DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 14(15-17), 2579-2603 (1988)

SALIVARY LEVELS OF QUININE IN PRESENCE OF SOME ANALGESICS, ANTIPYRETICS AND ANTIINFLAMMATORY AGENTS

B. Ramesh Rao and D. Rambhau

University College of Pharmaceutical Sciences
Kakatiya University, Warangal-India. 506 009.

ABSTRACT

A significant correlation ($r=0.6449$, $n=72$, $p < 0.01$) between saliva and serum levels of intact quinine was observed after a single oral administration of 500mg of quinine sulphate to six healthy male volunteers. The mean saliva/serum ratio obtained from the individual data of volunteers was 0.2497 (±0.0145 SEM). Salivary compartment was approached to study the interactions of quinine with aspirin or analgin or paracetamol or oxyphenbutazone in healthy human subjects. Salivary levels of quinine did not differ significantly in presence of the drugs co-administered, indicating absence of interactions. Comparison of various pharmacokinetic parameters also substantiated this.

INTRODUCTION

Quinine is a drug of choice in the treatment of chloroquin resistant falciparum malaria and in nocturnal leg cramps. In both these disease states analgesics, antipyretics and antiinflammatory

agents are commonly prescribed along with quinine. The inicidence of night cramps in arthritic patients was studied by pemberton[1], who found that among the patients with arthritis 62% of men and 43% of women had night cramps as a major complaint. In such cases an antiinflammatory drug shall have to be prescribed along with quinine.

Owing to certain distinct merits over blood or urine and significant correlations between saliva and plasma concentrations for intact drugs [5-9] and metabolites [4,6], salivary compartment could probably be exploited to study the pharmacokinetic interactions of drugs. We examine this issue for quinine for which interactions with some other drugs based on blood levels have been reported[2,3,28]. Purely from therapy monitoring stand point and from the significance of usage of quinine in the treatment of diseases stated above, We explore a correlation between saliva and serum concentrations for quinine, as a basis for studying its pharmacokinetic interactions with some anal gesics, antipyretics and antiinflammatory agents via salivary compartment.

MATERIALS AND METHODS

Subjects:-

The study was carried out in six healthy male volunteers, with their weight ranging between 46 - 70 kgs, height between 160 - 182.5 cms and age ranging between 23 - 26 years. They were non smokers, as tobacco smoking may affect the pharmacokinetics of some drugs[10-12]. They were in good health documented

by a complete medical examination by a physician, medical history and standard laboratory tests. Neither alcoholic beverages nor any medication was allowed for 2 weeks before the study and through out the duration of the study.

Protocol:

Six subjects were administered 500mg of quinine sulphate filled in a hard gelatin capsule along with 250 ml of water after an overnight fasting (10-12 hrs). No food and drinks were allowed to be taken by the subjects for 4 hours after drug administration. Non restricted regular meals taken before and after the selected times were balanced in terms of proteins, fats, carbohydrates and salts, and standardised for all subjects. All the subjects gave informed consent.

Blood samples (2ml) were withdrawn at intervals of 0.00, 0.5, 1, 1.5, 2, 3, 5, 7, 10, 12, 15, 24 and 36 hours from median cubital vien. Saliva (3ml) samples were also collected in vials at the same time intervals. All the samples were stored in vials in a refrigerator until assayed.

The pharmacokinetic interactions were undertaken in 5 of the six volunteers chosen for the saliva/serum correlation study. The sixth volunteer was deleted at random as the study involves only 5 treatments. Thus the study was performed according to a 5x5 balanced latin squared design, allowing a washout

period of one week between each treatment. The treatments given were:

1. Quinine sulphate 500mg capsule
2. Quinine sulphate 500mg capsule + 500mg Analgin tablet(a)
3. Quinine sulphate 500mg capsule + 500mg Paracetamol tablet(b)
4. Quinine sulphate 500mg capsule + 100mg Oxyphenbutazone tablet (c)
5. Quinine sulphate 500mg capsule + 600mg Aspirin (d) (as 2 tabs of 300mg each)

Food restrictions and the fasting period were similar to that followed in the saliva/serum correlation study. Saliva samples (3ml) were collected at intervals of 0.00, 0.5, 1, 1.5, 2, 3, 5, 7, 10, 12, 15, 24 and 36 hours following drug(s) administration in the morning. The samples thus collected were stored in a refrigerator until assayed.

Saliva Collection:

Some problems are associated with the collection of saliva samples. Several advantages and disadvantages have been reported by earlier workers[13-16] regarding the stimulation of salivary secretion. Taylor et al [17] observed adsorption of Propranolol and Indomethacin by parafilm and discouraged the use of such

---

a) Alkem Laboratories Pvt.Ltd., Bombay India(7.5±1 min)
b) Duphar-Interferan Ltd., Bombay (90±5 sec)
c) Pharmed Pvt.Ltd., Bombay, India (5±0.8 min)
d) Invinex pharma, Hyderabad, India (20±3 sec)

The values in the parenthesis indicate the mean disintegration time and standard deviation.

stimulants. Such losses were to the tune of 15-34% and 8-42% in case of 2 lipophilic drugs chlopromazine and butaperazine respectively [16]. In the present study saliva samples were collected without the aid of any stimulants, within 3 minutes, after cleaning the tongue debris and a thorough mouth wash every time prior to sampling.

Assay:

The quinine in the biological fluids was determined by Cramer and Isaksson's method [18] modified by Armand and Badinand[19]. In this method the biological sample was made alkaline with 0.1N NaoH to liberate free quinine. The quinine thus formed was extracted into benzene and the benzene extracts were washed twice with 0.1N NaoH solution to remove any metabolites present. The washed benzene extract was extracted with 0.1N H_2So_4 and the sulphuric acid extract was subjected to flourescence measurements on a sequoia-turner filter flourometer, Model 112, at an excitation wave length of 350 nmts and emission wave length of 450 nmts.

Hugnh-Ngoc and Sirois[20] compared two spectroflourimetric procedures for quinidine i.e., with and without alkaline washing of the benzene extract and observed that unwashed benzene extract assay averaged 18% higher than the washed benzene extract. This interference by quinidine metabolites has been demonstrated with specific techniques such as TLC [20,21] and HPLC[22,23].

In contrast to these results, Hufman and Hignite [24] obtained an excellent correlation between their GC-MS method and Cramer-Isaksson procedure. Sved et al [25] reported that comparison of values from the Armand-Badinand procedure with those obtained with a specific HPLC procedure indicated general agreement with some divergence (upto 15%) in the later 24-30 hrs samples, with HPLC values being lower, the area under the curve values were not affected markedly, although some pharmacokinetic parameters could be [22,23]. The previously reported comparison of results from the Armand - Badinand determination with those from a GLC procedure [26] tended to support this lack of interference in single dose studies. In that study, steady state samples from patients showed 30% greater values by flourimetric analysis than by GLC analysis, while single dose data were in excellent agreement. Very recently warburton et al (1987) reported that there was no significant difference in the serum quinine concentration when assayed by both flourimetric (double extraction technique) and HPLC methods.

Pharmacokinetic calculations:

The various pharmacokinetic parameters from both serum concentration vs time curves and saliva concentration vs time curves were obtained for each individual on an IBM personnal computer according to a one compartment open model. Thus the first order apparent over all elimination rate constant (Ke) was obtained from the slope of the terminal portion of the curve obtained when logarithm of the biological fluid concentration

was plotted against time, after subjecting it to linear regression analysis. The biological half life of the drug was determined from the formula $t_{\frac{1}{2}}=0.693/Ke$. The absorption rate constant (Ka) was calculated by the method of residuals i.e., by multiplying the slope of the residual concentration vs time curve with 2.303. The apparent volume of distribution (Vd) was calculated from the equation.

$$Vd = \frac{Dose}{(AUC)_0^{\infty} \times Ke}$$

Where $(AUC)_0^{\infty}$ is the total area under the concentration time curve.

Ke = over all apparent elimination rate constant.

In calculating Vd as above, an assumption was made i.e. 'F' the fraction of the dose absorbed as one. This assumption was made on the fact that quinine is almost completely absorbed on oral administration [28]. The systemic clearance (Cls) was calculated from the equation.

$$Cls = \frac{Dose}{(AUC)_0^{\infty}}$$

Again the fraction of the dose absorbed was considered as one. The area under the serum/saliva concentration time curves (AUC) upto 36 hours was calculated by the trapejoidal method. Total area under the curve i.e., upto infinite time was obtained by the summation of $(AUC)_0^{36}$ and the residual area obtained by dividing the concentrations at 36 hrs with Ke. The area thus calculated was expressed as a value corrected for body weight.

Statistical analysis:

Rejection of the discordant data:

All the biological fluid concentrations were subjected to 'Z' test to examine whether the deviated values can be discarded. The z values were calculated according to the equation.

$$Z = \frac{x - m}{s}$$

Where x is the value in question

m is the mean of all values

s is the standard deviation

It was observed that none of the values could be discarded with 95% confidence ($Z \geq 1.96$). A few values could be discarded with 90% confidence ($z \geq 1.645$). However deletion of such values did not alter the mean pharmacokinetic parameters significantly.

The various pharmacokinetic parameters obtained following administration of quinine alone and in combination with other drugs were validated using a 2-tailed students 't' - test for paired values. Analysis of variance was performed to find out interactions of quinine, intersubject and intrasubject variations in different pharmacokinetic parameters.

RESULTS AND DISCUSSION

Saliva/serum correlation study:

Secretion of quinine into different body fluids including saliva was reported earlier[28]. Serum and salivary concentration-time data are plotted in Fig.1. A significant correlation (r

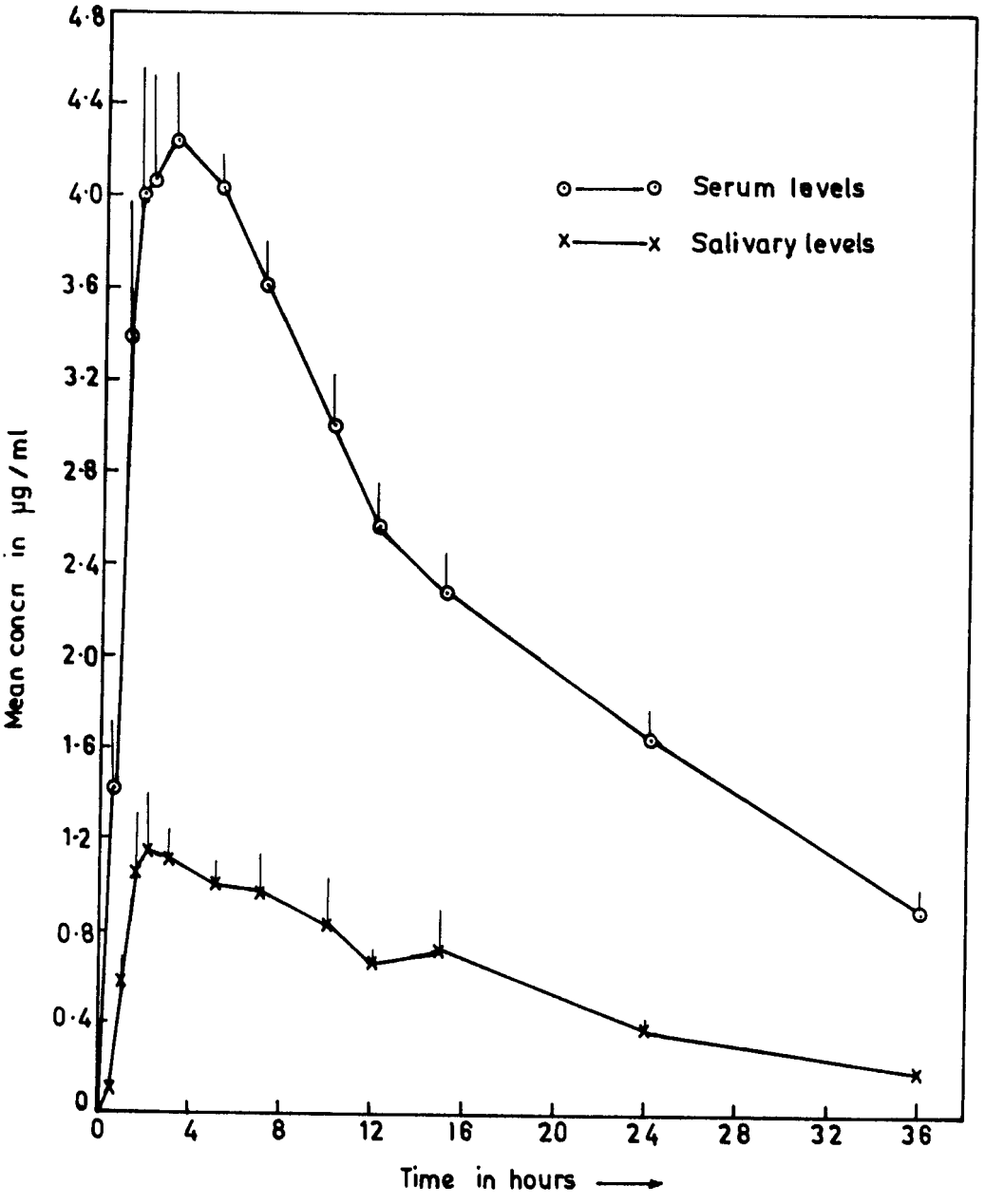

FIGURE 1: SERUM AND SALIVARY LEVELS OF QUININE FOLLOWING A SINGLE ORAL ADMINISTRATION OF 500 mg OF QUININE SULPHATE

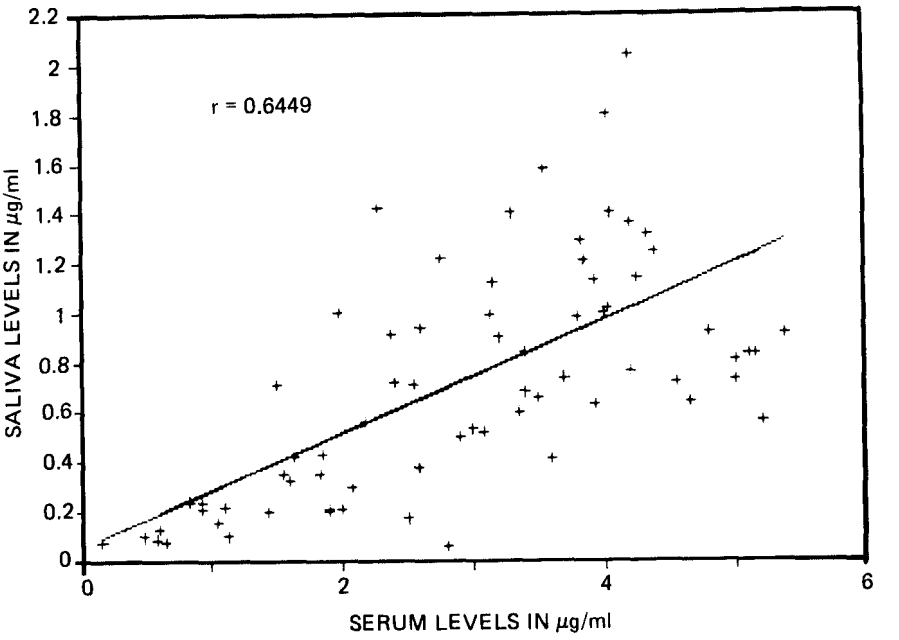

FIGURE 2: SCATTER DIAGRAM OF SALIVARY AND SERUM LEVELS OF QUININE

= 0.6449, n=72, $p < 0.01$) between saliva and serum quinine concentrations was observed(Fig.2). The equation for the line of intact of quinine as determined by the method of least squares is x = 0.228678 y+0.05707. The mean saliva/serum (SL/SR) ratio deduced from the individual data of the volunteers was 0.2497±0.0145(SEM). However the plot of mean SL/SR ratio from individual values vs time is not constant. (Fig.3). The mean SL/SR ratio increased upto 2 hrs. and thereafter remained approximately constant upto 12 hrs. Increase in the mean SL/SR ratio upto 2 hrs. may be due to the time required for attaining an equilibrium between serum and salivary compartments. Knop et al [29] have reported high S/P ratios for theophyline in the

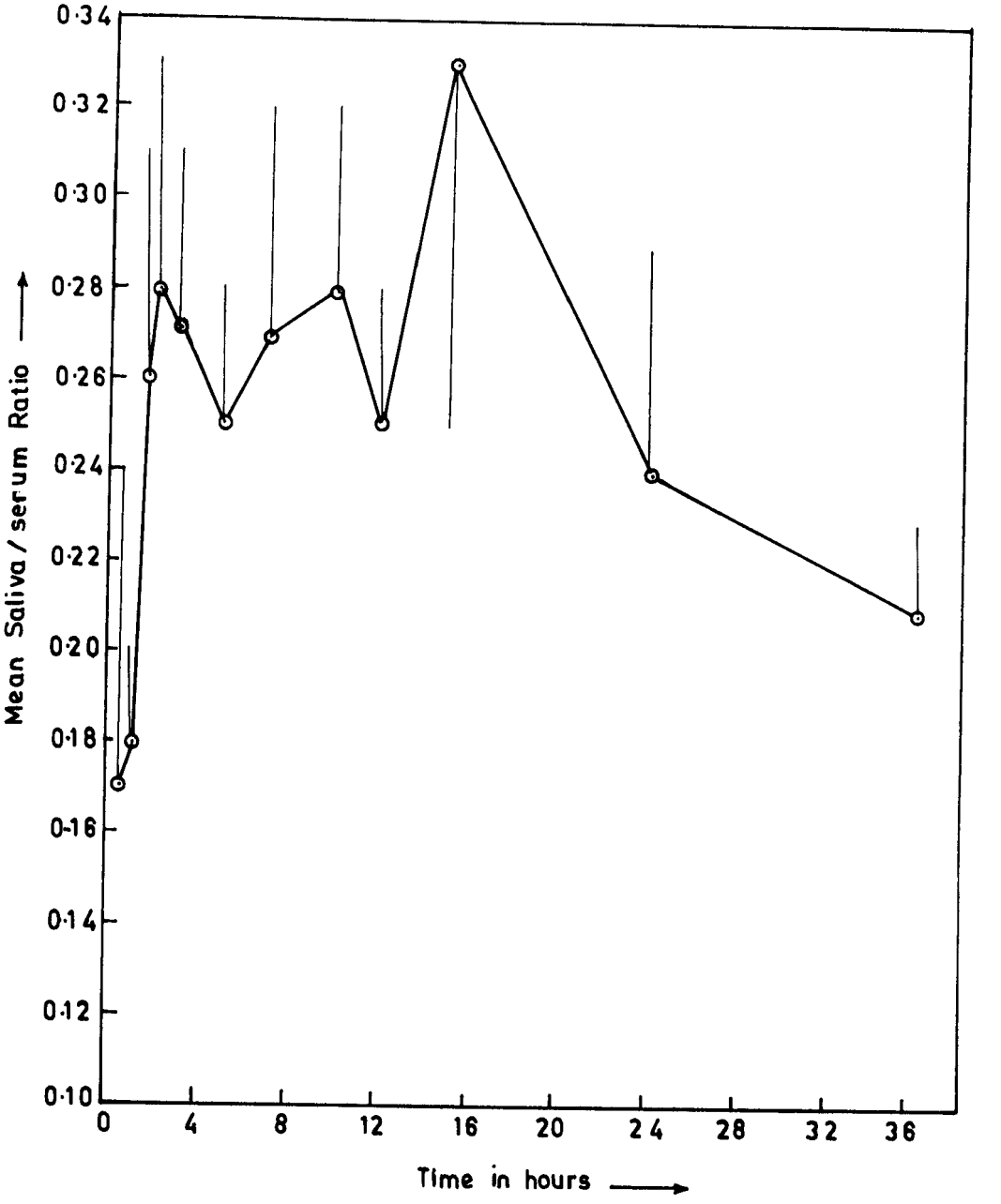

FIGURE 3: PLOT OF CHANGE IN MEAN SALIVA/SERUM RATIO OF SIX VOLUNTEERS WITH TIME

absorption phase. A maximum SL/SR ratio for quinine at 15th hour is note worthy. We believe that this may be due to circadian effects, as 15th hour sample falls in the dark period of circadian clock. This is in concurrence with our observations on diurnal oscillations of salivary levels of quinine.*

The values of various pharmacokinetic parameters calculated from serum or salivary levels are recorded in table 1. Use of salivary compartment to study the pharmacokinetics of quinine is indicated as the difference between the values of the various pharmacokinetic parameters obtained from saliva and serum was not stastically significant. ($P > 0.05$) The values are well in agreement with those reported earlier based on blood data [30].

Pharmacokinetic interactions of quinine:

Mean saliva quinine concentration vs time plots resulted following the administration of quinine alone or in combination with test drugs are shown in Figs.4 - 7. A careful examination of these and predictions based on the comparison of the various pharmacokinetic parameters (C_{max}, T_{max}, $T_{\frac{1}{2}}$, Ka and AUC - Table-2) it can be stated that salivary levels of quinine are unaffected (based on a student's 't' test at $P > 0.05$) by the presence of the test drugs in the body. This result is interesting at least in the context of oxyphenbutazone or aspirin as these have been reported to alter the pharmacokinetics of many drugs via competetive protein binding [31,32]. Since it is the free

*Paper to be presented in the 3rd international conference on chronopharmacology NICE (France)

TABLE-I

Pharmacokinetic parameters

Parameter	Serum	Saliva
C_{max} (ug/ml)	4.603 ±0.484	1.37 ±0.44
T_{max} (Hrs)	2.5 ±1.225	2.75 ±1.909
Ke	-0.049 ±0.0063	-0.0588 ±0.0096
Ka	0.9677 ±0.1658	0.8968 ±0.5264
$T_{\frac{1}{2}}$(Hrs)	14.375 ±1.845	12.155 ±2.178
Vd(Ltr/kg)	2.048 ±0.4196	1.896 ±0.5477
Cls (ml/min/kg)	1.7272 ±0.4725	1.9025 ±0.7847
AUC/kg	1.891 ±0.172	1.8807 ±0.7757

The values indicate the average of six readings along with standard deviation.

drug of plasma which is known to equilibriate with saliva any change in plasma concentration due to protein binding interaction shall be reflected on salivary levels. However our observations do not delineate this. Although oxyphenbutazone and aspirin are considerably bound to plasma proteins, they could not interfere with the binding of quinine probably due to their weak acidic nature. Weakly acidic drugs were reported to have binding affinity towards the albumin fraction of plasma proteins, where

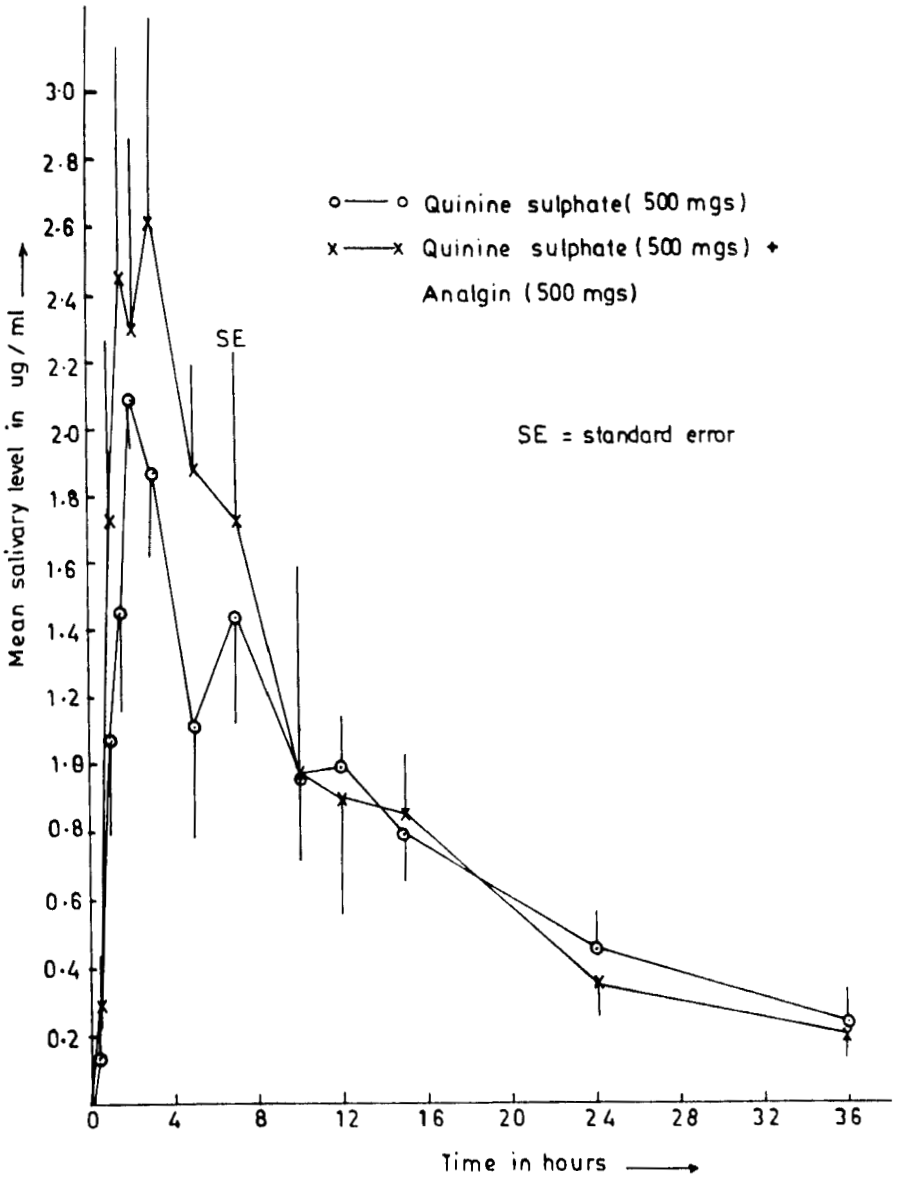

FIGURE. 4

MEAN SALIVARY LEVELS OF QUNINE SULPHATE AFTER IT'S ADMINISTRATION ALONE AND IN COMBINATION WITH ANALGIN Vs TIME PLOTS.

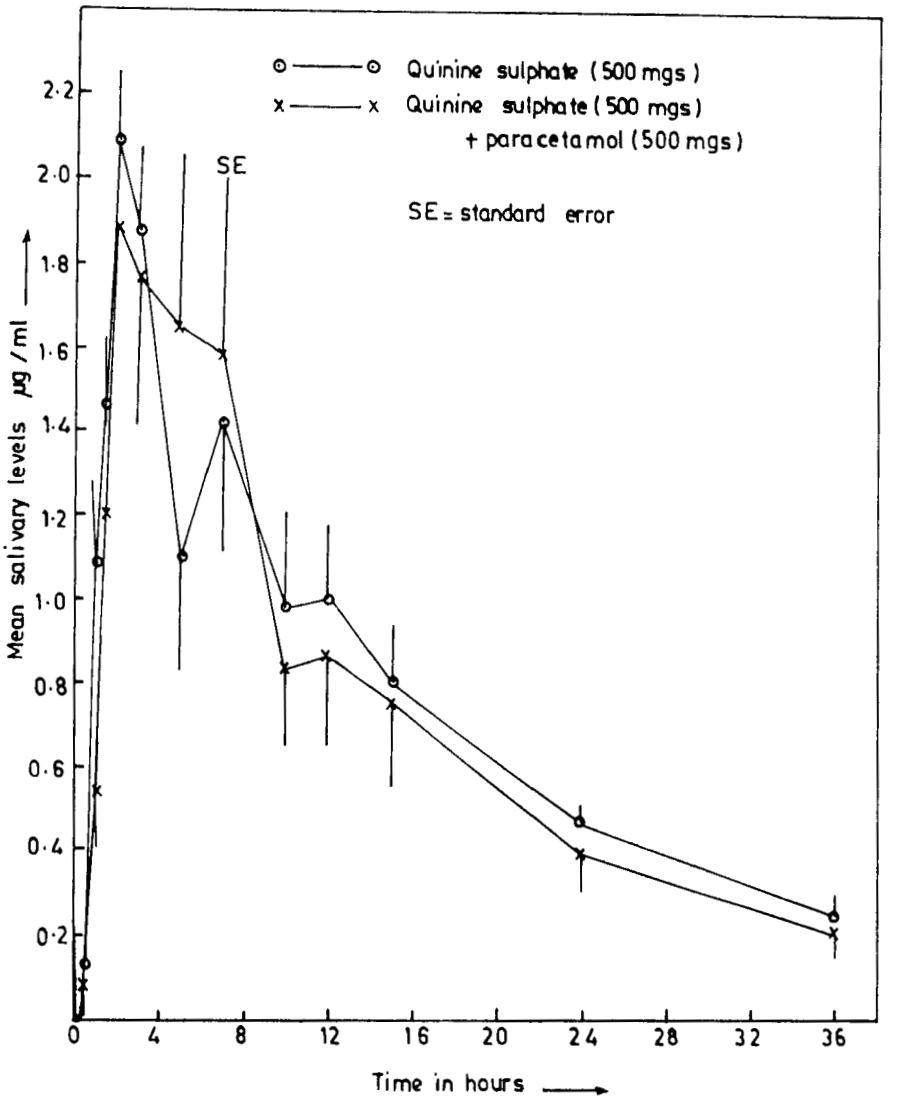

FIGURE. 5

MEAN SALIVARY LEVELS OF QUNINE SULPHATE AFTER IT'S ADMINISTRATION ALONE AND IN COMBINATION WITH PARACETAMOL Vs TIME PLOTS.

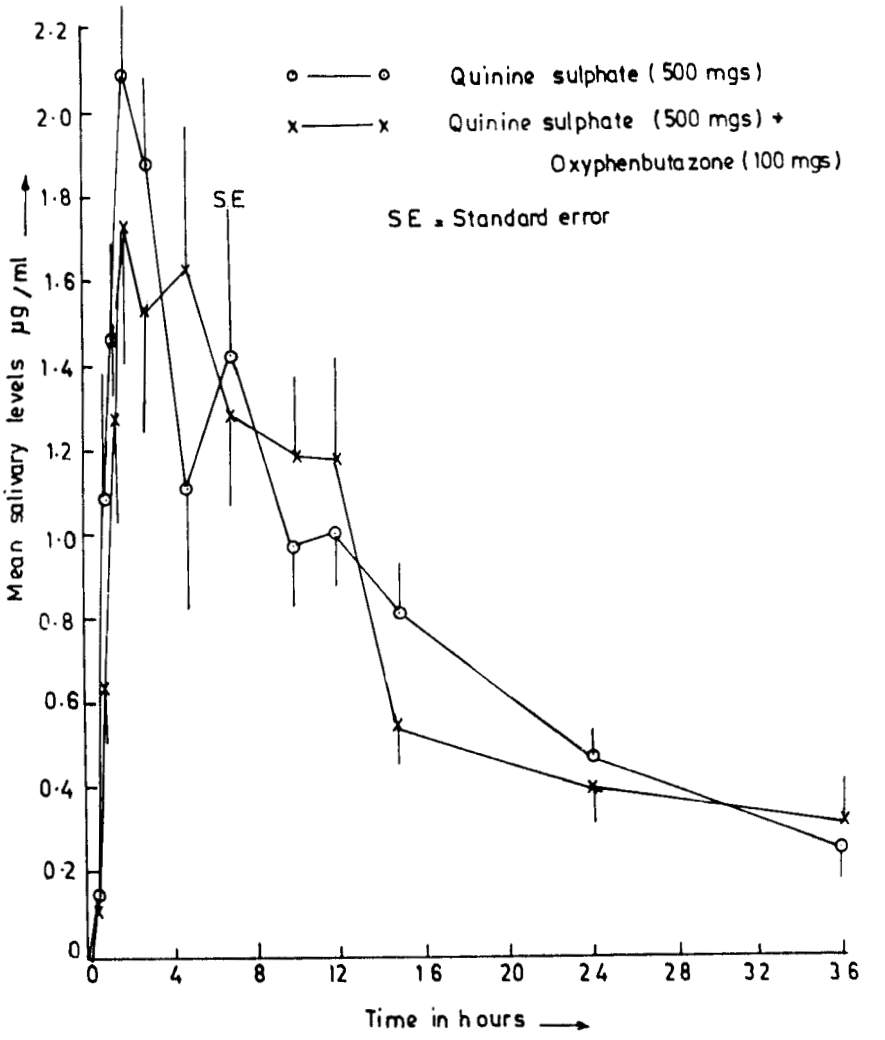

FIGURE. 6 :

MEAN SALIVARY LEVELS OF QUNINE SULPHATE AFTER IT'S ADMINISTRATION ALONE AND IN COMBINATION WITH OXYPHENBUTAZONE Vs TIME PLOTS.

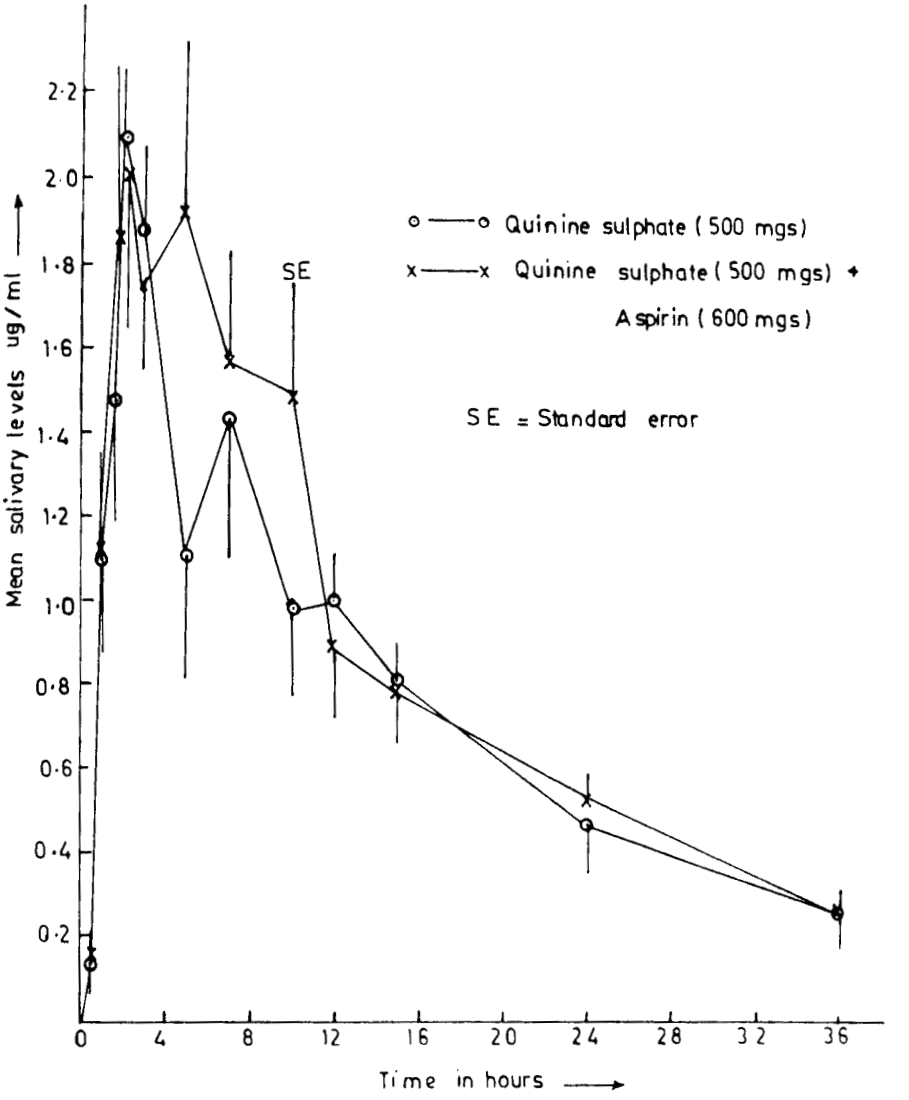

FIGURE. 7

MEAN SALIVARY LEVELS OF QUNINE SULPHATE AFTER IT'S ADMINISTRATION ALONE AND IN COMBINATION WITH ASPIRIN Vs TIME PLOTS.

TABLE-II

PHARMACOKINETIC PARAMETERS OF QUININE FOLLOWING ITS ADMINISTRATION ALONE AND INCOMBINATION WITH OTHER DRUGS OBTAINED FROM SALIVARY LEVELS

Parameter	Q	Q+AN	Q+P	Q+OP	Q+A
C_{max} (ug/ml)	2.108 ±0.345	2.95 ±1.229	2.202 ±0.640	1.852 ±0.646	2.424 ±0.778
T_{max} (Hrs)	2.2 ±0.4	2.7 ±0.6	3.3 ±1.47	3.4 ±1.357	2.9 ±1.2
Ke	-0.05906 ±0.0035	-0.0907 ±0.0375	-0.0697 ±0.0101	-0.0554 ±0.0184	-0.0464 ±0.0103
Ka	1.239 ±0.601	0.5676 ±0.13663	0.7434 ±0.58	0.9185 ±0.375	0.6224 ±0.3894
$T_{\frac{1}{2}}$ (Hrs)	11.78 ±0.755	8.766 ±2.821	10.194 ±1.685	14.594 ±6.548	11.134 ±1.891
Vd (Ltr/kg)	1.2069 ±0.4428	0.8851 ±0.2578	1.2072 ±0.3651	1.3839 ±0.4349	1.0841 ±0.3866
Cls (ml/min/kg)	1.279 ±0.3935	1.2459 ±0.3567	1.3738 ±0.3907	1.1594 ±0.2096	1.0973 ±0.2024
AUC/kg	2.2512 ±0.8463	2.5932 ±1.5136	2.184 ±1.0084	2.36 ±0.6301	2.536 ±0.8624

Q = quinine sulphate Q+AN = Quinine sulphate + analgin Q+P = Quinine sulphate + paracetamol

Q+OP = Quinine sulphate + Oxyphenbutazone Q+A = Quinine sulphate + aspirin

as weakly basic drugs seems to bound to lipoproteins and α_1 - acid glycoproteins. Affinity of quinidine (a stereo isomer of quinine) towards the plasma protein fractions other than albumin was reported [33,38].

The paracetamol with a high S/P ratio of 2 - 5 [34] could not in any way seems to affect the levels of quinine in saliva. Cotty et al (1977) have postulated that paracetamol may modify the metabolic extraction of aspirin on the basis that both the drugs are conjugated with glucuronic acid. Hydroxylation is also one of the path ways of paracetamol metabolism and this path way is reported to be of importance when the drug is administered in larger doses. In the present study only 500mg of paracetamol was administered along with 500mg of quinine sulphate whose main metabolic path way is hydroxylation [36]. The statistical analysis of the results based on a student's 't' test revealed no significant variation ($P > 0.05$) in any of the pharmacokinetic parameters of quinine due to a single dose paracetamol (500mg) administered concomitantly.

Oxyphenbutazone is capable of inducing liver microsomal enzymes [37], but such an affect may only be teneable on repeated administration and hence it could not interfere with the metabolism of quinine. The main metabolic pathway of quinine being hydroxylation and that of oxyphenbutazone the glucuronide conjugation, a metabolic interaction due to competition for a common path

way may not exist for these to account for a pharmacokinetic interaction between them.

The mean cumulative area under the curve vs time plots shown in Fig.8 indicate that aspirin and analgin increased the bioavailability of quinine to a little extent. However these changes are not statistically significant ($P > 0.05$). ANOVA calculations for intersubject, intrasubject variations and for variation within treatments revealed that there is no significant difference within treatments in any of the parameters studied at $P > 0.05$. However there was a significant ($P < 0.05$) intersubject variation in AUC and C_{max} and intrasubject variation in the absorption rate constant Ka. (Table-3)

The drug interaction studies based on salivary levels for quinine may be valuable since it is reported to be a useful drug to treat nocturnal leg cramps in geriatric patients. While showing a relation between plasma quinine concentration and attenuation of leg cramps, Warburton et al (1987) could not observe a quantifiable relation between placebo and quinine treatments possibly because the patients were consuming on an average 3.5 drugs other than quinine in a day, which might interfere with the drug levels in the plasma.

We therefore suggest that for children and geriatric patients saliva can be considered as a compartment of choice for interaction

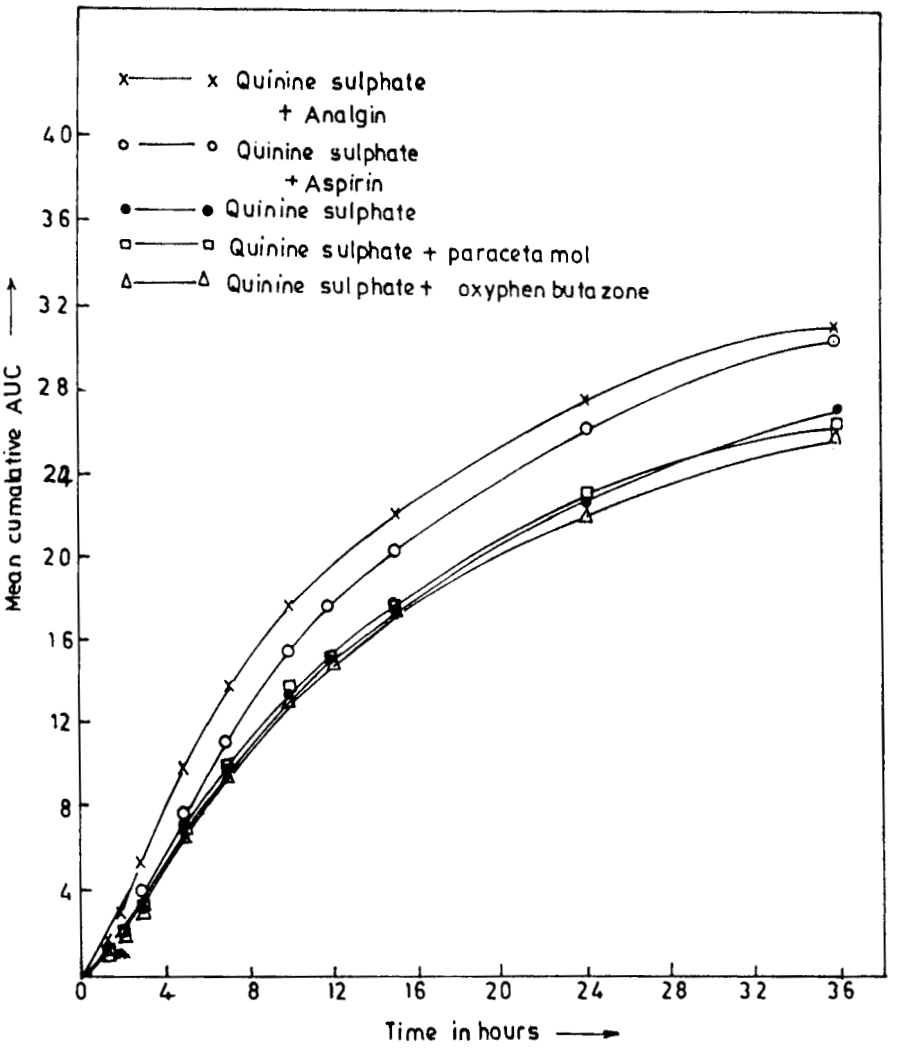

FIGURE. 8

MEAN CUMULATIVE AREA UNDER THE SALIVARY AVAILABILITY CURVES OBTAINED AFTER ADMINISTRATION OF QUININE SULPHATE ALONE AND IN COMBINATION WITH OTHER DRUGS Vs TIME PLOTS.

TABLE-III

ANOVA DATA

Source of variation	F values for parameters C_{max}	T_{max}	Ke	Ka	AUC
Volunteers	* 5.3801	0.3457	1.6212	0.7767	* 27.5595
Treatments	1.9067	0.705	2.5232	3.0652	0.7444
Weeks	0.141	1.125	1.7907	* 7.3451	0.4144

F = 3.36 at P = 0.05

* indicate the values which are significantly different

studies, even with limited observations including ours which have reported some discrepancies in S/P ratios in the absorption phase [29].

ACKNOWLEDGEMENTS

We are greatful to our volunteers for their enthusiastic participation in the study. We thank the M/s.Universal Pharmacy, Nagpur for providing a gift sample of quinine sulphate and Dr. Sunderesh Peri, for his help in computer analysis and typing.

REFERENCES

1. Pemberton, R. Arthritis and Rheumatoid conditions, Philadelphia, Lea & Febiger, 1935.p.53
2. Morelli, H.F., Melmon, K.L., Calif. Med. 109, 380 (1968)
3. Haag, H.B., Larson, P.S., Schwartz, J.J., J.Pharmacol. Exp. Ther. 79, 136 (1943).
4. Barchowsky, A; W.W. Stargel, D.G. Shand and P.A.Routledge. Ther. Drug Monit.4(4):335(1982)
5. Eatman, F.B., Moggio, A.C., J.Pharmacol and Bio pharm, 5 615 (1977)
6. Sa Dau Iguchi, Tsuyoshi Gonmara et al., J.pharm. Dyn.1 142(1978)
7. Amon, I., K.Amon, H.Scharp, G.Franke and F.Nagel. Eur.J.Clin. Pharmacol 24(1): 113 (1983)

8. Toback, Jamie, W., Peter Gal, N.Vildan Erkan, Craig Roop and Helen Robinson. Ther. Drug Monit. 5(2): 185(1983)

9. Laufen, H., M. Schmid and M.Leitold, J.Pharm. Sci 72(5): 496 (1983)

10. Jusko, W.J., J.Pharmacokinet and Biopharm 6: 7(1978)

11. Ollagnier, M., Queneau, P., Latour JF, Brazier, J.L., Decousus H., et al. Therapie 37: 549(1982)

12. Vestal, R.E., Wood, A.J.J., Clin. Pharmacokinet 5: 309(1980)

13. Feller, K., Le Petit, G. and Marx, U., Die Pharmazie 31, 745 (1976)

14. Mandel, I.W., J.Dent. Resear. 53: 246(1974)

15. Schmidt - Nielsen, B., Acta Physiol. Scand. 11: 104 (1946)

16. Chang, K. and Chiou, W.L., Chem.Pathol.Pharmacol 13: 357(1976)

17. Taylor, E.A., Tasoulla., Kaspi, T.L. and Turner, P.J.Pharm. Pharmac., 30: 813 (1978)

18. Cramer, G. and Isaksson, B., Scand J.Clin.Lab. Invest., 15: 553 (1963)

19. Armand, J. and Badinand, A., Ann. Biol. Clin. 30 : 599 (1972)

20. Hugnh-Ngoc, T. and Sirois, G., J.Pharm. Sci. 66(4): 591 (1977)

21. Hartel, G. and Korhonen, A; J. Chromatogr;49: 70(1968)

22. Guentert, T.W., Holford, N.H.G. Coates, P.E., Upton, R.A and Riegelman, S., J.Pharmacokinet. Biopharm, 7: 315(1979)

23. Guentert, T.W., Upton, R.A., Holford, N.M.G. and Riegelman,S., J.Pharmacokinet. Biopharm 7 303(1979)

24. Huffman, D.H. and Hignite, C.E., Clin. Chem., 22 810(1976)

25. Sved, S., MC Gilveray, I.J. and Beandoin, N., J.Chromatoge 145 437(1978)

26. Midha, K.K., MC Gilveray, I.J., Charette, C. and Rowe, M., Can.J.Pharm. Sci., 12 41 (1977)

27. Warburton, A., Royston, J.P., O Neill, C.J.A., Nicholson P.W., Jee, R.D., Denham, M.J., DOBBS, S.M and Dobbs, R.J., Br.J. Clin. Pharmac 23 459(1987)

28. Goodman, Gilman's The phaarmacological basis of therapeutics A. Mac Millan publishing Co., Inc. New York, 4th Ed (1970)

29. Knop, H.J., Kalafusz, R., Knols, A.J.F., and Vander Keleijn, E., Pharmaceutisch Weekblad, 110 1297 (1975)

30. Goodman and Gilman's The Pharmacological Basis of Therapeutics Mac Millan Publishing Co. New York, 7th Ed (1985)

31. Prescott L.F: pharmacokinetic drug interactions. Lancet 2 1239 (1969)

32. Segre E.J., Chaplin, M. et al, Clin. pharmacol ther. 15 (4) : 374 (1974)

33. Piafsky, K.M. Clin. Pharmacokinet; 5: 246(1980)

34. Glynn, J.P. and Bastain, W., J.Pharm. Pharmac 25 420(1973)

35. Cotty VG et al. Toxicol Appl pharmacol 41 7 (1977)

36. Brodie, B.B., Bear, J.E. and Craig, L.C., J.Biol. Chem. 188 567 (1951)

37. Chen, W.et al, Life Sci. 2 35 (1962)

38. Vallner, J.J., J.Pharm. Sci., 66 447 (1977)