Compound B was also obtained as a colourless solid. It also responded to Mg/HCl test. Its UV spectrum ($\lambda_{max}$ 259 and 304 nm) showed the compound to be a furanoflavane. The IR spectrum showed the presence of a carbonyl group (1627 cm$^{-1}$). Its $^1$H NMR spectrum, showed two doublets ($J$ 2.0 Hz) at $\delta$ 7.75 and 7.16 for H-5$^a$ and H-4$^a$ furano protons. Another doublet ($J$ 10.0 Hz) at $\delta$ 8.19, for one proton was assignable to H-5. Two multiplets at $\delta$ 8.12 (two protons) and 7.52 (three protons), were assignable to H-2', H-6' and H-6; H-4' and H-5' respectively. Singlets at $\delta$ 7.25 (one proton) and 3.93 (three protons), were attributable to H-3 and methoxy protons. The compound B could thus characterized as 3'-methoxy (2",3";7,8) furanoflavone (2). The RDA fission in the MS fragmentation pattern suggested the presence of methoxy groups in the B-ring by showing peaks at 160 (A-ring fragment) and 132 (B-ring fragment).

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We are grateful to Landscape, HAU, Hisar, for the supply of plant material; Mr. Avtar Singh for providing $^1$H NMR data; and Mr. Rakesh Sharma for providing MS data.

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Pharmacokinetic Interaction between Sparfloxacin and an Antacid in Normal Volunteers

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This Study was conducted to examine if antacid alters the pharmacokinetics of sparfloxacin by microbiological assay using plate diffusion technique using Klebsiella pneumoniae. The plasma concentration of sparfloxacin was determined in six healthy male volunteers at 1, 2, 3, 4, 5, 6, 24 and 36 h, after administration of 200 mg single oral dose of sparfloxacin. The effect of concurrent administration of 100 mg of antacid on sparfloxacin kinetics was then determined. Co administration of antacid significantly reduced the C$_{max}$ [0.338±0.158 $\mu$g/ml Vs. 0.8715±0.85 $\mu$g/ml; p<0.05] and AUC$_{24-h}$ [3.670±2.12 $\mu$g/ml.h Vs 8.3732±4.03 $\mu$g/ml.h; p<0.05] of sparfloxacin. Concomitant administration of antacid containing aluminum and magnesium reduces the bioavailability of sparfloxacin in normal volunteers.

Sparfloxacin is a difluoroquinolone with a dimethyl piperazinyl group. It has two methyl groups in the piperazinyl ring and an additional fluorine atom at position 8, which enhances its activity against Gram-positive or Gram-negative organisms. Also sparfloxacin has approximately four and
tenfold greater activity against most ciprofloxacin susceptible, methicillin-resistant S. aureus. Sparfloxacin is almost used as drug of choice in respiratory tract infections. This condition when associated with acid peptic disease (APD) necessitated the concurrent use of sparfloxacin with antacids. Hence in this investigation, the pharmacokinetic interaction of antacid with sparfloxacin was studied. Identification of such interactions may prevent complications during infection and alternative drug to reduce acid secretion may be recommended.

Gift sample of pure sparfloxacin was obtained from Torrent Pharmaceuticals, Ahmedabad. Muller Hinton broth (beef infusion-800 g/l, casein enzyme hydrolysate-17.5 g/l, and soluble starch-1.5 g/l) Muller Hinton Agar (beef infusion-3 g/l, casein enzyme hydrolysate-17.6 g/l, soluble starch-1.5 g/l). All other chemicals used were of analytical grade. The strain of Klebsiella pneumoniae used was NCIM 2719 obtained from The National collection of industrial microorganisms (NCIM) Pune.

The study was carried out in 6 healthy male volunteers. The Ethical Committee of Sri Ramakrishna Institute of Paramedical Sciences approved the protocols of these experiments. The liver and kidney functioning of the human volunteers assessed to be normal by clinical and standard biochemical investigation. None of the subjects consumed alcohol, tobacco or any medication a week prior and during the study. After administering 200 mg of sparfloxacin to volunteers, blood samples of 5 ml were withdrawn at 1, 2, 3, 4, 5, 6, 24 and 36 h. A flush out period of 1 w was given before interaction study so that the whole drug gets excreted from the body. Then an antacid 100 mg (Gelusil MPS Tablets, Parke Davis India Limited, Mumbai) was administered and the blood samples were drawn at similar time intervals as with sparfloxacin alone. Plasma was separated and stored at −20°C until the assay is carried out.

Peak plasma concentration (Cmax) and time to reach the peak plasma concentration (Tmax) were calculated from the actual plasma data. Rate constant for elimination i.e. K12 was calculated by regression analysis of the mono exponential declining line of the log plasma drug concentration versus time graph, while elimination half life (t1/2el) was obtained from the formula \( t_{1/2}\text{el}=0.693/\text{K}_{12} \). Area under the plasma drug concentration versus time curve (AUC0–24h) was calculated by trapezoidal rule. Extension of the AUC data to infinity (AUC0–\(\infty \)) was done by dividing the last observed concentration of drug in plasma by the elimination rate constant (Ke). Students ‘t’ test was used to determine the statistical significance of the data.

K. pneumoniae culture was maintained in Muller Hinton Broth. Stock culture of K. pneumoniae were maintained on Muller Hinton agar plates and stored at 4°C. Before each assay run, the organism was sub-cultured onto Muller Hinton Broth. At least two transfers in Muller Hinton broth that made before the test assay. The Muller Hinton broth cultures were kept at 37°C for 24 h.

Muller Hinton agar (10.5 g) was dissolved in 500 ml of distilled water and pH adjusted to 7.2 using 0.1 M Sodium hydroxide solution. The Muller Hinton agar solution was autoclaved for 15 min at 15 lbs pressure. The agar solution was then brought to 37°C to 40°C and the organism kept in Muller Hinton broth was seeded (0.4 ml culture/500 ml solution). This was then poured into sterile Petri plates. Wells of 2 mm diameter were cut. Stock solution of sparfloxacin (1 mg/ml) was prepared by dissolving it in a minimal amount of sterile 0.1N hydrochloric acid solution (0.2 ml acid/10mg sparfloxacin). Sparfloxacin in concentrations of 0.5, 1.0, 2.0, 4.0, 5.0, 8.0 and 10.0 µl/ml in pooled plasma were set up in quadruplicate. Ten micro liters of the standard solutions were loaded onto the wells. The plates were incubated at 37°C for 24 h. The zone of inhibition was measured and its diameter was found to be proportional to the amount of the drug. The concentration of the drug in unknown samples can be calculated from the regression line of the log concentration of

**TABLE 1: SERIAL SPARFLOXACIN CONCENTRATIONS BY MICROBIOLOGICAL METHOD**

<table>
<thead>
<tr>
<th>Concentrations of Sparfloxacin (µg/ml)</th>
<th>Zone of inhibition* of K. pneumoniae against Sparfloxacin in plasma (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>8.52±0.56</td>
</tr>
<tr>
<td>0.5</td>
<td>15.4±1.03</td>
</tr>
<tr>
<td>1.0</td>
<td>17.0±1.04</td>
</tr>
<tr>
<td>2.0</td>
<td>21.3±0.97</td>
</tr>
<tr>
<td>4.0</td>
<td>24.0±0.04</td>
</tr>
<tr>
<td>5.0</td>
<td>27.9±1.02</td>
</tr>
<tr>
<td>8.0</td>
<td>31.6±0.69</td>
</tr>
<tr>
<td>10.0</td>
<td>33.5±0.56</td>
</tr>
</tbody>
</table>

*Values are the results of 5 different occasions (mean±SEM) and the samples on each occasion in duplicate (n=10).
TABLE 2: SPARFLOXACIN KINETICS WITH AND WITHOUT ANTACID TREATMENT IN HEALTHY VOLUNTEERS

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Sparfloxacin</th>
<th>Sparfloxacin + Antacid</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{\text{max}} (\mu g/ml)</td>
<td>0.87±0.85</td>
<td>0.34±0.158</td>
</tr>
<tr>
<td>T_{\text{max}} (h)</td>
<td>4.00±0.07</td>
<td>4.00±0.07</td>
</tr>
<tr>
<td>K_{e1} (h^{-1})</td>
<td>0.04±0.04</td>
<td>0.06±0.04</td>
</tr>
<tr>
<td>t_{1/2e} (h)</td>
<td>15.7±0.57</td>
<td>12.0±1.12</td>
</tr>
<tr>
<td>AUC_{\text{36h}} (\mu g/ml.h)</td>
<td>8.84±4.03</td>
<td>3.67±2.12</td>
</tr>
</tbody>
</table>

Values represent mean±SEM, n=6; P<0.05 when compared to sparfloxacin alone.

the standard and the diameter of the zone of inhibition.

The mean zones of inhibition obtained with plasma are presented in Table 1. Results showed that the zones of inhibition increased with increasing concentrations of sparfloxacin over the range tested. The mean plasma concentration of sparfloxacin with antacid was lower as compared to sparfloxacin alone. The difference was statistically significant at 1, 2, 3, 4, 5, 6, 24 and 36 h (P<0.05). Table 2 compares various pharmacokinetic parameters (mean±SEM) of sparfloxacin before and after single oral dose of antacid. C_{\text{max}} and AUC_{\text{36h}} of sparfloxacin with antacid were significantly, lower as compared to sparfloxacin alone (P<0.05).

When antacid was administered along with sparfloxacin the C_{\text{max}} and AUC_{\text{36h}} of sparfloxacin has been significantly lowered. The bioavailability of sparfloxacin is reduced due to the presence of aluminum and magnesium ions.

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Effect of Cholesterol on Size Distribution of Freeze-thaw Extruded Liposomes

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The effect of cholesterol on vesicle size during freezing and thawing process of extruded egg phosphatidylcholine liposomes were studied by varying the length and number of freeze-thaw cycles. Laser diffraction particle size analysis showed that the volume median diameter of freeze-thawed egg phosphatidylcholine multilamellar vesicles was increased when cholesterol was included in the bilayers. Using a freeze-thaw cycle of 3 min freezing in liquid nitrogen at −196°C followed by 3 min thawing at 50°C resulted in an anomalously large particle size for egg phosphatidylcholine/cholesterol formulations. When egg phosphatidylcholine/cholesterol multilamellar

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