellow product with an absorbance maximum at 478 nm. When the medium was made alkaline, bathochromic, and hypochromic shifts occurred; the new maximum was located at 525 nm. The mean percentage recoveries for authentic samples amounted to 100 and 100.21 by the acid and alkaline procedures, respectively ($P = 0.05$). Both reactions could be used to determine primaquine salts in pharmaceutical preparations. The results obtained were in good agreement with those of the official methods. Recoveries were quantitative by both methods.

Sastry *et al.* [40] described a spectrophotometric method for the determination of primaquine and other antimalarial drug using quinones. The drug was determined as a (1:1) complexes with embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone), 2,5-dihydroxy-1,4-benzoquinone, or chloranilic acid. Samples of tablets, injection, or syrup containing 50 mg of the drug were dissolved in or diluted with 10 mL of water and the solution was filtered and mixed with 2 mL of 1 M sodium hydroxide and the precipitated free base were extracted into chloroform (4 × 20 mL). The combined chloroform extracts were washed with water (2 × 20 mL), dried over sodium sulfate and diluted to 100 mL with chloroform, then diluted with chloroform to contain 100 μg/mL of the drug. Aliquots of these solutions (containing 20–200 μg of the free base) were mixed with 2 mL of a 0.5% solution of embelin, 2,5-dihydroxy-1,4-benzoquinone or chloranilic acid in chloroform at 30 ± 5 °C and diluted with chloroform and after 2 min, the absorbance was measured at 520 nm for embelin, 500 nm for 2,5-dihydroxy-1,4-benzoquinone or 540 nm for chloranilic acid. The Beer's law ranges, regression equations, correlation coefficient, and coefficient of variation were 1.99% for complexes of primaquine with embelin and were tabulated.

Sastry *et al.* [41] used a new spectrophotometric method for the estimation of primaquine, using 3-methylbenzothiazolin-2-one hydrazone. An aqueous extract of the sample of powdered tablets (containing 50 μg/mL of primaquine phosphate was mixed with 1 mL each of aqueous 8.5 mM 3-methylbenzothiazolin-2-one hydrazone and 11.84 mM CeIV (in 0.72 M sulfuric acid), the mixture was diluted to 10 mL, and the absorbance was measured at 510 nm versus a reagent blank. Beer's law was obeyed for 0.7–12 μg/mL of the drug; and for 50 μg, the coefficient of variation was 0.52% ($n = 8$). Other antimalarials and pharmaceutical adjuvants did not interfere.

Sastry *et al.* [42] reported the use of an extractive spectrophotometric method for the determination of primaquine and other antimalarial agents using Fast Green FCF (C.I. Food Green 3) or Orange II (C.I. Acid Orange 7). Sample solution

containing 20 μg/mL of primaquine was mixed with phthalate buffer solution (pH 5) and aqueous 0.5% C.I. Food Green 3. After dilution with water, the aqueous solution was extracted with chloroform and the absorbance of the chloroform layer was measured at 630 nm. Instead of C.I. Food Green 3 and phthalate buffer solution, aqueous 0.5% C.I. Acid Orange 7 and 0.1 M hydrochloric acid were also used with absorbance measurement at 495 nm.

Mahrous *et al.* [43] determined primaquine and other antimalarials by use of chloranilic acid for the colorimetry. Primaquine was treated with 0.2 chloranilic acid solution in acetonitrile to give a purple solution with absorption maximum at 522 nm. Beer's law was obeyed from 0.04 to 0.2 mg/mL. Analysis of pharmaceutical formulation by this method is as accurate as the official method.

El-Kommos and Emara [44] described a spectrophotometric method, for the determination of primaquine and other secondary aromatic amines pharmaceuticals, using 3-methylbenzothiazolin-2-one hydrazone. The method is based on oxidative coupling reaction of 3-methylbenzothiazolin-2-one hydrazone.

Rao *et al.* [45] determined primaquine phosphate, in pharmaceutical dosage forms, by using a colorimetric method. Powdered tablets containing the equivalent of 100 mg of primaquine phosphate were heated with 25 mL of water for 10 min, the solution was cooled and filtered, and 10 mL portion of the filtrate was diluted 10-fold with water. A 5-mL portion of this solution was mixed with 5 mL of pH 5 buffer solution, 1 mL of 0.08% amidopyrine solution in aqueous 95% alcohol and 2 mL of aqueous 0.1% sodium periodate. After 10 min, 0.5 mL of aqueous sodium metabisulfite solution was added and the absorbance was measured at 580 nm. Beer's law was obeyed between 4 and 43 μg/mL of primaquine phosphate. Recoveries were quantitative.

Rao *et al.* [46] reported the use of a spectrophotometric method for the determination of primaquine phosphate with ninhydrin. Standard solution of 0.01% primaquine phosphate solution (3 mL) or solution prepared from the drug or its tablets was mixed with 2 mL of water, 5 mL of 2-methoxyethanol and then with 4 mL of ninhydrin reagent. The mixture was boiled for 35 min, and, after cooling and dilution to 25 mL with water, the absorbance was measured at 570 nm versus a reagent blank. Beer's law was obeyed from 4 to 20 μg/mL with recoveries of 99.4–100.2% for 25 mg of primaquine phosphate.

El-Kommos and Emara [47] determined primaquine and other secondary aromatic amines pharmaceuticals by a spectrophotometric method using 4-dimethyl amino cinnamaldehyde. The reaction of the reagent with primaquine and with the other amines was investigated. Powdered tablets were extracted with methanolic 0.1 M perchloric acid. The extract was mixed 1:1 with methanolic 0.2% of 4-dimethyl amino cinnamaldehyde and the mixture was diluted with methanol before measurement of the absorbance at 670 nm for primaquine phosphate. Beer's law was obeyed for 2–20 μg/mL of primaquine. The pink and green color formed with primaquine was stable for at least 24 h. Recoveries were good. Amodiaquine did not interfere with the determination of primaquine.

Sastry *et al.* [48] described a new spectrophotometric method for the estimation of primaquine, using 4-aminophenazone (4-aminoantipyrine). Aliquots of primaquine solution were mixed with 2 mL of methanolic 0.5% of 4-aminoantipyrine. After 5 min, 5 mL of 0.1% sodium periodate was added, and, after 3 min, the solution was

diluted to 25 mL with water. The absorbance was measured at 580 nm versus a reagent blank within 30 min. Beer's law was obeyed from 2 to 34 μg/mL of primaquine. The coefficient of variation ($n = 6$) was 0.9% at 600 μg of primaquine.

Ibrahim *et al.* [49] described a spectrophotometric method for the determination of primaquine and other antimalarial agents in pharmaceuticals. Powdered tablet or ampule contents containing 25 mg of primaquine phosphate, was dissolved in water and the solution was made alkaline with 6 M ammonia before extraction with chloroform. The extract was evaporated to dryness and the residue was dissolved in acetonitrile. A portion of the solution was mixed with 0.04% tetracyanoethylene solution in acetonitrile and diluted to volume with acetonitrile. After 10 min, the absorbance was measured at 415 nm for primaquine. Beer's law was obeyed from 2 to 12 mg/mL. The results agreed well with those of the United State Pharmacopoeia XX method.

Sastry *et al.* [50] estimated primaquine in its tablet formulation. Powdered tablets equivalent to 100 mg of primaquine phosphate were dissolved in water, filtered, and filtrate was diluted to 100 mL with water. Portions of the solution were shaken with 3 mL of 5 mM brucine–0.16 M sulfuric acid, 1.5 mL of 5 mM sodium periodate and 2 mL of 1.2 M sulfuric acid and diluted to 9 mL with water. The solution was set aside for 20 min in a boiling water bath, cooled, and diluted to 10 mL with water. The absorbance was measured at 510 nm versus a reagent blank. Beer's law was obeyed from 20 to 140 μg/mL of primaquine phosphate. The coefficient of variation was 1.56% ($n = 8$). Recovery was 99.2%.

El-Brashy [51] reported the determination of primaquine and other antimalarials via charge-transfer complexes. Powdered sample of primaquine phosphate was dissolved in water and the solution was adjusted to an alkaline pH with 6 M ammonia and extracted with chloroform. The extract was dried with anhydrous sodium sulfate, filtered, and evaporated to dryness under nitrogen and the residue was dissolved in acetonitrile. Portions of the solution were mixed with 0.2% 7,7,8,8-tetracyanoquinodimethane, diluted with acetonitrile, and set aside for 10 min before the absorbance was measured at 845 nm versus a reagent blank. The calibration graphs were linear from 0.4 to 3 μg/mL and recovery was 98%.

Baker *et al.* [52] reported the use of a colorimetric method for the determination of primaquine metabolites, in urine.

4.2.7. Nuclear magnetic resonance spectrometric methods

Clark *et al.* [53] subjected primaquine to metabolic studies using microorganisms. A total of 77 microorganisms were evaluated for their ability to metabolize primaquine, of these, 23 were found to convert primaquine to one or more metabolites (thin-layer chromatography analysis). Preparative scale fermentation of primaquine with four different microorganisms resulted in the isolation of two metabolites, identified as 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline and 8-(4-acetamido-1-methylbutylamino)-6-methoxyquinoline. The structures of the metabolites were proposed, based primarily on a comparison of the ^{13}C NMR spectra of the acetamido metabolite and the methyl ester of the carboxy metabolite with that of primaquine. The structures of both metabolites were confirmed by direct comparison with authentic samples.

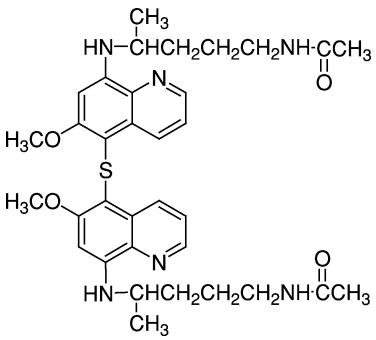
Singh *et al.* [54] used a ^{13}C NMR spectrometric method and reported the chemical shifts of primaquine and chloroquine. The signals are assigned on the basis of substituent effects on benzene shifts, intensities, multiplicities in single-frequency off-resonance decoupled and the comparison with structurally related compounds.

Hufford *et al.* [13] used a ^{13}C NMR spectroscopic method for the assignments of dissociation constants of primaquine. The first and second dissociation constants of primaquine were determined by titration with 0.1 N hydrochloric acid in acetonitrile–water mixtures and values were extrapolated to water by using linear regression analysis. The assignments of the dissociation constants were unambiguously achieved by studying the ^{13}C NMR spectral data obtained with monohydrochloride, dihydrochloride, and trihydrochloride salts.

McChesney and Sarangan [55] reported that primaquine undergoes unexpectedly rapid aromatic proton exchange with the medium. At pH <4, the exchange of carbon number 5 in the quinoline ring system is so fast as to be unmeasurable by proton NMR methods. At pH 6–6.5 the half-life for exchange is 4–5 min. This unexpectedly high rate of proton exchange with a medium may provide an important clue to the biological activity of primaquine.

Hufford and Baker [56] reported the assignments of the ^{13}C NMR spectra of three derivatives of primaquine namely 4-methylprimaquine, 5-methoxy-4-methylprimaquine, and 5-methoxyprimaquine. These assignments were based on comparison with those of primaquine, proton-coupled data, and selective long-range proton decoupling.

Hufford *et al.* [57] used proton and ^{13}C NMR spectrometric data to establish the novel sulfur-containing microbial metabolite of primaquine. Microbial metabolic studies of primaquine using *Streptomyces roseochromogenus* produced an *N*-acetylated metabolite and a methylene-linked dimeric product, both of which have been previously reported, and a novel sulfur-containing microbial metabolite. The structure of the metabolite as an S-linked dimer was proposed on the basis of spectral and chemical data. The molecular formula $C_{34}H_{44}N_6O_4S$ was established from field-desorption mass spectroscopy and analytical data. The ^{1}H- and ^{13}C NMR spectra data established that the novel metabolite was a symmetrical substituted dimer of primaquine *N*-acetate with a sulfur atom linking the two units at carbon 5. The metabolite is a mixture of stereoisomers, which can equilibrate in solution. This observation was confirmed by microbial synthesis of the metabolite from optically active primaquine.



Perussi *et al.* [58] studied the interaction of primaquine and chloroquine with ionic micelles as studied by ^{1}H NMR and electronic absorption spectroscopy. The characteristic of binding of primaquine and chloroquine to the micelles of surfactants with different charge of head groups were studied by ^{1}H NMR and optical absorption of spectroscopy. Cetyltrimethylammonium chloride was used as a cationic surfactant, sodium dodecyl sulfate as an anionic surfactant and *N*-hexadecyl-*N,N*-dimethyl-3-ammonio-1-propanesulfonate as zwitterionic. The pK values and binding constants were estimated. Interaction with sodium dodecyl sulfate significantly increases an apparent pK of primaquine and chloroquine. The chemical shift patterns and values of binding constants in the presence of different surfactants show that the mode of interaction of charged drugs with micelles is nonspecific, since the complexes formed are similar for different types of surfactants.

4.2.8. Culometric method

Berka *et al.* [59] described an accurate and reproducible coulometric method, with chlorine electrogenerated at the anode, for the determination of micro quantities of primaquine phosphate. Titration was carried out in an anode compartment with a supporting electrolyte of 0.5 M sulfuric acid–0.2 M sodium chloride and methyl orange as indicator. One coulomb was equivalent to 1.18 mg of primaquine phosphate. The coefficients of variation for 0.02–0.5 mg of primaquine phosphate were 1–5%. Excipients did not interfere.

4.2.9. Polarographic methods

Zhan and Mao [60] used a simple, fast, and selective alternating current oscilloscopic polarographic titration method for the determination of primaquine and other alkaloid phosphate in pharmaceutical preparation. The titration was carried out with a standard lead solution in hexamethylene tetramine buffer containing 1 M sodium chlorate (pH 5.5). The results obtained by this method are comparable to those obtained by pharmacopoeial method.

Zhan [61] reported the use of an oscillopolarographic method for the determination of primaquine phosphate and other drugs in pure form and in pharmaceutical preparations. The sample solution was mixed with potassium bromide and 6 M hydrochloric acid and the mixture was titrated with 0.1 M sodium nitrite. A micro platinum electrode and a platinum electrode were used as indicators and reference electrodes, respectively. The mean recoveries were 96.88–99.88%. Results agreed well with those obtained by the Chinese Pharmacopoeia method.

Saad *et al.* [62] reported that electrodes based on polyvinyl chloride matrix membranes containing one of three macrocyclic crown ethers with dioctyl phenyl phosphonate solvent mediator and, in some instances, anion excluder have been studied for their potentiometric response to the primaquinium cation. Primaquine-selective electrodes based on dibenzo(18-crown-6) gave sub-Nernstian responses while those based on dibenzo(24-crown-8) and dibenzo(30-crown-10) exhibited good electrochemical characteristics, such as Nernstian responses, fast dynamic response times (30 s), a wide range of working pH (pH 4–10) and good selectivity over many metal cations, chloroquine, and sulfonamide drugs. The addition of 50% mole ratio anion excluder, relative to the sensor, not only led to an improvement in

the electromotive force stability, but also produced, in most instances, improved selectivity characteristics for both the dibenzo(24-crown-8) and dibenzo(30-crown-10) based electrodes. Determination of primaquine diphosphate (4.5–453 μg/cm^3) using the standard additions method resulted in a mean recovery and relative standard deviation of 107% and 8%, respectively. Determination of primaquine in pharmaceutical preparation is also described.

Mohamed [63] investigated the complexation behavior of amodiaquine and primaquine with Cu(II) by a polarographic method. The reduction process at dropping mercury electrode in aqueous medium is reversible and diffusion controlled, giving well-defined peaks. The cathodic shift in the peak potential (E_p) with increasing ligand concentrations and the trend of the plot of $E_{½}$ versus log C_x indicate complex formation, probably more than one complex species. The composition and stability constants of the simple complexes formed were determined. The logarithmic stability constants are: log $B_1 = 3.56$; log $B_2 = 3.38$, and log $B_3 = 3.32$ [Cu(II)–primaquine at 25 °C].

4.2.10. X-ray analysis

Yu *et al.* [64] established the S-configuration for (+)-primaquine, prepared from the racemate by chemical resolution, by solid-state X-ray analysis of the (+)-1-phenylethylurea obtained with R-(+)-1-phenylethylisocyanate.

4.2.11. Atomic absorption spectroscopy

Amin and Issa [15] described an indirect atomic absorption spectrometric method for the determination of primaquine and other antimalarials. The method is based on the formation of their ion-associates with [Cd^{2+}, Co^{2+}, Mn^{2+}, and Zn^{2+1}] thiocyanate, ammonium reineckate, and/or sodium cobaltinitrate.

Hassan *et al.* [65] used a method for the determination of primaquine and other antimalarials, through ternary complex formation. The analytical aspects of the reaction between the widely used antimalarial drugs with cobalt and thiocyanate to form ternary complexes are described. Alternatively, determination of the cobalt content of the nitrobenzene extract using atomic absorption spectroscopy provided an indirect method for the determination of the drugs. Both methods are applied to the analysis of pharmaceutical preparation and the results obtained agreed well with those obtained with official methods.

4.2.12. Chromatographic methods

4.2.12.1. Thin-layer chromatography methods

Fusari *et al.* [66] described a qualitative thin-layer chromatography (TLC) elution technique, for the assay of primaquine and other pharmaceuticals.

Zheng and Sun [67] used a thin-layer chromatographic method for the analysis of primaquine and other quinoline derivatives. The drug and other compounds were chromatographed on silica gel GF_{254} plate, with methanol:aqueous 25–28% ammonia (200:3) and chloroform:dichloromethane:diethylamine (4:3:1), as mobile phases. Spots were located under ultraviolet radiation. The detection limit was 1–2 μg/mL. Total separation could be achieved by the use of two plates and the respective mobile phase.

Dwivedi *et al.* used a thin-layer chromatographic densitometric and ultraviolet spectrophotometric methods for the simultaneous determination of primaquine and a new antimalarial agent, CDRI compound number 80/53 [68]. The new antimalarial agent, compound 80/53 is unstable in acidic conditions where it is converted into primaquine. To conduct stability studies of this compound, thin-layer chromatography densitometric and ultraviolet spectrophotometric determination methods were developed. These methods are also suitable of the determination of compound 80/53 or primaquine in bulk and pharmaceutical dosage forms.

Dwivedi *et al.* [69] used a high-performance thin-layer chromatographic method for the simultaneous analysis of primaquine, bulaquine, and chloroquine. The high performance thin-layer chromatography workstation comprised of Automatic TLC sampler-III equipped with 50 μL Hamilton syringe, TLC Scanner-3, equipped with mercury, tungsten, and deuterium lamp for scanning of TLC plates. The separation was achieved on thin-layer plates of precoated Si-gel 60 F_{254} (20 cm × 20 cm, 0.2 mm). The plates were scanned at wavelength of 254 nm. Data was acquired and processed using a CATS4-HPTLC software. The solvent system used was hexane–diethylether–methanol–diethylamine in the ratio of (37.5: 37.5:25:0.5). TLC was performed at 27 ± 3 °C in a Camag TLC twin trough glass chamber.

Clarke [2] recommended the following three thin-layer chromatography systems [70].

System 1

Plates. Silica gel G, 250 μm thick, dipped in, or sprayed with, 01 M potassium hydroxide in methanol, and dried.

Mobile phase. Methanol:strong ammonia solution (100:1.5).

Reference compounds. Diazepam R_f 75, chlorprothixene R_f 56, codeine R_f 33, atropine R_f 18.

$R_f = 19$ Acidified iodoplatinate solution, positive [70].

System 2

Plates. This system uses the same plates as system 1 with which it may be used because of the low correlation of R_f values.

Mobile phase. Cyclohexane:toluene:diethylamine (75:15:10).

Reference compounds. Dipipanone R_f 66, pethidine R_f 37, desipramine R_f 20, codeine R_f 06.

$R_f = 13$ Acidified iodoplatinate solution, positive [70].

System 3

Plates. This system uses the same plates as systems 1 and 2 with which it may be used because of the low correlation of R_f values.

Mobile phase. Chloroform:methanol (90:10).

Reference compounds. Meclozine R_f 79, caffeine R_f 58, dipipanone R_f 33, desipramine R_f 11.

$R_f = 05$ Acidified iodoplatinate solution, positive [70].

4.2.12.2. Gas chromatographic methods

Clarke [2] reported the following gas chromatographic system [71].

Column. 2.5% SE-30 on 80–100 mesh chromosorb G (acid-washed and dimethyldichlorosilane-treated), 2 cm × 4 mm internal diameter glass column, it is essential that the support is fully deactivated.

Column temperature. Normally between 100 and 300 °C. As an approximate guide, the temperature to be used is the retention index ÷10.

Carrier gas. Nitrogen at 45 mL/min.

Reference compounds. *n*-Alkanes with an even number of carbon atoms. Retention index: 2314 [71].

Rajagopalan *et al.* [72] described an electron-capture gas chromatographic assay method for the determination of primaquine in blood. The method involves derivatization with heptafluorobutyric anhydride to form the diheptafluorobutyramide derivative after a single extraction at alkaline pH. The derivatives are quantitated by electron-capture gas chromatography. Blood levels of primaquine as low as 8 ng/mL can be measured with good precision.

4.2.12.3. Gas chromatography–mass spectrometric methods

Baty *et al.* [73] studied several substituted 8-aminoquinoline related to primaquine and the known antimalarial drugs, by gas chromatography–mass spectrometry method. 5,6-Dihydroxy-8-aminoquinoline, a possible metabolite of primaquine, can be detected by single ion-monitoring after conversion to trimethylsilyl ether derivative. The mass spectra obtained in this study indicate that there are certain ions which are characteristic of trimethylsilyl ethers of hydroxylated 8-aminoquinolines and 5,6-dimethoxy-8-aminoquinolines. These compounds should thus be amenable to analysis if they were produced during *in vivo* metabolism studies. Using selected ion-monitoring, the derivatized compounds can be detected at submicrogram levels.

Greaves *et al.* [74] used a selected ion-monitoring assay method for the determination of primaquine in plasma and urine using gas chromatography–mass spectrometric method and a deuterated internal standard. After freeze-drying and extraction with trichloroethylene, the sample plus internal standard was reacted with Tri Sil TBT (a 3:3:2 by volume mixture of trimethylsilylimidazole, *N,O*-bis-(trimethylsilylacetamide and trimethylchlorosilane) and an aliquot injected to the gas chromatograph-mass spectrometer. The gas chromatographic effluent was monitored at *m*/*z* 403, and *m*/*z* 406, the molecular ions of the bis-tetramethylsilane ethers of primaquine and 6-trideuteromethoxy primaquine.

4.2.12.4. High performance liquid chromatography–mass spectrometric methods

Nitin *et al.* [75] developed and validated a sensitive and selective liquid chromatography–tandem mass spectrometric method (LC–MS–MS) for the simultaneous estimation of bulaquine and its metabolites primaquine in monkey plasma. The mobile phase consisted of acetonitrile–ammonium acetate buffer (20 mM, pH 6) (50:50, v/v) at a flow rate of 1 mL/min. The chromatographic separations were achieved on two Spheri cyano columns (5 μm, 30 cm × 4.6 mm), connected in

series. The quantitation was carried out using a Micromass LC–MS–MS with an electrospray source in the multiple reaction monitoring mode. The analytes were quantified from the summed total ion value of their two most intense molecular transitions. Another method was also used by the same authors. A simple liquid–liquid extraction method with 2 × 1 mL *n*-hexane-ethyl acetate–dimethyl octyl amine (90:10:0.05, v/v) was utilized. The method was validated in terms of recovery, linearity, accuracy, and precision (within- and between-assay variation). The recoveries from spiked control samples were ≥90% and 50% for bulaquine and primaquine, respectively. Linearity in plasma was observed over a dynamic range of 1.56–400 and 3.91–1000 ng/mL for bulaquine and primaquine, respectively.

4.2.12.5. High-pressure liquid chromatographic methods

Dua *et al.* [76] carried out some chromatographic studies on the isolation of peroxydisulfate oxidation products of primaquine. A 100 μL sample (0.25 mg/mL) of primaquine and 100 μL of potassium peroxydisulfate (0.24 mg/mL) were mixed and 5 μL portions injected onto a μ-Bondapak C_{18} (5 μm) column (30 cm × 3.9 mm) column 5, 15, 30, and 40 min after the start of oxidation. Analytes were eluted (1 mL/min) with acetonitrile–methanol–1 M perchloric acid (30:7:1.95) and detected at 254 nm. The product of a semipreparative oxidation performed on a 10-fold scale were crudely separated on a column (100 cm × 2 cm) filled with Bio-Gel P-2 and each colored band, identified visually, analyzed by high performance liquid chromatography. Fractions with similar peak profiles were pooled, rechromatographed on Bio-Gel P-2 and checked by high performance liquid chromatography until pure compounds were obtained. Eight oxidation compounds were obtained at 90% purity and the structures of five major products were determined by infrared, nuclear magnetic resonance, and mass spectrometry. Two products, 6-methoxy-5,8-bis-(4-amino-1-methylbutylamino)quinoline and 5,5-bis-[6-methoxy-8-(4-amino-1-methylbutylamino)quinoline] exhibited a threefold to fourfold increase in gametocytocidal activity of primaquine.

Dua *et al.* [77] also develoed a reversed-phase high performance liquid chromatographic method using acetonitrile–methanol–1 M perchloric acid–water (30:9:1:95, v/v) at a flow rate of 1.5 mL/min on a μ-Bondapak C_{18} column with an ultraviolet detection at 254 nm, for the separation of primaquine, its major metabolite carboxyprimaquine and other metabolites such as *N*-acetylprimaquine, 4-hydroxyprimaquine, 5-hydroxy primaquine, 5-hydroxy-6-methoxy primaquine and demethyl primaquine, and also other antimalarials. The calibration graphs were linear in the range 0.025–100 μg/mL for primaquine and 4−1000 μg/mL for caroxyprimaquine. The within-day and day-to-day coefficients of variation averaged 3.65 and 6.95%, respectively, for primaquine; and 3 and 7.52%, respectively, for carboxyprimaquine in plasma. The extraction recoveries for primaquine and carboxyprimaquine were 89% and 83%, respectively.

Jane *et al.* [78] used a high performance liquid chromatographic method for the analysis of primaquine and other basic drugs on silica columns using nonaqueous ionic eluents. Unmodified silica columns together with nonaqueous ionic eluents give stable and flexible systems for the analysis of primaquine and the other basic drug compounds by high performance liquid chromatography. Low wavelength ultraviolet and fluorescence detection are used and fluorescence is optimized by

postcolumn pH change or derivatization of some primary aliphatic amines with *o*-phthaldialdehyde. A novel feature is that electrochemical oxidation can be used for the detection of most analytes. Retention and relative response data (UV, 254 nm and electrochemical, +1.2 V) were generated for primaquine and 161 other compounds, using a 125-mm Spherisorb S5W silica column and methanolic ammonium perchlorate (10 mM, pH 6.7) as eluent. This system can be used isocratically in qualitative analyses as well as for quantitative work, when either the wavelength or the applied potential can be adjusted to optimize the response.

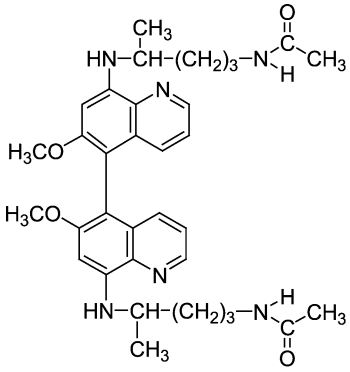
Parkhurst *et al.* [79] described a high performance liquid chromatographic method for the simultaneous determination of primaquine and its metabolites from plasma and urine samples, utilizing acetonitrile deproteinization, and direct injection onto a cyano column. Levels of 100 ng/mL per 20 μL injection could be quantitated. Preliminary pharmacokinetic analysis is reported for two human subjects after oral doses of 60–90 mg primaquine diphosphate. Two apparent plasma metabolites and two possible urinary metabolites are also reported.

Wheals used isocratic multicolumn high performance liquid chromatography as a technique for qualitative analysis and its application to the characterization of primaquine and other basic drugs using an aqueous methanol solvent [80]. A variety of high performance liquid chromatographic packing materials were prepared and their chromatographic properties compared for the separation of primaquine and the other basic drugs using a single solvent system. The three most promising packing materials (silica, a mercapto propyl modified silica and a propyl sulfonic acid modification) were subsequently used to provide retention volume data for a large number of drugs.

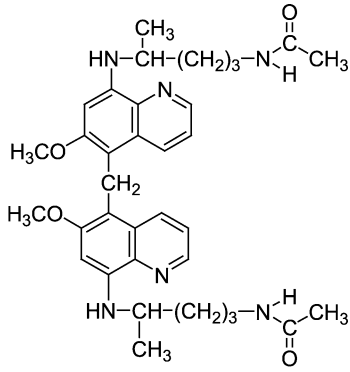
Clark *et al.* [81] determined the time course of *N*-acetylation of primaquine by *Streptomyces roseochromogenous* and *Streptomyces rimosus* by quantitative high performance liquid chromatographic analyses of the culture broths. The *N*-5-bistrifluoroacetyl derivative of primaquine was used as an internal standard in the analysis for the quantitation of primaquine *N*-acetate in microbial culture broths. *S. roseochromogenous* forms the highest level of primaquine *N*-acetate at 24–36 h after substrate addition, while *S. rimosus* is slower in its acetylation, peaking at 3 days after substrate addition. The formation of a novel dimeric compound from the reaction of primaquine with 8-(4-phthalimido-1-methylbutylamino)-6-methoxy quinoline is also reported.

Baker *et al.* [82] used high performance liquid chromatographic method for the analysis of the metabolism of primaquine. Using rats, dosed with 20 mg/kg of primaquine diphosphate (11.4 mg/kg), it was found that the drug underwent a metabolic oxidative deamination to give 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline. The presence of this new mammalian metabolite was verified using high performance liquid chromatography, gas chromatography, and mass spectral methods. A quantitative high performance liquid chromatographic method for the determination of primaquine and the carboxylic acid metabolite in plasma using only 50 μL of whole blood from the rat was developed and the method could be used to detect levels as low as 0.05 μg/mL of the metabolite. Following intravenous administration of the drug, the plasma levels of primaquine fell very rapidly and after 30 min, the levels of the metabolite was much higher than those of primaquine.

Hufford *et al.* [83] studied the stereochemistry of dimeric microbial metabolites of primaquine. The dimeric microbial metabolite **1** of (±)-primaquine was shown by high performance liquid chromatography to be composed of a mixture of three compounds, which were separated. Heating of these three compounds demonstrated that they exhibit atropisomerism. Synthesis of the dimeric metabolite **1** from (−)-primaquine showed only two compounds by high performance liquid chromatography. These experiments demonstrated the presence of three enantiomeric pairs of diastereoisomers of the dimeric metabolite **1**. The other dimeric microbial metabolite **2** was shown by high performance liquid chromatography to be composed of a mixture of two compounds, which could be separated but equilibrated back to the original mixture. Synthesis of metabolite **2** from optically active primaquine produced only the compound with a longer retention time in the high performance liquid chromatography. Mixing of metabolite **2** prepared from (+)-primaquine with that prepared from (−)-primaquine produced the two compounds. The experiments demonstrate that the two compounds are an enantiomeric pair and meso-isomer, which equilibrate through a novel mechanism.



Metabolite **1**



Metabolite **2**

Katori *et al.* [84] used a high performance liquid chromatographic method for the determination of primaquine phosphate tablets. Chromatography on a TSK Gel-4 (octadecylsilanized) column was used to determine the active ingredient in tablets and injections for treating tropical diseases. The mobile phase used is 26% acetonitrile in 0.05-M pH-3 phosphate buffer and the drug was detected at 260 nm. The peak area and peak height reproducibilities were $\leq$1.69%, and results compared well with those of other methods.

Okamoto *et al.* [85] performed the optical resolution of primaquine and other racemic drugs by high performance liquid chromatography using cellulose and amylose tris-(phenylcarbamate) derivatives as chiral stationary phases. Primaquine and other compounds were effectively resolved by cellulose and/or amylose derivatives having substituents such as methyl, tertiary butyl, or halogen, on the phenyl groups.

Sidhu *et al.* [86] developed a reliable and simple high performance liquid chromatographic method for the routine analysis of pharmaceutical dosage forms using a C_{18} Bondapak reversed-phase column with a binary solvent system consisting of

acetonitrile and 0.05 M potassium dihydrogen phosphate. Standardized extraction procedures for drugs in various dosage forms were developed and applied to a wide range of current pharmaceutical formulations.

Rao *et al.* [87] developed a high performance liquid chromatographic method for the determination of primaquine phosphate in pharmaceutical dosage form. A μ-Bondapak NH_2 column with chloroform–methanol (60:40) as eluent was selected with sulfalene as internal standard. The method was convenient with recovery of approximately 100% for primaquine.

Taylor *et al.* [88] developed a general separation strategy, involving solid-phase extraction followed by reversed-phase ion-pairing high performance liquid chromatography with an organic counter ion for primaquine and a set of 10 widely used antimalarial drugs and metabolites. The basis underlying the separation has been explored and work, including quantitative data, has been carried out on illustrative separations, which form the basis of novel quantitative assays of groups of antimalarials that are relevant to current prophylaxis and treatment of malaria.

Liu and Wu [89] used a high performance liquid chromatographic method for the simultaneous determination of primaquine and pyronaridine in plasma. The two drugs were extracted with ethyl acetate from alkalinized plasma or whole blood of rabbits and determined by high performance liquid chromatography, using methanol–0.05 M citric acid–disodium hydrogen phosphate pH 3/buffer solution (10:100) and detection at 254 nm. The detection limit was 3 ng. The calibration curve was linear at 0.05−0.50 μg/mL. The average recovery was 83.21 and 65.23 for pyronaridine and primaquine, respectively, and the coefficient of variation was $<5\%$.

Li *et al.* [90] used a normal phase high performance liquid chromatographic process to separate and detect primaquine in blood and liver after a single intravenous dose of the hepatic targeting agent neoglycoalbumin–primaquine conjugate and primaquine phosphate in mice. 6-Methoxy-8-(4-aminobutyrylamino)quinoline, synthesized and identified by the authors, was used as an internal standard to be added to the biological samples obtained from mice at different times after giving neoglycoalbumin–primaquine or primaquine phosphate. The mixture was extracted with ether after alkalinization in the primaquine phosphate group. In the neoglycoalbumin–primaquine group, the biological samples must be hydrolysed by heating under nitrogen and acid condition in a domestic pressure cooker before extraction. The extracts were evaporated to dryness under nitrogen, and then dissolved in the mobile phase (chloroform–methanol–ammonium hydroxide, 86.8:12.5:0.7). The results showed that the hepatic primaquine collecting ratio and the retention time of primaquine in liver in the neoglycoalbumin–primaquine group were higher and longer than those of the primaquine phosphate group. The results also point out that neoglycoalbumin–primaquine has lower targeting property.

Fang *et al.* [91] studied the enantiomeric separation of primaquine and 10 other drugs having a primary amino group using high performance liquid chromatography with a chiral Crownpak CR (+) column. The enantiomers of primaquine and the other drugs were separated by high performance liquid chromatography with Crownpak CR (+) column. By decreasing the column temperature, the elution of the analytes was delayed and enantioselectivity (resolution) was improved, but by increasing the methanol content, the elution of the analytes became more rapid, and the enantioselectivity was decreased.

Dwivedi *et al.* [69] used high performance liquid chromatographic assay method for the simultaneous analysis of primaquine, bulaquine, and chloroquine. The high performance liquid chromatographic system was equipped with 250 binary gradient pumps, a Rheodyne model 7125 injector with a 20-μL loop, and 235 diode-array detectors. The high performance liquid chromatographic separation was achieved on a reversed-phase select-B C_8 Lichrospher analytical column (25 cm × 4 mm, 5 μm). Column effluent was monitored at 265 nm. Data was acquired and processed using an IndTech HPLC interface and software. The mobile phase was 0.01 M sodium acetate buffer pH 5.6 and acetonitrile (45:55). Both the solutions were filtered in degassed before use. Chromatography was performed at 27 ± 3 °C at a flow rate of 1 mL/min.

Jawahar *et al.* [92] developed and validated a precise and reproducible high performance liquid chromatographic method for simultaneous determination of bulaquine and its metabolite primaquine in rabbit plasma. The method, applicable to 0.5 mL plasma, involves double extraction of samples with *n*-hexane:isopropanol (98:2) containing dimethyloctylamine (0.1%). Separations were accomplished by reversed-phase liquid chromatography using a Spheri-5-cyano column with a low pressure gradient with mobile phase consisting of ammonium acetate buffer (50 mM, pH 6) and acetonitrile with dimethyloctylamine. The method was sensitive with a limit of quantitation of 20 ng/mL in rabbit plasma for both bulaquine and primaquine and the recoveries were >85% and >45%, respectively.

Dean *et al.* [93] used a high performance liquid chromatographic method for the simultaneous determination of primaquine and carboxyprimaquine in plasma with electrochemical detection. After the addition of the internal standard, plasma was deproteinized by the addition of acetonitrile. Nitrogen-dried supernatants, resuspended in mobile phase were analyzed on a C_8 reversed-phase column. Limits of detection for primaquine and carboxyprimaquine were 2 and 5 ng/mL with quantitation limits of 5 and 20 ng/mL, respectively. The assay sensitivity and specificity are sufficient to permit quantitation of the drug in plasma for pharmacokinetics following low dose (30 mg, base) oral administration of primaquine, typically used in the treatment of malaria and *P. carinii* pneumonia.

The other high performance liquid chromatographic systems [94–105] are shown in Table 6.

4.2.12.6. Capillary electrophoresis methods

Fan *et al.* [106] developed a high performance capillary electrophoresis method for the analysis of primaquine and its trifluoroacetyl derivative. The method is based on the mode of capillary-zone electrophoresis in the Bio-Rad HPE-100 capillary electrophoresis system; effects of some factors in the electrophoretic conditions on the separation of primaquine and trifluoroacetyl primaquine were studied. Methyl ephedrine was used as the internal standard and the detection was carried out at 210 nm. A linear relationship was obtained between the ratio of peak area of sample and internal standard and corresponding concentration of sample. The relative standard deviations of migration time and the ratio of peak area of within-day and between-day for replicate injections were <0.6% and 5.0%, respectively.

Ng *et al.* [107] described a simple and rapid systematic optimization scheme, known as overlapping resolution mapping scheme, which make use of nine

Table 6. High performance liquid chromatography conditions of the methods used for the determination of primaquine diphosphate

Column	Mobile phase [Flow rate]	Detection	Remarks	Reference
20 cm × 6 mm of Partisil ODS III (10 μm)	pH 3.5, water–acetonitrile–methanol (6:3:1), containing 0.5 mM octanesulfonic acid [1.5 m/min]	Not given	Reversed-phase HPLC for analysis of the drug in biological fluids. Detection limit of the drug in plasma 1 ng/mL.	[94]
25 cm × 3.9 mm of μ-Bondapak CN	Acetonitrile–0.08 M citrate (77:23, pH 5), [1 mL/min]	Dual electrode electrochemical detection	Analysis of the drug and metabolites in human plasma and urine.	[95]
Fused-silica column (15 cm × 4.6 mm) of Spherisorb S5W	0.1 mL chloroform–methanol–aqueous 25% ammonia (868:125:7) [1.5 mL/min]	254 nm	Normal phase HPLC analysis in whole blood and urine.	[96]
Stainless steel column (25 cm × 4.6 mm) of YWG-$C_{18}H_{37}$ (10 μm)	Aqueous 85% methanol; [1.5 mL/min]	267 nm	Reversed-phase HPLC analysis in rabbit serum.	[97]
Whatman PX-5/25 ODS 25 cm × 4.6 mm Pirkle type 1A column	Hexane–propan–2-ol-acetonitrile (170:21:15), [1 mL/min]	Not given	Separation of enantiomers and diastereoisomers of the drug and its metabolites.	[98]
30 cm × 3.9 mm of μ-Bondapak phenyl	Acetonitrile–0.01 M KH_2PO_4 (pH 3.8)–anhydrous acetic acid (58:141:1) [2 mL/min]	278 nm	Analysis in tablets. Dextromethorphan used as internal standard.	[99]
30 cm × 3.9 mm of Bondapak NH_2	Chloroform–methanol (3:2) [1 mL/min]	254 nm	In tablets dosage forms.	[100]
15 cm × 6 mm of ODS (5 μm)	Methanol–water–anhydrous acetic acid (800:200:5) containing 0.5 mM–sodium dodecyl sulfate [1 mL/min]	340 nm	Determined primaquine with chloroquine simultaneously.	[101]
25 cm × 4 mm of Lichrosorb RP-8 (10 μm), fitted with a precolumn of the same material	0.2062 g of KH_2PO_4 and 0.2625 g of K_2HPO_4 in water–acetonitrile–methanol (1:1:2) [1 mL/min]	254 nm	Analysis of tablets. Results agreed well with B. P. method. Recovery was 102%.	[102]
15 cm × 4.6 mm of Asahipak ODP-50 at 40°C (0.45 μm)	70 mM phosphate buffer of pH 5.8–acetonitrile (77:23) containing 10 mg/L of Na_2–EDTA [0.8 mL/min]	Electrochemical	Analysis of primaquine and carboxyprimaquine in calf plasma.	[103]
Cartridge column (10 cm × 4.6 mm) of C_{18} (5 μm) equipped with a pre-column (3 cm × 4.6 mm) of C_{18}	Acetonitrile–0.05 K_2HPO_4 of pH 6–tetrahydrofuran (60:39:1) containing *N*, *N*′-dimethyl octylamine (0.5 mL/L) [0.7 mL/min]	269 nm	Determined the drug as a metabolite of a new drug, CDRI compound 8053, simultaneously, in serum.	[104]
Normal phase silica column	Chloroform–methanol–ammonia solution (86.8:12.5:0.7)	254 nm	Assay of primaquine and hepatic targeting neoglycoalbumin–primaquine in whole blood and liver of mouse by reversed-phase HPLC.	[105]

preliminary experiments to predict the optimum capillary electrophoresis separation conditions for primaquine and other antimalarial agents. In this investigation, the validity of the overlapping resolution mapping scheme is verified by choosing the optimum separation conditions predicted by the scheme in the separation of a group of 12 amino acids derivatized with the *N*-tert-butoxycarbonyl functional group, primaquine and a group of six other antimalarial agents. The effect of the buffer composition on the separation is discussed.

Taylor and Reid [108] reported that the separations of primaquine and 10 other antimalarial drugs and metabolites are shown by capillary-zone electrophoresis at low pH and by micellar electrokinetic chromatography at high pH. Capillary-zone electrophoresis is shown to be superior to micellar electrokinetic chromatography in resolution capability for these compounds under the conditions examined. Both are shown to provide different selectivities to those obtained by ion-pair reversed-phase high performance liquid chromatography. The effect of sample injection solvent is examined in capillary-zone electrophoresis and it is shown that field-amplified sample injection is effective for these compounds. It is shown that injection of sample in an organic solvent such as methanol augments the stacking of analytes resulting in lower detection limit. The limit of detection of some of the antimalarial agents in a urine matrix is reported.

Stalcup and Agyei [109] investigated heparin, the naturally occurring polydisperse and polyanionic glycosaminoglycan, as a chiral selector for capillary-zone electrophoresis. Baseline separations were obtained for a variety of underivatized drugs including primaquine and other antimalarial agents. Analysis was carried out at a pH of 4.5 or 5 using 2% heparin (w/w) in a 10-mM phosphate buffer under an applied voltage of 15 kV. All of the solutes successfully resolved contained at least two nitrogens with one of the nitrogens incorporated in a heterocyclic aromatic ring. Furthermore, successful resolution seemed to require that the nitrogens be somewhat distant from each other in the molecule. In addition to electrostatic interactions, solute size may also play a role in the heparin chiral recognition mechanism.

Nishi *et al.* [110] used dextran and dextrin as chiral selectors in capillary-zone electrophoresis. Polysaccharides such as dextrins, which are mixtures of linear α-(1,4)-linked D-glucose polymers, and dextrans, which are polymers of D-glucose units linked predominantly by α-(1,6) bonds, have been employed as chiral selectors in the capillary electrophoretic separation of enantiomers. Because these polymers are electrically neutral, the method is applicable to ionic compounds. The enantiomers of basic or cationic drugs such as primaquine were successfully separated under acidic conditions. The effects of molecular mass and polysaccharide concentration on enantioselectivity were investigated.

Phinney *et al.* [111] investigated the application of citrus pectins, as chiral selectors, to enantiomer separations in capillary electrophoresis. Successful enantioresolution of primaquine and other antimalarials, was achieved by utilizing potassium polypectate as the chiral selector. Changes in pH, chiral additive concentration, and capillary type were studied in relation to chiral resolution. The effect of degree of esterification of pectin materials on chiral recognition was evaluated.

Bortocan and Bonato [112] presented a capillary electrophoresis method for the enantioselective analysis of primaquine and its metabolite carboxyprimaquine. The method is used for the simultaneous determination of the drug and its metabolite, in

rat-liver mitochondrial fraction, suitable for *in vitro* metabolic studies. The drug and the metabolite were extracted by liquid–liquid extraction using ethyl ether. The enantiomers were resolved in a fused-silica capillary, 50 μm inside diameter and 24 cm of effective length, using an electrolyte solution consisting of 20 mmol/L sodium phosphate solution, pH 3, and 10% (w/v) maltodextrin. Hydrodynamic sample injection was used with a 10 s injection time at a pressure of 50 mbar. The applied voltage was 22 kV and the capillary temperature was controlled at 20°. Detection was carried out at 264 nm. Under these conditions, the enantiomeric fractions of the drug and of its metabolite were analyzed within 6 min. The extraction procedure was efficient in removing endogenous interferents and low values (<10%) for the coefficients of variation and deviation from theoretical values were demonstrated for both within-day and between-day assays. The method allows the analysis of primaquine and carboxyprimaquine enantiomers as low as 100 and 40 ng/mL, respectively. After validation, the method was used for an *in vitro* metabolic study of primaquine. The results showed the enantiomer (−)-primaquine was preferentially metabolized to (−)-carboxyprimaquine.

5. STABILITY

Kristensen *et al.* [113] studied the photochemical degradation of primaquine in an aqueous medium. The investigation was done using a medium simulating *in vivo* conditions, therefore, the effect of light of wavelength 320–600 nm on primaquine in phosphate buffer solution of pH 7.4 was studied. Primaquine formed seven major and several minor decomposition products. At concentrations above 4×10^{-5} secondary reactions seemed to occur, leading to the formation of a very high number of degradation products. The isolated degradation products emphasized that the quinoline ring remained intact after exposure of the drug to light, however, irradiation caused various cleavages of the side chain. It was concluded that degradation products of primaquine are not photochemically inert but may participate in secondary reactions and the two main photodecomposition products are not likely to be formed *in vivo* but may be formed *in vitro* after light exposure of drug formulations. The main photochemical degradation products were isolated by means of preparative thin-layer chromatography. The samples were identified by mass spectrometry (chemical ionization, electron impact and high resolution), ^{1}H NMR spectroscopy, and gas chromatography–mass spectrometry.

Chyan *et al.* [114] studied and compared the photophysical and redox properties of primaquine and dibucaine. Absorption and emission spectra and amperometric cyclic voltammograms of the two drugs were recorded at different concentrations and pH values. Primaquine at 77 K aggregates to form an excited-state double-proton-transfer dimer at concentrations higher than 5×10^{-5} M and in the pH range 2.5−5.5. The electrochemical instability of primaquine is probably associated with the observation that the 1×10^{-4} M primaquine in acetonitrile displays a highly accentuated signal of cyclic voltammogram in the reduction reaction H^{+}. The proton dissociative nature of primaquine supports the nuclear magnetic resonance measurement that the exchange of carbon-5 aromatic proton in primaquine at pH <4 is unusually fast. The acidic nature of primaquine, resulting from the

dissociation of carbon-5 aromatic proton, could assist the uptake of the antimalarial drugs. The spectroscopic investigations of native bacteriorhodopsin and delipidated deionized bacteriorhodopsin incorporated with the two drugs show that primaquine affects its lipid and denatures the purple membrane. The results are discussed in light of the pharmacological actions of the two drugs.

Gaudette and Coatney [115] reported that primaquine phosphate was unstable when subjected to dry heat of 100 °C in the presence of sodium chloride for 24 h, when boiled in water for 24 h and when heated for 24 h at 100 or 200 °C in melted hydrogenated vegetable oil. These findings exclude the use of primaquine phosphate as a salt additive in cooking. Primaquine phosphate was isolated from the test preparations at alkaline pH by extraction into ethylene chloride, after which primaquine phosphate was returned to an aqueous phase by shaking with 0.1 N sulfuric acid; the concentration of primaquine phosphate was then determined spectrophotometrically. The ultraviolet absorption curve of primaquine phosphate has maxima at 224, 266, 282, and 300 nm, and minima at 216, 250, 276, and 310 nm. A solution containing 10 γl/mL has an optical density of 0.375 at 282 nm; optical densities were proportional to concentrations.

Cheng *et al.* [116] reported that the structure of primaquine phosphate irradiated with 0.7−10 Mrad remained unchanged. The energy transfer action of the quinolyl group was considerable due to its resonance stabilization. Radiation-induced degradation of polyl(vinyl alcohol) decreased in the presence of primaquine phosphate but the degradation mechanism was unaffected. The content of primaquine phosphate showed linear relationship with degradation parameters of poly(vinyl alcohol).

Kristensen [117] studied the photochemical stability and phototoxicity of primaquine *in vitro*. The irradiation of primaquine in aqueous mediums causes cleavage of the side chain, leaving the quinoline structure unchanged. Both primaquine and the photodecomposition products absorb ultraviolet–visible light and might cause phototoxic reactions. Primaquine binds to melanin *in vitro*. Primaquine has photohemolytic properties in the ultraviolet–visible region, indicating that it is a potential photosensitizer. Hence, the *in vivo* hemolysis might be light induced.

Kristensen *et al.* [118] studied the influence of molecular oxygen and oxygen radicals on the photoreactivity of primaquine. Oxygen is directly involved in the photodecomposition of the drug. Flushing with helium gas prior to and during irradiation to suppress the oxygen level of the medium, retards the degradation rate of primaquine (followed by high performance liquid chromatography) and leads to the formation of only two degradation products (identified by mass spectrometry) compared to eight main and several minor products under normal atmospheric conditions. Flushing with oxygen gas, prior to and during irradiation to increase the oxygen content of the medium, accelerates the degradation rate of primaquine. Primaquine produces oxygen radicals (hydroxyl and superoxide) during photolysis, while the photoproducts of primaquine seem likely to induce singlet oxygen formation (detected by addition of radical scavenger). Sensitization reactions involving singlet oxygen lead to decomposition of primaquine (followed by high performance liquid chromatography). On the basis of these results, photochemical reaction mechanisms of primaquine are postulated and discussed. At physiological conditions (aqueous, neutral pH, oxygen rich), primaquine has large potential to

decompose after light absorption. The photoreaction seems to be initiated at the quinoline nitrogen atom. The ability to form intramolecular hydrogen bond seems to be essential for the luminescence properties of the drug. Phosphorescence lifetime of primaquine is about 5 μs. Fast chemical reactions may occur from the short-lived triplet state of the drug, but the excited compound can diffuse only a limited distance prior to de-excitation. This can be important concerning light-induced adverse effects that may appear after medication with primaquine.

Moore and Hemmens [119] studied the photosensitization of primaquine and other antimalarial agents. The drugs were tested for *in vitro* photosensitizing capability by irradiation with 365 nm ultraviolet light in aqueous solutions. The ability of these compounds to photosensitize the oxidation of 2,5-dimethylfuran, histidine, trypotophan, or xanthine, and to initiate the free radical polymerization of acrylamide was examined in the pH range 2–12. Primaquine does not have significant photochemical activity in aqueous solution.

Motten *et al.* [120] carried out some photophysical studies on primaquine and other antimalarial drugs. The drugs were irradiated with light ($\lambda < 300$ nm) and electron paramagnetic resonance and laser flash photolysis studies were conducted to determine the possible active intermediates produced. Each antimalarial drug produced at least one electron-paramagnetic resonance adduct with the spin-trap 5,5-dimethyl-1-pyrroline N-oxide in benzene or a nitrogen-centered radical adduct only (primaquine). In ethanol, only primaquine quenched singlet oxygen efficiently (2.6×10^8 M^{-1} s^{-1} in D_2O, pD7).

6. PHARMACOKINETICS

Greaves *et al.* [121] studied the plasma kinetics and urinary excretion of primaquine phosphate in volunteers after single and multiple dose regimes. The kinetic parameters were similar in glucose 6-phosphate dehydrogenase normal subjects from Thailand, glucose-6-phosphate dehydrogenase deficient subjects from Thailand, and in Caucasians. In the Caucasians subjects, approximately 1% of the dose was excreted in the urine. The kinetic parameters obtained from multiple dose studies were very similar to those obtained from single-dose studies.

Greaves *et al.* [122] also studied urinary primaquine excretion and red cell methemoglobin levels in man following a primaquine:chloroquine regimen. Red cell methemoglobin levels were found to be significantly raised in healthy subjects given a 14-day course of primaquine with chloroquine on the first 3 days. The methemoglobin levels were not related the quantity of primaquine excreted. No primaquine could be detected in plasma at 24 h following the last three daily doses.

Fletcher *et al.* [123] used a sensitive and specific gas chromatography–mass spectrometry method for the assay of primaquine in plasma and urine for studying the plasma kinetics. Preliminary studies on the effects of single and multiple oral doses were carried out. In both cases, the drug was completely removed from plasma in 24 h. The concentration of primaquine in plasma usually reached a peak 1–2 h after oral administration. The plasma elimination half-life was about 4 h.

Ward *et al.* [124] studied the pharmacokinetics of primaquine after acute and chronic administration of the drug to healthy Thai volunteers. After acute dosage

(15 mg, orally) mean peak plasma concentrations of 65 ng/mL were achieved within 2 h. Thereafter plasma drug concentrations declined monoexponentially with a mean elimination half-life of 4.4 h. The mean oral clearance was 37.6 L/h. These values are in broad agreement with values obtained in healthy Caucasians after administration of an equivalent dose of primaquine. The carboxylic acid metabolite of the drug accumulated in plasma after repeated dosing such that by day 14 of chronic dosing, the mean area under the concentration curve (measured from 0 to 24 h) for this metabolite was 74% greater than that obtained after acute administration of the drug.

Ward *et al.* [125] investigated the disposition of ^{14}C-radiolabeled primaquine in the isolated perfused rat liver preparation, after the administration of 0.5, 1.5, and 5 mg doses of the drug. The pharmacokinetics of primaquine in the experimental model was dependent on dose size. Increasing the dose from 0.5 to 5 mg produced a significant reduction in clearance from 11.6 to 2.9 mL/min. This decrease was accompanied by a disproportionate increase in the value of the area under the curve from 25.4 to 1128.6 μg/mL, elimination half-life from 33.2 to 413 min, and volume of distribution from 547.7 to 1489 mL. Primaquine exhibited dose dependency in its pattern of metabolism. While the carboxylic acid derivative of primaquine was not detected perfusate after the 0.5 mg dose, it was the principal perfusate metabolite after 5 mg dose. Primaquine was subject to extensive biliary excretion at all doses, the total amount of ^{14}C-radioactivity excreted in the bile decreased from 60 to 30% as the dose of primaquine was increased from 0.5 to 5 mg.

Prasad *et al.* [126] developed and used a sensitive and specific spectrophotometric method for the estimation of primaquine to study the plasma kinetics of primaquine in Rhesus monkeys. It was observed that the drug completely disappeared from the plasma in 24 h after a single oral dose. Its concentration in the plasma reached a peak at 2 h of administration. The mean absorption and elimination half-lives were 0.36 ± 0.08 and 3.44 ± 0.37 h, respectively.

Mihaly *et al.* [127] examined the pharmacokinetics of primaquine in healthy volunteers who received single oral doses of 15, 30, and 45 mg of the drug, on separate occasions. Each subject received an intravenous tracer dose of ^{14}C-primaquine (7.5 μCi), simultaneously with 45 mg oral dose. Absorption of primaquine was virtually complete with a mean absorption bioavailability of 0.96. Elimination half-life, oral clearance, and apparent volume of distribution for both primaquine and the carboxylic acid metabolite were unaffected by either dose size or route of administration.

Mihaly *et al.* [128] identified the carboxylic acid derivative of primaquine as a major plasma metabolite. After oral administration of 45 mg of primaquine to healthy volunteers, absorption of the drug was rapid, with peak primaquine levels of 153.3 ng/mL at 3 h, followed by an elimination half-life of 7.1 h, systemic clearance of 21.1 L/h, volume of distribution of 205 L and cumulative urinary excretion of 1.3% of the dose. Primaquine was converted rapidly to the carboxylic acid metabolic, which achieved peak levels of 1427 ng/mL at 7 h.

Ma *et al.* [129] prepared a new pharmaceutical form, primaquine–liposome and determined the toxicity and the therapeutic efficacy of the primaquine–liposome in mice. The LD_{50} of intravenous primaquine–liposome in mice was 2–3 times less than that of free primaquine. Primaquine–liposome concentration in blood was higher

than free primaquine in blood (at the beginning of 2 h). After intravenous administration in mice, primaquine–liposome showed a high concentration in the spleen and liver, and lesser uptake by the lung and heart.

Ward *et al.* [130] studied the pharmacokinetics of (+)- and (−)-primaquine in the isolated perfused rat liver preparation. The perfusate plasma concentrations of primaquine in the isolated, perfused rat liver, declined biexponentially following the addition of either (+)- or (−)-primaquine at doses 0.5–2.5 mg in the perfusate reservoir. There were no differences between pharmacokinetic profiles of the two isomers at the 0.5 mg dose. By contrast, the elimination of (−)-primaquine was greater than (+)-primaquine when either was added in a dose of 2.5 mg; also, the clearance of the (−)-isomer was greater, the half-life was shorter, and the area under the plasma concentration curve was shorter. The volume of distribution was similar for the two isomers. These results are relevant to both the therapeutic efficacy and toxicity of primaquine.

Singhasivanon *et al.* [131] investigated the pharmacokinetics of primaquine in eight healthy subjects (four males and four females). The volunteers received 15 mg base of primaquine daily for 14 days. The results showed that the concentration–time profiles in whole blood and in plasma were similar. The mean values (±SD) of the area under the curve of the last dose were significantly decreased when compared to the values of the first dose both in the whole blood and in plasma.

Yoshimura *et al.* [132] studied the pharmacokinetics of primaquine in calves of 180−300 kg live weight. The drug was injected at 0.29 mg/kg (0.51 mg/kg as primaquine diphosphate) intravenously or subcutaneously and the plasma concentrations of primaquine and its metabolite carboxyprimaquine were determined by high performance liquid chromatography. The extrapolated concentration of primaquine at zero time after the intravenous administration was 0.5 ± 0.48 μg/mL which decreased with an elimination half-life of 0.16 ± 0.07 h. Primaquine was rapidly converted to carboxyprimaquine after either route of administration. The peak concentration of carboxyprimaquine was 0.5 ± 0.08 μg/mL at 1.67 ± 0.15 h after intravenous administration. The corresponding value was 0.47 ± 0.07 μg/mL at 5.05 ± 1.2 h after subcutaneous administration. The elimination half-lives of carboxyprimaquine after intravenous and subcutaneous administration were 15.06 ± 0.99 h and 12.26 ± 3.6 h, respectively.

Kim *et al.* [133] studied the pharmacokinetics of primaquine and carboxyprimaquine in Korean patients with *vivax* malaria. Thirty *vivax* malaria patients were given 15 mg primaquine daily for 14 days after 3 days of chloroquine treatment. Plasma samples were taken at intervals after each daily dose of primaquine for 3 days. Plasma concentrations of primaquine and carboxyprimaquine were measured by high performance liquid chromatography. The primaquine concentrations reached a maximum of 0.28 ± 0.18 μg/mL at 1.5 h after the first dose. The maximum concentration of carboxyprimaquine was 0.32 ± 0.13 μg/mL at 4 h. Higher drug concentrations with repeated dosing were observed for carboxyprimaquine, but not for the parent drug primaquine. The elimination half-life was 3.76 ± 1.8 h and 15.7 ± 12.2 h, for the drug and the metabolite, respectively.

Arica *et al.* [134] reviewed and discussed the pharmacokinetics and bioavailability of primaquine diphosphate.

7. METABOLISM

Clark *et al.* [53] used ^{13}C NMR spectrometry to study the metabolism of primaquine by microorganism. A total of 77 microorganisms were evaluated for their ability to metabolize primaquine. Of these, 23 were fund to convert primaquine to one or more metabolites as studied by thin-layer chromatography analysis. Preparative scale fermentation of primaquine with four different microorganisms resulted in the isolation of two metabolites identified as 8-(4-acetamido-1-methylbutylamino)-6-methoxyquinoline and 8-[3-carboxy-1-methylpropyl amino]-6-methoxyquinoline. The structures of the two metabolites were proposed primarily on a comparison of the ^{13}C NMR spectra of the first metabolite and the methyl ester of the second metabolite with that of primaquine. The structures of both metabolites were confirmed by direct comparison with authentic samples.

Strother *et al.* [135] studied the metabolism of primaquine in adult dogs dosed with tritium-labeled primaquine diphosphate and were observed to excrete approximately 16% of the injected radioactivity in the urine within 8 h. Organic extracts of the urine were fractionated by thin-layer chromatography and the metabolic pattern obtained. Some of primaquine was excreted along with at least five metabolites including: 5-hydroxy-6-methoxy-8-[4-amino-1-methylbutylamino)quinoline and a small amount of 6-hydroxy-8-(4-amino-1-methylbutylamino)quinoline. The first metabolite could form a quinoneimine-type compound, which may induce methemoglobin formation. This metabolite and the other metabolites isolated from urine were active methemoglobin inducers *in vitro*. *In vitro* metabolism of primaquine by mouse liver enzymes also produced compounds capable of methemoglobin formation.

Clark *et al.* [136] studied the excretion, distribution, and metabolism of primaquine in rats. The drug was administered intravenously, intraperitoneally, and orally and blood samples were collected at various time intervals. Primaquine was metabolized by oxidative deamination to give 8-(3-carboxy-1-methylpropylamino)-6-methoxy quinoline. The plasma levels of both primaquine and its metabolites were determined by high performance liquid chromatography.

Baker *et al.* [137] reported that Rhesus monkeys were administered primaquine (6–10.5 mg as the phosphate/kg intravenously) and plasma samples were analyzed by high performance liquid chromatography for the presence of the unchanged drug and the major metabolite, 8-[3-carboxy-1-methylpropylamino)-6-methoxyquinoline. Primaquine had an unusually high affinity for tissue compartments, which produced a rapid initial drop in plasma concentration. Within 15 min, the plasma concentration of the metabolite far exceeded that of primaquine. Thirty-five to eighty-three percent of the primaquine dose was converted to the major metabolite. This metabolite possessed much lower affinity for the tissues compartments than the drug itself.

Baker *et al.* [138] studied the excretion of metabolites in bile following the administration of primaquine in rats. Six metabolites of primaquine were found in the bile of rats. Quantitative high performance liquid chromatography analysis of the metabolites revealed that the sum of the six metabolites excreted in the bile represented quantitative recovery of the dose of primaquine.

Price and Fletcher [139] studied the metabolism and toxicity of primaquine in human and monkeys. Results of a number of studies by the authors on primaquine

metabolism and pharmacokinetics are summarized. Other studies on primaquine metabolism and toxicity in man as well as *in vitro* studies are reviewed.

Baker *et al.* [140] characterized the urinary metabolites of primaquine in rats. Following the synthesis of reference standards of primaquine metabolites, a high performance liquid chromatographic analytical method for carboxyprimaquine, its glycine conjugate, and its glucuronide conjugate in urine samples were developed. After administration of the drug only trace quantities of primaquine and carboxyprimaquine (each $<1\%$ of dose) and no significant quantity of the two conjugates of carboxyprimaquine were excreted in the urine. When carboxyprimaquine was administered to rats, only 0.3% of the dose was excreted in the urine. When carboxyprimaquine glycinate was administered, the compound was found in the 700–1300 μg/mL concentration range in the urine within the first few hours. With the use of ^{14}C-labeled primaquine, six new metabolites were found in the urine.

Hufford *et al.* [57] used proton and ^{13}C NMR spectrometric data to establish the novel sulfur containing microbial metabolite of primaquine. Microbial metabolic studies of primaquine using *S. roseochromogenus* produced an *N*-acetylated metabolite and a methylene linked dimeric product, both of which have been previously reported, and a novel sulfur containing microbial metabolite.

Frischer *et al.* [141] studied the biotransformation of primaquine *in vivo* with human erythroleukemic K562 cells and bone marrow cells. The biotransformation of primaquine was investigated *in vitro* in serum-supplemented liquid cultures of partially synchronized and exponentially growing human erythroleukemic K562 cells and in bone marrow cells. Primaquine was rapidly and predominantly converted *in vitro* into carboxyprimaquine in a quantitative manner and without further modification. In addition to this metabolite, a compound Xc that was not 6-methoxy-8-aminoquinoline, and was not derived from carboxyprimaquine appeared in minor amounts in a delayed fashion. With erythroleukemic K562 cells as well as the bone marrow cells the formation of carboxyprimaquine from primaquine could be totally blocked by large concentrations of the nitrosourea, 1,3-bis(2-chloroethyl) nitrosourea. With bone marrow, increasing blockade of carboxyprimaquine formation by 1,3-bis(2-chloroethyl)nitrosourea led invariably to a progressive and striking accumulation of Xc.

Bangchang *et al.* [142] studied a number of antimalarial drugs for their effect on the metabolism of primaquine by human liver microsomes ($N = 4$) *in vitro*. The only metabolite generated was identified as carboxyprimaquine by cochromatography with the authentic standard.

Ni *et al.* [143] investigated the profile of the major metabolites of primaquine produced by *in vitro* liver microsomal metabolism, with silica gel thin-layer and high performance liquid chromatography analysis. Results indicated that the liver microsomal metabolism could simultaneously produce both 5-hydroxyprimaquine (quinoline ring oxidation product) and carboxyprimaquine (side-chain oxidative deamination product). However, the quantitative comparative study of microsomal metabolism showed that the production of 5-hydroxyprimaquine was far much higher than that of carboxyprimaquine.

Ni *et al.* [144] also investigated the profiles of major metabolites of primaquine produced from liver microsomal and mitochondrial metabolism, *in vitro* by silica gel thin-layer and reversed-phase high performance liquid chromatography. The results

indicated that 5-hydroxyprimaquine and 5-carboxyprimaquine were simultaneously produced by either microsomes or pure mitochondrial preparations. However, the quantitative study showed that microsomes produced approximately 19 times more 5-hydroxyprimaquine and only 1/34 of the carboxyprimaquine produced by mitochondria.

Constantino *et al.* [145] studied the role of monoamine oxidase and cytochrome P450 in the oxidative deamination of primaquine by rat liver fractions. Rat liver fractions including liver homogenate, mitochondria, microsomes, and 100,000 g supernatant fractions were prepared from a pool of rat livers and characterized using benzylamine as a probe for monoamine oxidase activity and *N,N*-dimethylbenzamide as a probe for cytochrome P450 *N*-dealkylation activity. Incubation of all fractions with primaquine yielded carboxyprimaquine as the only metabolite detectable by high performance liquid chromatography. The mitochondrial fraction, which contained monoamine oxidase activity but no cytochrome P450 activity, presented the highest $V_{max}/K(M)$ value for the formation of carboxyprimaquine.

Bergqvist and Churchill [146] reviewed the methods used for the detection and determination of primaquine and other antimalarial drugs and their metabolites in body fluids.

Wernsdorfer and Trigg [147] reviewed the pharmacokinetics, metabolism, toxicity, and activity of primaquine.

8. BINDING TO DNA

Whichard *et al.* [148] studied the binding of primaquine and three other aminoquinoline antimalarial agents to native and denatured calf thymus DNA, by equilibrated dialysis and direct spectrophotometry. The binding of these drugs to DNA is (1) accompanied by a decrease in absorbance of the ligand, (2) decreased by an increase in ionic strength, (3) decreased by addition of magnesium ion to a greater extent than would be expected from ionic strength effects alone, and (4) decreased under some conditions by the presence of four molar urea. In 0.01 M potassium phosphate (pH 6), the total binding of primaquine and the other drugs to various DNA preparations at DNA nucleotide-to-amioquinoline ratios $\geq$6 occurs in the following order: native DNA = denatured DNA > native DNA in 4 M urea > denatured DNA in 4 M urea.

Morris *et al.* [149] studied the binding of primaquine and pentaquine, some of their potential metabolites and chloroquine to DNA, RNA, and various polydeoxyribonucleotides and polyribonucleotides, by equilibrium dialysis and direct spectrophotometry. The extent of binding of the three drugs to native calf thymus DNA, as measured by equilibrium dialysis, does not vary in the range of pH 6–8. A major portion of each 6-hydroxy derivative of the 8-aminoquinolines binds to DNA, although, in the two examples studied, the extent of binding is somewhat less than for the corresponding 6-methoxy parent compound. Removal of the protonated terminal nitrogen of the 8-diamino side chain decreases the binding of the 8-aminoquinolines to very slow level.

9. IMMUNOASSAY

Al-Abdulla *et al.* [150] developed a magnetisable solid-phase fluorimmunoassay method for the analysis of primaquine and carboxyprimaquine. Primaquine coupled to keyhole limpet hemocyanin was used as an immunogen to produce antiprimaquine antibodies in three sheep. The antisera obtained were characterized by the increase in fluorescence polarization found upon binding to fluorescein-labeled primaquine prepared through same route. All sheep showed a good antibody response and one antiserum was coupled to megnetisable solid-phase particles to facilitate the separation of the antibody bound from free labeled antigen and the removal of interfering components which may be present in the sample. The fluoroimmunoassay requires addition of 100 μL of standard or sample (urine or serum) to 100 μL tracer (150 nmol/L) followed by 100 μL of megnetisable solid-phase particles (12.2 g/L). After 1-h incubation followed by the usual washing and eluting procedures, using a magnetic rack, the fluorescence of the supernatant was measured directly in a fluorimeter. Sodium salicylate was incorporated in the tracer solution to block the nonspecific binding of tracer to the protein in serum samples. Cross-reactivity studies showed that the antibodies have high specificity for the 8-amioquinoline nucleus but not to the 8-*N*-aminobutyl side chain. Thus, carboxyprimaquine cross-reacted equally with primaquine and the assay can be used to measure their combined level. After extraction of primaquine from a basified sample with methylene chloride, the assay may be applied for the quantitation of either primaquine (in the organic phase) or its acidic metabolites including carboxyprimaquine (in the aqueous phase) separately. This approach was applied for the determination of total primaquine (primaquine and its metabolites) and extracted primaquine in urine samples following a single oral dose of 45 mg primaquine.

Al-Abdulla *et al.* [151] presented a comparison between the three different activation methods for coupling antibodies to magnetisable cellulose particles. The periodate and 1,1′-carbonyldiimidazole activation methods were compared with the cyanogen bromide procedure for coupling antibodies to magnetisable cellulose/iron oxide solid-phase particles. Fluoroimmunoassay for primaquine and other compounds were employed to assess the binding properties of such coupled solid-phases. The cyanogen bromide and 1,1′-carbonyldiimidazole methods gave similar products in most cases, while the specific binding capacity of periodate coupled particles was between two and five times lower. Nevertheless, comparable standard curves could be obtained with solid-phase coupled by each method. The periodate and 1,1′-carbonyldiimidazole methods are acceptable alternatives, notably for laboratories lacking the facility to handle the toxic cyanogens bromide.

10. PHARMACOLOGY

Olenick [152] presented a review on primaquine. Fernex and Leimer [153] reviewed the pharmacology and clinical properties of primaquine and other antimalarial agents.

Baird *et al.* [154] reported that primaquine base (30 mg/day) had protective efficacy against *Plasmodium falciparum* and *P. vivax* of 85–93%. Among 339

children with an age of less than 8 years and adults taking this regimen for 12–52 weeks, there was no greater risk of adverse symptomatic events among primaquine users than among recipients of placebo in double-blind studies. Among 151 subjects evaluated after 20–52 weeks of daily primaquine therapy, methemoglubinemia was mild (<13%; typically <6%) and transient (duration, <2 weeks). The authors considered primaquine base (0.5 mg/kg/day consumed with food) to be safe, well-tolerated, and effective prophylaxis against malaria for nonpregnant persons and those with normal glucose-6-phosphate dehydrogenase levels. Primaquine's major advantage over most drugs for chemoprophylaxis is that it does not have to be taken before entering or beyond 3 days after leaving a malarious area.

Baird and Hoffman [155], in a review, examined the wide range of clinical applications of primaquine described in the medical literature between 1946 and 2004. The risk of relapse of *P. vivax* malaria without primaquine therapy ranged from 5% to 80% or more, depending largely upon geographical location. Supervision of therapy profoundly impacts the risk of relapse, and almost all reports of malaria resistant to primaquine are associated with lack of such supervision. The authors suspect that there is widespread resistant to the stated course of primaquine therapy, which is 15 mg primaquine base daily for 14 days. Clinical evidence confirms that the course of 15 mg daily for just 5 days, a regimen widely used in areas where malaria is endemic, has no discernible efficacy. The authors recommended a regimen of 0.5 mg/kg primaquine daily for 14 days, on the basis of superior efficacy and good tolerability and safety in nonpregnant persons without glucose-6-phosphate dehydrogenase deficiency.

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REFERENCES

[1] Budavari (ed.), *The Merck Index*, 12th edn., Merck and Co., NJ, 1996, p. 1330.
[2] A. C. Moffat (ed.), *Clarke's Isolation and Identification of Drugs*, 2nd edn., Pharmaceutical Press, London, 1986, p. 921.
[3] *British Pharmacopoeia*, 16th edn., Vol. 1, Her Majesty Stationary Office Publication Ltd., London, 2000 (CD-Rom).
[4] *United States Pharmacopoeia*, 23rd edn., United States Pharmaceutical Conversion, Inc., Rockville, MD, 2003.
[5] Sean C. Sweetman (ed.), *Martindale, The Complete Drug Reference*, 33rd edn., Pharmaceutical Press, 2002, p. 434.
[6] Swiss Pharmaceutical Society (ed.), *Index Nominum 2000, International Drug Directory*, 17th edn., Medpharm GmbH Scientific Publisher, Stuttgart, 2000, p. 875.
[7] R. C. Elderfield, H. E. Mertel, R. T. Mitch, I. M. Wempen and E. Werble, *J. Am. Chem. Soc.*, 1955, **77**, 4816.
[8] T. H. Chu, C. K. Liu, C. S. Yang, K. T. Lu and C. C. Chang, *Yaoxue Xuebao*, 1956, **4**, 197.
[9] P. Nickel, *Pharmazeutische Zeitung*, 1968, **113**, 1609.

[10] M. I. Walash, M. Rizk, A. A. Abou-Ouf and F. Belal, *Anal. Lett.*, 1983, **16**, 129.
[11] S. J. Ahmad and I. C. Shukla, *Analyst*, 1984, **109**, 1103.
[12] C. X. Yang, Y. G. Li, Z. F. Xiao, H. J. Liu and P. X. Liu, *Yaoxue Xuebao*, 1991, **26**, 531.
[13] C. D. Hufford, J. D. McChesney and J. K. Baker, *J. Heterocyclic Chem.*, 1983, **20**, 273.
[14] R. B. Papovic, K. I. Nikolic and M. D. Bodiroga, *Glasnik Hemijskog Drustva Beograd*, 1980, **45**, 373.
[15] A. S. Amin and Y. M. Issa, *J. Pharm. Biomed. Anal.*, 2003, **31**, 785.
[16] A. A. Abou-Ouf, S. M. Hassan and M. E. S. Metwally, *Analyst*, 1980, **105**, 1113.
[17] R. N. Prasad, S. K. Garg, R. C. Mahajan and N. K. Ganguly, *Indian J. Med. Res.*, 1983, **77**, 388.
[18] S. M. Hassan, M. E. S. Metwally and A. M. Abou-Ouf, *J. Assoc. Off. Anal. Chem.*, 1983, **66**, 1433.
[19] S. T. Sulaiman and D. Amin, *Int. J. Environ. Anal. Chem.*, 1985, **20**, 313.
[20] P. K. Chatterjee, C. L. Jain and P. D. Sethi, *Indian Drugs*, 1986, **23**, 563.
[21] M. Abdel-Salam, A. S. Issa and H. Lymona, *J. Pharm. Belg.*, 1986, **41**, 314.
[22] H. S. G. Vishwavidyalaya, N. Talwar, P. J. Gogoi, S. P. Vyas and N. K. Jain, *Indian Drugs*, 1990, **28**, 156.
[23] Q. Min, P. Chen, C. Li, J. Wang and G. Liu, *Yaowu Fenxi Zazhe*, 1992, **12**, 110.
[24] I. H. Refaat, M. E. El-Kommos, H. H. Farag and N. A. El-Rabat, *Bull. Pharm. Sci. Assiut Univ.*, 1987, **10**, 85.
[25] S. S. Artemchenko, V. V. Petrenko and V. A. Tsilinko, *Patent No*. SU 1113722, USSR, 1984.
[26] N. Talwar, P. J. Gogoi, S. P. Vyas and N. K. Jain, *Indian Drugs*, 1990, **28**, 156.
[27] Z. Gu, H. He, D. Rong, G. Yao, Z. Qin, D. Li, H. Xu and M. Cao, *Yao Wu Fen Hsi Tsa Chih*, 1982, **2**, 9.
[28] J. J. Aaron, S. A. Ndiaye and J. Fidanza, *Analusis*, 1982, **10**, 433.
[29] M. Tsuchiya, E. Torres, J. J. Aaron and J. D. Winefordner, *Anal. Lett.*, 1984, **17**, 1831.
[30] F. A. Ibrahim, F. Belal and A. El-Brashy, *Microchem. J.*, 1989, **39**, 65.
[31] Y. Cheng, N. N. Que, S. L. Li and L. Li, *Yaowu Fenxi Zazhi*, 1993, **13**, 167.
[32] G. Fuhrmann and K. Werrbach, *Z. Tropenmedizin Parasitologie*, 1965, **16**, 397.
[33] Y. P. Rao and G. R. Rao, *Indian Drugs*, 1981, **18**, 152.
[34] A. S. Issa, M. S. Mahrous, M. Abdel-Salam and M. Abdel-Hamid, *Journal de Pharmacie de Belgique*, 1985, **40**, 339.
[35] D. L. A. De Faria and P. S. Santos, *Mol. Biomol. Spectrosc.*, 1991, **47A**, 1653.
[36] S. M. El-Ashry, F. A. Aly and A. M. El-Brashy, *Arch. Pharmac. Res.*, 1994, **17**, 415.
[37] T. John, S. Rani and R. Hiremath, *Indian Drugs*, 1996, **33**, 293.
[38] A. S. Amin and Y. M. Issa, *Mikrochim. Acta*, 2000, **134**, 133.
[39] S. M. Hassan, M. E. S. Metwally and A. M. Abou-Ouf, *Anal. Lett.*, 1982, **15**, 213.
[40] B. S. Sastry, E. V. Rao, M. V. Suryanarayana and C. S. P. Sastry, *Pharmazie*, 1986, **41**, 739.
[41] B. S. Sastry, E. V. Rao, M. K. Tummuru and C. S. P. Sastry, *Indian J. Pharm. Sci.*, 1986, **48**, 190.
[42] B. S. Sastry, E. V. Rao, M. K. Tummuru and C. S. P. Sastry, *Indian Drug*, 1986, **24**, 105.
[43] M. S. Mahrous, M. Abdel-Salam, A. S. Issa and M. Abdel-Hamid, *Talanta*, 1986, **33**, 185.
[44] M. E. El-Kommos and K. M. Emara, *Bull. Pharm. Sci. Assiut Univ.*, 1988, **11**, 18.
[45] G. R. Rao, S. S. N. Murthy and I. R. K. Raju, *Indian Drugs*, 1988, **25**, 475.
[46] G. R. Rao, S. S. N. Murthy and I. R. K. Raju, *Indian Drugs*, 1988, **26**, 86.
[47] M. E. El-Kommos and K. M. Emara, *Alexandria J. Pharm. Sci.*, 1988, **2**, 171.
[48] B. S. Sastry, J. V. Rao, T. N. V. Prasad and C. S. P. Sastry, *Indian Drugs*, 1989, **26**, 194.
[49] F. A. Ibrahim, A. El-Brashy and F. Belal, *Mikrochem. Acta*, 1989, **1**, 321.
[50] B. S. Sastry, E. V. Rao, M. V. Suryanarayana and C. S. P. Sastry, *Indian Drugs*, 1990, **27**, 260.
[51] A. M. El-Brashy, *Anal. Lett.*, 1993, **26**, 2595.
[52] J. K. Baker, J. D. McChesney and L. Jorge, *Bull. World Health Organ.*, 1985, **63**, 887.
[53] A. M. Clark, C. D. Hufford and J. D. McChesney, *Antimicrob. Agents Chemother.*, 1981, **19**, 337.
[54] S. P. Singh, S. S. Parmar and V. I. Stenberg, *J. Heterocyclic Chem.*, 1978, **15**, 9.
[55] J. D. McChesney and S. Sarangan, *Pharm. Res.*, 1984, **4**, 184.
[56] C. D. Hufford and J. K. Baker, *Spectrosc. Lett.*, 1989, **19**, 595.
[57] C. D. Hufford, J. K. Baker, J. D. McChesney and A. M. Clark, *Antimicrob. Agent Chemother.*, 1986, **30**, 234.
[58] J. R. Perussi, V. E. Yushmanov, S. C. Monté, H. Imasato and M. Tabak, *Physiol. Chem. Phys. Med. NMR*, 1995, **27**, 1.

[59] A. Berka, K. Nicolic and K. Velasevic, *Acta Pol. Pharm.*, 1990, **47**, 7.
[60] Y. F. Zhan and J. Z. Mao, *Huaxue Shijie*, 1992, **33**, 165.
[61] Y. Zhan, *Fenxi Huaxue*, 1992, **20**, 493.
[62] B. B. Saad, Z. A. Zahid, S. A. Rahman, M. N. Ahmad and A. H. Husin, *Analyst*, 1992, **117**, 1319.
[63] G. B. Mohamed, *Alexandria J. Pharm. Sci.*, 1989, **3**, 180.
[64] Q. S. Yu, A. Brossi and J. L. Flippen-Anderson, *FEBS Lett.*, 1987, **221**, 325.
[65] S. M. Hassan, M. E. S. Metwally and A. M. Abou-Ouf, *Analyst*, 1982, **107**, 1235.
[66] S. A. Fusari, I. J. Holcomb and R. B. Luers, *J. Chromatogr. Sci.*, 1975, **13**, 589.
[67] H. Zheng and H. Sun, *Yaowu Fenxi Zazhi*, 1983, **3**, 113.
[68] A. K. Dwivedi, M. Khanna, R. Pal and S. Singh, *Indian J. Pharm. Sci.*, 1997, **59**, 321.
[69] A. K. Dwivedi, D. Saxena and S. Singh, *J. Pharm. Biomed. Anal.*, 2003, **33**, 851.
[70] A. H. Stead, R. Gill, T. Wright, J. P. Gibbs and A. C. Moffat, *Analyst*, 1982, **107**, 1106.
[71] R. E. Andrey and A. C. Moffat, *J. Chromatogr.*, 1981, **220**, 195.
[72] T. G. Rajagopalan, B. Anjaneyulu, V. D. Shanbag and R. S. Grewal, *J. Chromatogr.*, 1981, **224**, 265.
[73] J. D. Baty, D. A. Price-Evan, H. M. Gilles and J. Greaves, *Biomed. Mass Spectrom.*, 1978, **5**, 76.
[74] J. Greaves, D. A. Price-Evans, H. M. Gilles and J. D. Baty, *Biomed. Mass Spectrom.*, 1979, **6**, 109.
[75] M. Nitin, M. Rajanikanth, J. Lal, K. P. Madhusudanan and R. C. Gupta, *J. Chromatogr.*, 2003, **793**, 253.
[76] V. K. Dua, S. N. Sinha and V. P. Sharma, *J. Chromatogr. Biomed. Appl.*, 1998, **708**, 316.
[77] V. K. Dua, P. K. Kar, R. Sarin and V. P. Sharma, *J. Chromatogr. Biomed. Appl.*, 1996, **675**, 93.
[78] I. Jane, A. McKinnon and R. J. Flanagan, *J. Chromatogr.*, 1985, **323**, 191.
[79] G. W. Parkhurst, M. V. Nora, R. W. Thomas and P. E. Carson, *J. Pharm. Sci.*, 1984, **73**, 1329.
[80] B. B. Wheals, *J. Chromatogr.*, 1980, **187**, 65.
[81] A. M. Clark, S. L. Evans, C. D. Hufford and J. D. McChesney, *J. Natural Products*, 1982, **45**, 574.
[82] J. K. Baker, J. D. McChesney, C. D. Hufford and A. M. Clark, *J. Chromatogr.*, 1982, **230**, 69.
[83] C. D. Hufford, A. M. Clark, J. D. McChesney and J. K. Baker, *J. Org. Chem.*, 1984, **49**, 2822.
[84] N. Katori, T. Shibazaki and M. Uchiyama, *Iyakuhin Kenkyu*, 1985, **16**, 1407.
[85] Y. Okamoto, R. Aburatani, K. Hanato and K. Hatada, *J. Liq. Chromatogr.*, 1988, **11**, 2147.
[86] A. S. Sidhu, J. M. Kennedy and S. Deeble, *J. Chromatogr.*, 1987, **391**, 233.
[87] G. R. Rao, S. S. N. Murty, I. R. K. Raju and C. M. R. Srivastava, *Indian Drugs*, 1989, **26**, 430.
[88] R. B. Taylor, R. G. Reid, R. H. Behrens and I. Kanfer, *J. Pharm. Biomed. Anal.*, 1992, **10**, 867.
[89] X. Liu and R. Wu, *Zhongguo Yaoke Daxue Xuebao*, 1989, **20**, 240.
[90] T. L. Li, Q. J. Pang, Y. L. He and P. Wang, *Yaoxue Xuebao*, 1995, **30**, 721.
[91] J. Fang, X. Xia and Y. Wu, *Zhongguo Yaowu Huaxue Zazhi*, 2003, **13**, 48.
[92] L. Jawahar, M. Nitin and G. R. Chandra, *J. Pharm. Biomed. Anal.*, 2003, **32**, 141.
[93] R. A. Dean, W. Ochieng, J. Black, S. F. Queener, M. S. Bartlett and N. G. Dumaual, *J. Chromatogr. Biomed Appl.*, 1994, **655**, 89.
[94] S. A. Ward, G. Edwards, M. L. E. Orme and A. M. Breckenridge, *J. Chromatogr.*, 1984, **305**, 239.
[95] M. V. Nora, G. W. Parkhurst, R. W. Thomas and P. E. Carson, *J. Chromatogr.*, 1984, **307**, 451.
[96] S. C. Bhatia, S. N. Revankar, E. D. Bharucha and K. J. Doshi, *Anal. Lett.*, 1985, **18**, 1671.
[97] X. Fang, S. Dong, P. Zhou, R. Wu and D. An, *Nanjing-Yaoxueyuan-Xuebao*, 1985, **16**, 43.
[98] J. K. Baker, A. M. Clark and C. D. Hufford, *J. Liq. Chromatogr.*, 1986, **9**, 493.
[99] V. Das-Gupta, *Anal. Lett.*, 1986, **19**, 1523.
[100] G. R. Rao, S. S. N. Murty, I. R. K. Raju and C. M. R. Srivastava, *Indian Drugs*, 1989, **26**, 430.
[101] S. K. Sanghi, A. Verma and K. K. Verma, *Analyst*, 1990, **115**, 333.
[102] N. Talwar, A. K. Shakya, P. J. Gogoi, S. P. Vyas and N. K. Jain, *Indian Drugs*, 1991, **28**, 437.
[103] Y. S. Endoh, H. Yoshimura, N. Sasaki, Y. Ishihara, H. Sasaki, S. Nakamura, Y. Inoue and M. Nishikawa, *J. Chromatogr. Biomed. Appl.*, 1992, **117**, 123.
[104] J. K. Paliwal, R. C. Gupta and P. K. Grover, *J. Chromatogr. Biomed. Appl.*, 1993, **127**, 155.
[105] T. Li, G. Pang, Y. Jia, P. Wang, T. Ma, J. Meng and S. Zhao, *Hua Hsi I Ko Ta Hsueh Hsueh Pao.*, 1995, **26**, 109.
[106] G. Fan, J. Hu, M. Lin, Z. Zhang and D. An, *Yaowu Fenxi Zazhi*, 1999, **19**, 84.
[107] C. L. Ng, Y. L. Toh, S. F. Y. Li and H. K. Lee, *J. Liq. Chromatogr.*, 1993, **16**, 3653.
[108] R. B. Taylor and R. G. Reid, *J. Pharm. Biomed. Anal.*, 1993, **11**, 1289.
[109] A. M. Stalcup and N. M. Agyei, *Anal. Chem.*, 1994, **66**, 3054.

[110] H. Nishi, S. Izumoto, K. Nakamura, H. Nakai and T. Sato, *Chromatographia*, 1996, **42**, 617.
[111] W. K. Phinney, L. A. Jinadu and L. C. Sander, *J. Chromatogr.*, 1999, **857**, 285.
[112] R. Bortocan and P. S. Bonato, *Electrophoresis*, 2004, **25**, 2848.
[113] S. Kristensen, A. L. Grislingaas, J. V. Greenhill, T. Skjetne, J. Karlsen and H. H. Toennesen, *Int. J. Pharm.*, 1993, **100**, 15.
[114] Y. G. Chyan, M. W. Hsiao, C. M. Catuara and C. T. Lin, *J. Phys. Chem.*, 1994, **98**, 10352.
[115] L. E. Gaudette and G. R. Coatney, *Am. J. Trop. Med. Hygiene*, 1960, **9**, 532.
[116] K. Cheng, C. Yang and M. Wang, *Fushe Yanjiu Yu Fushe Gongyi Xeubao*, 1986, **4**, 34.
[117] S. Kristensen, *Biol. Eff. Light 1993*, *Proceedings of the 3rd Symposium, 1994*, p. 144.
[118] S. Kristensen, K. Nord, A. L. Orsteen and H. H. Tonnesen, *Pharmazie*, 1998, **53**, 98.
[119] D. E. Moore and V. J. Hemmens, *Photochem. Photobiol.*, 1982, **36**, 71.
[120] A. G. Motten, L. J. Martinez, N. Holt, R. H. Sik, K. Reszka, C. F. Chignell, H. H. Tonnesen and J. E. Roberts, *Photochem. Photobiol.*, 1999, **69**, 282.
[121] J. Greaves, D. A. P. Evans, H. M. Gilles, K. A. Fletcher, D. Bunnag and T. Harinasuta, *Br. J. Clin. Pharmacol.*, 1980, **10**, 399.
[122] J. Greaves, D. A. Evans and K. A. Fletcher, *Br. J. Clin. Pharmacol.*, 1980, **10**, 293.
[123] K. A. Fletcher, D. A. P. Evans, H. M. Gilles, J. Greaves, D. Bunnag and T. Harinasuta, *Bull. World Health Org.*, 1981, **59**, 407.
[124] S. A. Ward, G. W. Mihaly, G. Edwards, S. Looareesuwan, R. E. Phillips, P. Chanthavanich, D. A. Warrell, M. L. Orme and A. M. Breckenridge, *Br. J. Clin. Pharmacol.*, 1985, **19**, 751.
[125] S. A. Ward, G. W. Mihaly, D. D. Nicholl, G. Edwards and A. M. Breckenridge, *Drug Metabolism Disposition*, 1985, **13**, 425.
[126] R. N. Prasad, S. K. Garg, R. C. Mahajan and N. K. Ganguly, *Int. J. Clin. Pharmacol., Therapy Toxicol.*, 1985, **23**, 45.
[127] G. W. Mihaly, S. A. Ward, G. Edwards, D. D. Nicholl, M. L. Orme and A. M. Breckenridge, *Br. J. Clin. Pharmacol.*, 1985, **19**, 745.
[128] G. W. Mihaly, S. A. Ward, G. Edwards, M. L. Orme and A. M. Breckenridge, *Br. J. Clin. Pharmacol.*, 1984, **17**, 441.
[129] Y. Ma, Y. Jiang, H. Xu, B. Shao, X. Ye, D. Shen and X. Zhao, *Shanghai Yike Daxue Xuebao*, 1987, **14**, 21.
[130] S. A. Ward, G. W. Mihaly, D. D. Nicholl, A. M. Breckenridge and G. Edwards, *Biochem. Pharmacol.*, 1987, **36**, 2238.
[131] V. Singhasivanon, A. Sabcharoen, P. Attanath, T. Chongsuphajaisiddhi, B. Diquet and P. Turk, *Southeast Asian J. Tropical Med. Public Health*, 1991, **22**, 527.
[132] H. Yoshimura, Y. S. Endoh, Y. Ishihara, S. Nakamura and Y. Inoue, *Vet. Res. Commun.*, 1993, **17**, 129.
[133] Y. R. Kim, H. J. Kuh, M. Y. Kim, Y. S. Kim, W. C. Chung, S. Kim II and M. W. Kang, *Arch. Pharm. Res.*, 2004, **27**, 576.
[134] B. Arica, A. Ozer, H. Yekta and A. Atilla, *FABAD Farmasotik Bilimler Dergisi*, 1992, **17**, 149.
[135] A. Strother, I. M. Fraser, R. Allahyari and B. E. Tilton, *Bull. World Health Org.*, 1981, **59**, 413.
[136] A. M. Clark, J. K. Baker and J. D. McChesney, *J. Pharm. Sci.*, 1984, **73**, 502.
[137] J. K. Baker, J. A. Bedford, A. M. Clark and J. D. McChesney, *Pharm. Res.*, 1984, **2**, 98.
[138] J. K. Baker, J. D. McChesney and L. A. Walker, *Pharm. Res.*, 1985, **5**, 231.
[139] A. H. Price and K. A. Fletcher, *Prog. Clin. Biol. Res.*, 1986, **214**, 261.
[140] J. K. Baker, J. D. McChesney and L. F. Jorge, *Pharm. Res.*, 1986, **3**, 132.
[141] H. Frischer, T. Ahmad, M. V. Nora, P. E. Carson, M. Sivarajan, R. Mellovitz, L. Ptak, G. W. Parkhurst, H. S. Chow and H. Kaiser, *J. Lab. Clin. Med.*, 1987, **109**, 414.
[142] K. N. Bangchang, J. Karbwang and D. J. Back, *Biochem. Pharmacol.*, 1992, **44**, 587.
[143] Y. C. Ni, M. J. Wang, Y. Q. Xu and Hu-Ling, *Chinese J. Parasitol. Parasitic Dis.*, 1992, **10**, 275.
[144] Y. Ni, Y. Xu and M. Wang, *Zhongguo Yaoli Xueboa*, 1992, **13**, 431.
[145] L. Constantino, P. Paixoa, R. Moreira, M. J. Portela, V. E. Do Rosario and J. Iley, *Exp. Toxicol. Pathol.*, 1999, **51**, 299.
[146] Y. Bergqvist and F. C. Churchill, *J. Chromatogr.*, 1988, **434**, 1.
[147] W. H. Wernsdorfer and P. I. Trigg (eds), *Proceedings of a Meeting of the Scientific Working Group on the Chemotherapy of Malaria*, Geneva, Suitz 27–28 February 1984, John Wiley & Sons, Chichester, UK, 1987, p. 164.

[148] L. P. Whichard, C. R. Morris, J. M. Smith and J. D. Holbrook Jr., *Mol. Pharmacol.*, 1968, **4**, 630.
[149] C. R. Morris, L. V. Andrew, L. P. Whichard and D. J. Holbrook Jr., *Mol. Pharmacol.*, 1970, **6**, 240.
[150] I. H. Al-Abdulla, A. M. Sidki, J. Landon and F. J. Rowell, *Southeast Asian J. Trop. Med. Public Health*, 1989, **20**, 361.
[151] I. H. Al-Abdulla, G. W. Mellor, M. S. Childerstone, A. M. Sidki and D. S. Smith, *J. Immunol. Methods*, 1989, **122**, 253.
[152] J. G. Olenick, *Antibiotics*, 1975, **5**, 516.
[153] M. Fernex and R. Leimer, *Medecine et Hygiene*, 1986, **44**, 2113, 2117, 2120.
[154] J. K. Baird, D. J. Fryauff and S. L. Hoffman, *Clin. Infect. Dis.*, 2003, **37**, 1659.
[155] J. K. Baird and S. L. Hoffman, *Clin. Infect. Dis.*, 2004, **39**, 1336.