7. IN VIVO PHARMACOKINETIC STUDY IN RATS

7.1 In Vivo Studies of Risedronate and its Formulations

7.1.1 Experimental Animals

The experimental protocol in the present study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC), SBKS Medical College and Research Institute, Sumandeep Vidyapeeth, Vadodara bearing registration number (SVU/DP/IAEC/2014/03/19). The experiment was carried out on healthy female Wistar rats weighing 200-250 g. Rats were housed in polypropylene cages, maintained under standardized condition (12 h light/dark cycle, 24°C, 35-60 % humidity) and allowed free access to diet (Nav Maharashtra oil mills ltd, Pune) and purified drinking water.

7.1.2 Bioanalytical Method

In the present work, chromatographic separation was carried out on a Shimadzu UFLC prominance liquid chromatographic system, controlled by LC solution software. It was equipped with LC 20AD Binary pump, a manual injector, a column and a photo diode array (PDA) detector (SPD 20A). The mobile phase consisted of a mixture of Phosphate Buffer pH 6.8, Acetonitrile, methanol in the ratio 450:480:70 respectively. The mobile phase was prepared daily and degassed by sonication and filtered through a 0.45μm membrane filter before use. The column was maintained at room temperature.

The mobile phase was delivered isocratically with a flow rate of 1 mL min\(^{-1}\), the injection volume was 20 μL and the wavelength for UV detection was 262 nm. For chromatographic separation, Enable C18 250mm × 4.6 mm column, 5 μm was used. All the chromatograms were analyzed by LCs solution.

7.1.3 Experimental Design

The animals were fasted at least 12 h prior to dose administrations and for 4 h after dosing with free access to water. Animals were divided into four groups each consisting of six animals. All animals were given different formulations group wise as described underneath.
Group I: Control group (Plain RIS suspension in 0.5% w/v sodium CMC, 3mg/kg, p.o.).

Group II: Formulation 1 (optimized Risedronate Nanoparticles equivalent to 3mg/kg, p.o.).

Group III: Formulation 2 (optimized Risedronate SLN equivalent to 3 mg/kg, p.o.).

Serial blood samples (0.5ml) were withdrawn through capillary inserted in to retro orbital plexus under mild ether anesthesia at a time interval of predose 1, 2, 4, 8, 12 and 24 h post dose. Blood samples were collected in micro centrifuge tubes containing anticoagulant (3.8% w/v sodium citrate). The plasma samples were collected immediately from aforementioned samples after centrifugation at 5,000 rpm at 4°C for 10 minutes and stored immediately at -20°C until further analysis. Samples were analyzed by standard HPLC method after sample extraction procedure as discussed in section 7.1.2.

7.1.4 Pharmacokinetic Data Analysis

Pharmacokinetic parameters were estimated by using Microsoft Excel 2007 add in PK solver. Various parameters like maximum plasma concentration ($C_{max}$), time for achieving maximum plasma concentration ($T_{max}$), mean residence time (MRT) and relative bioavailability (F) were calculated.

7.1.5 Statistical Analysis

Statistical analysis of the obtained data was carried out by using data analysis feature of Microsoft Excel 2007. The student’s $t$ – test was calculated with the level of significance, $P<0.05$.

7.2 In Vivo Studies of Alendronate and its Formulations

7.2.1 Experimental Animals

The experimental protocol in the present study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC), SBKS Medical College and Research Institute, Sumandeep Vidyapeeth, Vadodara bearing registration number (SVU/DP/IAEC/2014/03/19). The experiment was carried out on healthy female Wistar rats weighing 200-250 g. Rats were housed in polypropylene cages,
maintained under standardized condition (12 h light/dark cycle, 24°C, 35-60 % humidity) and allowed free access to diet (Nav Maharashtra oil mills ltd, Pune) and purified drinking water.

### 7.2.2 Bioanalytical Method

In the present work, chromatographic separation was carried out on a Shimadzu UFLC prominance liquid chromatographic system, controlled by LC solution software. It was equipped with LC 20AD Binary pump, a manual injector, a column and a photo diode array (PDA) detector (SPD 20A). The mobile phase consisted of a mixture of 0.05 M sodium citrate and sodium phosphate buffer (pH 8) acetonitrile methanol (75:20:5 v/v/v). The mobile phase was prepared daily and degassed by sonication and filtered through a 0.45μm membrane filter before use. The column was maintained at room temperature.\(^{[46]}\)

The mobile phase was delivered isocratically with a flow rate of 1 mL min\(^{-1}\), the injection volume was 20 μL and the wavelength for UV detection was 266 nm. For chromatographic separation, Enable C18 250mm × 4.6 mm column, 5 μm was used. All the chromatograms were analyzed by LCs solution.

### 7.2.3 Experimental Design

The animals were fasted at least 12 h prior to dose administrations and for 4 h after dosing with free access to water. Animals were divided into four groups each consisting of six animals. All animals were given different formulations group wise as described underneath.

**Group IV:** Control group (Plain ALD suspension in 0.5% w/v sodium CMC, 3mg/kg, p.o.).

**Group V:** Formulation 3 (optimized Alendronate Nanoparticles equivalent to 3mg/kg, p.o.).

**Group VI:** Formulation 4 (optimized Alendronate SLN equivalent to 3 mg/kg, p.o.).

Serial blood samples (0.5ml) were withdrawn through capillary inserted in to retro orbital plexus under mild ether anesthesia at a time interval of predose 1, 2, 4, 8, 12 and 24 h post dose. Blood samples were collected in micro centrifuge tubes containing anticoagulant (3.8% w/v sodium citrate). The plasma samples were
collected immediately from aforementioned samples after centrifugation at 5,000 rpm at 4°C for 10 minutes and stored immediately at -20°C until further analysis. Samples were analyzed by standard HPLC method after sample extraction procedure as discussed in section 7.2.2.

7.2.4 Pharmacokinetic Data Analysis

Pharmacokinetic parameters were estimated by using Microsoft Excel 2007 add in PK solver. Various parameters like maximum plasma concentration ($C_{\text{max}}$), time for achieving maximum plasma concentration ($T_{\text{max}}$), mean residence time (MRT) and relative bioavailability (F) were calculated.

7.2.5 Statistical Analysis:

Statistical analysis of the obtained data was carried out by using data analysis feature of Microsoft Excel 2007. The t – test was calculated with the level of significance, $P<0.05$. 