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In vitro Absorption Studies of A New Antimalarial in the Gastro-Intestinal Tract*

G. K. JAIN & S. Singh

Divn. of Pharmaceutics, Central Drug Research Institute, Lucknow.

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N'-(3'-Acetyl-4',5'-dihydro-2' - furanyl)-N⁴-(6-methoxy-8 - quinolinyl) - 1,4 - pentane diamine 1, a new anti-relapse antimalarial primaquine derivative developed at C.D.R.I. (compound No. 80/53) is less toxic than the parent compound. Its in vitro diffusion and absorptiin rate constants in simulated body fluids have been determined using Sartorius Absorptiion Simulator.

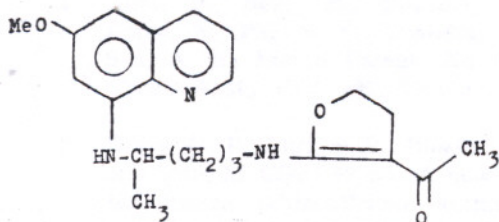
A Primaquine derivative N'-(3-Acetyl-4', 5'-dihydro-2'-furanyl)-N⁴ - (6 - methoxy-8-quinolinyl)-1,4-pentane diamine **1**, is a new antimalarial developed at CDRI.¹ It has equivalent tissue schizontocidal activity but is far less toxic than primaquine.²

With a view to develop a suitable pharmaceutical dosage form of **1**, it was considered of interest to determine its *in vitro* diffusion and absorption rate constant in GI tract using simulated body fluids and to compare them with that of Primaquine diphosphate. This communication describes the related findings.

Sartorius absorption simulator model SM 16750 was used to determine the diffusion and absorption rate constants in GI-tract. This model consists of (a) two thermostatically controlled vessels phase I and phase II, (b) the diffusion cell with synthetic lipoidal barrier and (c) a hose pump which circulates the simulated biological fluids through the vessels and the corresponding compartments of the diffusion cell.

Phase I was either filled with 100 ml of simulated gastric or duodenal fluid with 0.05 mg/ml of drug 80/53 (**1**) or 0.05 mg/ml of primaquine diphosphate dissolved into it and the phase II with 100 ml with 100 ml of simulated blood plasma which

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are then circulated through the lipoidal barrier (Area 40 cm²) and the samples were collected hourly.

In case of samples from simulated duodenal fluid and simulated plasma the concentration of the compound was determined spectrophotometrically at 302 nm against the corresponding blank. In case of simulated gastric fluid i.e. acidic buffer of pH 1.1 to 2.2, the rate constants could not be determined because the compound converts almost immediately into primaquine and α -acetyl- γ -butyrolactone. The presence of both the primaquine and α -acetyl- γ -butyrolactone was confirmed by TLC and Co-TLC with an authentic sample where as the formation of α -acetyl- γ -butyrolactone was also confirmed by its IR, NMR and Mass spectral data. The concentration of primaquine was determined at 265 nm in case of samples from simulated gastric juice and at 259 nm in case of samples from simulated duodenal fluid.

Diffusion rate constant (Kd):^{3,4} This constant was calculated from the initial diffusion rate according to following equation :

$$Kd = \frac{CII_2 - CII_1}{T_2 - T_1} \cdot \frac{VII_0}{CI_0} \cdot F \quad [Cm. \text{min}^{-1}]$$

CII_x : Substance concentration in Phase II at instant T_x (mg/ml)

VII₀ : Volume of Phase II at instant T₀ (ml)

F : Barrier area (cm²)

T_x : Time (min)

CI₀ : Extrapolated initial concentration in Phase I (mg/ml)

Absorption rate constant K_i : This was calculated by using the following equation :

$$Ki = G (Kd - Kdo) \quad (\text{min}^{-1})$$

G = a constant (For Man duodenum it is 10.0)

The results obtained are as follows :

Table 1 — Rate constants in duodenum

Compound	Diffusion rate constant (Kd) cm min ⁻¹	Absorption rate constant (Ki) min ⁻¹
80/53	2.9976 x 10 ⁻⁴	11.70 x 10 ⁻⁴
Primaquine diphosphate	1.593 x 10 ⁻³	14.13 x 10 ⁻³

The diffusion and absorption rate constant of primaquine at pH 1.1 was found to be 0.83 x 10⁻³ cm min⁻¹ and 3.3 x 10⁻³ min⁻¹ respectively.

The observation that 1 is liberating primaquine under acid pH reveals that the antimalarial activity of this compound is due to primaquine. However, minimized side effect like less met-haemoglobin accumulation was possibly due to α -acetyl- γ -butyrolactone liberated during acid treatment. It can also be correlated with the finding that *in vitro* sodium formate inhibits the accumulation of met-haemoglobin formed due to primaquin.⁵

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Simultaneous High Performance Liquid Chromatographic Determination of Bromhexine Hydrochloride and Salbutamol Sulphate from Pharmaceutical Preparations

R. T. SANE*, D. P. GANGAL, R. V. TENDOLKAR, R. M. KOTHURKAR,
K. D. LADAGE AND LEELA JOSHI

S.P. Mandali's TDM Laboratory, Scheme 6, Road No. 15, Sion-Koliwada, Sion (East), Bombay-400 022.

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A reversed-phase HPLC method for the simultaneous determination of bromhexine hydrochloride and salbutamol sulphate from their combined formulations on a C₁₈ column using a mobile phase consisting of acetonitrile : water : glacial acetic acid : triethylamine in the volume ratio of 55 : 45 : 1 : 0.1 is described. Phenylbutazone was used as an internal standard. The method was applied to market formulations and statistically validated.

SALBUTAMOL is official in the IP,¹ the BP² and the USP³ (albuterol), whereas bromhexine hydrochloride is official only in the BP.² The pharmacopoeias describe non-aqueous potentiometric titration methods for the determination of the drugs from bulk drug and uv spectrophotometric methods for the determination from formulations.

Only one HPLC method⁴ is reported for the simultaneous determination of bromhexine hydrochloride and salbutamol from their combined formulation; though

there are various methods for the determination of bromhexine hydrochloride^{5,6} and salbutamol⁷⁻¹² from body fluids and formulations either individually or in combination with other drugs.

Perkin Elmer HPLC equipped with Series 410 LC pump, LC-235 diode array detector and Rheodyne injector. The instrument was coupled to a LCI-100 laboratory computing integrator, was used.

Chromatographic conditions : Column — Econosphere C₁₈ (250 mm x 4.6