

## SHORT COMMUNICATION

### **Simultaneous Determination of a New Antimalarial Agent, CDRI Compound No 80/53 and Primaquine by TLC Densitometry and UV Spectrophotometry\***

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Compound 80/53 is a new antimalarial agent developed at C.D.R.I. It is unstable in acidic conditions where it is converted into primaquine. To conduct stability studies of this compound, TLC densitometric and U.V. spectrophotometric estimation methods have been developed. These methods are also suitable for the estimation of 80/53 or primaquine in their dosage forms and bulk drug samples.

**P**RIMAQUINE (I) is a potent antimalarial agent<sup>1</sup>. Its derivative, N-3-acetyl+ dihydro-2-furanyl), N-(6-methoxy-8-quinolinyl)-4-pentanediamine [80/53] (II)<sup>2</sup> shows radical curative and causal prophylactic activities against sporozite- induced *Plasmodium cyanomolgi* infection in rhesus monkeys<sup>3,4</sup>. It is safer than primaquine<sup>5</sup>. It is also found safe in sub-acute toxicity studies in rats and rhesus monkeys and has no teratogenic action<sup>6</sup>. Currently it is under phase II clinical trials. A number of estimation methods for I<sup>7-11</sup> and for II<sup>12,13</sup> have been reported. Owing to the unstable nature of furanyl derivation of Primaquine (80/53) in an aqueous systems below pH7, leading to its conversion into I<sup>14,15</sup>, it is of interest to develop a simple assay method for the simultaneous determination of I and II to conduct the stability studies and for quantitation of I and/or II in their dosage forms and bulk drug samples.

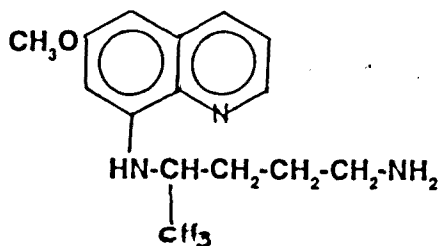
Primaquine diphosphate was obtained from M/s Sigma Chemical Co., U.S.A. and 80/53<sup>1</sup> free base was obtained from Chemical Technology Division of this Institute. Methanol and chloroform used were of A.R. grade. Dual wavelength TLC scanner (Shimadzu model CS-910) fitted with a Shimadzu U-235 data recorder, precoated silicagel plates

60F254 with a layer thickness 0.25 mm [E. Merck] and micro syringe (50  $\mu$ L, Top) were used. The U.V. absorbances were recorded on a Perkin Elmer Lambda-15 UV/Vis spectrophotometer.

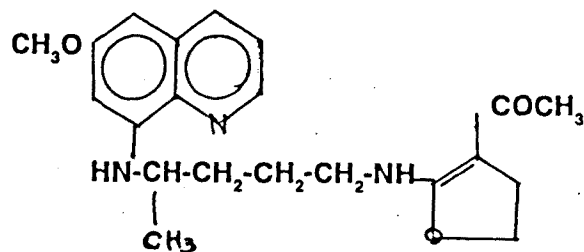
Standard compound 80/53 [m.p. 105<sup>o</sup>, soluble in methanol, chloroform and benzene] and primaquine diphosphate 2.5 mg each were dissolved separately in 25 ml methanol containing 50  $\mu$ l of dimethyloctyl amine (DMOA) to get a solution with concentration of 0.1  $\mu$ g/ $\mu$ l. DMOA was used to prevent degradation of 80/53 in methanolic solution. Stability of 80/53 in the given conditions was also checked. It was observed that not more than 5% of 80/53 was decomposed when kept in its solution form for 24 h at room temperature.

About 2.5 mg of II or mixed contents of 20 capsules of 80/53 equivalent to 2.5 mg of II was dissolved in 25 ml of methanol to get the test solution. Twenty  $\mu$ l of the above sample solution, 20  $\mu$ l of blank methanol and four spots each (5  $\mu$ L, 10  $\mu$ L and 30  $\mu$ L of II and I standard solutions were loaded on the TLC plate, chromatography was carried out in glass TLC tank saturated with chloroform:methanol:ammonia (92:8:0.5) as mobile phase and run to a height of at least 15 cm. Plates were removed, dried, used

\*For correspondence



I - Primaquine

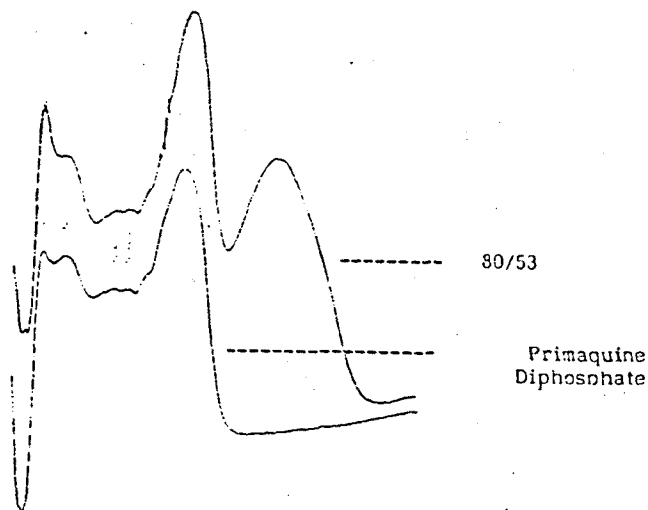


II - 80/53

for the quantitation of 80/53 free base [Rf-0.77] and primaquine diphosphate [Rf-0.22]

The TLC plate was placed on the stage of dual wavelength, TLC scanner, chromatographic zones on the TLC plate were scanned at 265 nm using dual wavelength transmission mode with back ground substration and using a light beam of 1 x 10 mm. Chromatograms were plotted with the chart speed of 40 mm/min. and peak areas were calculated. The method was calibrated using methanol containing known amount of authentic 80/53 free base and primaquine diphosphate. The concentrations of 80/53 and primaquine were determined by using standard curves. Recoveries were calculated by adding known concentration of 80/53 free base or primaquine diphosphate to preanalysed samples.

The standard solutions and the sample solutions of I or II equivalent to about 4 ug/ml were scanned at 305 nm [specific peak of 80/53] and 265 [common peak present in 80/53 and primaquine] under U.V. light and absorbances of the solutions were recorded. In the TLC densitometric method, the calibration curves for 80/53 and primaquine were linear in the range from 0.5 ug to 10 ug with correlation coefficient of 0.9955 and 0.9976 respectively. Reproducibility of the method was determined by inter and intra assay variations in replicate (n=4) analysis of standard fortified samples (% C.V < 6 %) and the reproducibility was determined by calculating % deviation from actual concentration (% D.F.A. < 6 %). Results indicate high accuracy and precision of the method.



UV Spectra of 80/53 and primaquine

With the U. V. method the amount of 80/53 and primaquine in the mixture was calculated by the following equations:

$$\text{Amount of 80/53} = \frac{x - 0.0163}{0.0506}$$

$$\text{Amount of primaquin} = \frac{y - 1.45x - 0.00729}{0.0588}$$

Where x is the absorbance of the mixture at 305 nm and y is the absorbance of the mixture at 265 nm. The equation (1) and (2) gave very good results

**Table - 1**  
**Estimation of primaquine diphosphate (I) and 80/53 (II) in a mixture by the UV method**

Added ( $\mu\text{g}$ )	Amount of 80/53		Added ( $\mu\text{g}$ )	Amount of primaquine	
	Found ( $\mu\text{g}$ )	% C.V.		Found ( $\mu\text{g}$ )	% C.V.
	Mean $\pm$ SD (n=4)			Mean $\pm$ SD (n=4)	
1	0.96 $\pm$ 0.04	4.25	1	1.023 $\pm$ 0.063	6.29
2	2.09 $\pm$ 0.075	3.61	2	2.122 $\pm$ 0.097	4.59
3	3.13 $\pm$ 0.038	1.21	3	2.993 $\pm$ 0.109	3.64
4	4.13 $\pm$ 0.035	0.84	4	4.006 $\pm$ 0.187	4.66
5	5.16 $\pm$ 0.01	0.22	5	4.981 $\pm$ 0.0	0.00

in the mixture containing 1 to 5  $\mu\text{g}/\text{ml}$  solution of both the drugs with recoveries of 96-104%. Table 1 shows the analysis results of 80/53 free base and primaquine diphosphate in their mixture by the U. V. method.

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