CHAPTER V

CONCLUSIONS

In this study, the feasibility of developing stable and effective salmon CT nasal spray preparations has been explored. The results can be summarized as follows:

1. Two strengths of salmon CT nasal spray containing 100 and 200 IU of active peptide per actuation were prepared. The preparations contained isotonic solution of synthetic salmon CT (BP 2002 and EP 2002) with appropriate preservative, buffer and tonicity adjuster.

2. The purity of salmon CT raw material, which was available in a lyophilized powder, acetate salt form, was determined by reverse-phase gradient HPLC according to the method of BP 2002 (or EP 2002). The percent assay was 99.59 $\pm$ 1.18 %, which was within the limit of 90.0 – 105.0 % of the peptide $C_{145}H_{240}N_{44}O_{48}S_2$, calculated with reference to the anhydrous, acetic acid-free substance (salmon calcitonin EPCRS).

3. Four batches of salmon CT nasal sprays were prepared (two batches each for 100 and 200 IU strengths) using an imported, special spray pump that was able to provide an accurate spray volume of 0.09 ml after each actuation. Each bottle was filled with 2.0 ml of peptide solution, which could deliver more than the conventional 14-dose bottle made by the innovator.

4. The appearance of all nasal solutions was clear and colorless. The solutions had a pH of about 3.5 and the osmolarity was within the isotonic range of 290 - 310 mOsm/kg. These two properties did not change during the stability evaluation either at the recommended storage temperature (4 ^{o}C) or under the accelerated condition (30 ^{o}C).

5. The percent labeled amount of all four batches was within 90.0 – 110.0 % ranges. This was the same limit required for salmon CT injection BP 2002. The assay

content was also stable for all batches for at least 4 months at 30° C (accelerated condition) and for 12 months at 4° C (real-time storage condition).

6. The level of calcitonin C, a major heat-generated degradation product of salmon CT solution, was also less than 7% limit after HPLC analysis (the same limit required for salmon CT injection BP 2002) regardless of the formulation (100 or 200 IU) and storage conditions (30° C or 4° C). The four batches also met the BP requirements for related peptide, which allow not more than 5% of the total peak area for the combined secondary peaks and not more than 3% of the total peak area for the individual secondary peak. Stability with respect to the related peptide content was also observed at both storage temperatures.

7. The four batches were also evaluated for leakage, droplet size distribution and spray pattern. Negative leak test results confirmed the ability of the crimping machine to tightly seal the bottle with the spray pump. The droplet size distribution and the spray pattern was evaluated by Pfeiffer GmbH, the manufacturer of the pump assembly. Closeness in the results of the two representative batches indicated the reproducibility of the spray pump performance with respect to the size and shape of the emitted spray.

8. Test on uniformity of mass (weight per spray) showed that all four batches passed the BP 2002 general requirements for solution nasal spray regarding the pump-to-pump reproducibility of the delivered weight per actuation. None of the individual values from ten bottles were more than 25 percent of the average value, even when stored at an elevated temperature (30 °C). The results thus reflected the pump performance with respect to the dose uniformity among individual bottles, i.e., the products were able to deliver the desired amount of the solution accurately and reproducibly.

9. The four batches also passed other *in vitro* tests such as the clarity and sterility tests under both storage conditions (4 ° and 30 °C). The overall *in vitro* results thus pointed to the suitability of the prepared nasal sprays for further *in vivo* bioequivalence evaluation.

10. Batch III of the prepared nasal spray (200 IU per spray) was selected as a representative batch to test for *in vivo* bioequivalence in comparison with the innovator product in twelve healthy male volunteers. Two sprays of 200 IU of salmon CT (total dose 400 IU) was administered to each subject in a two-way crossover study. Plasma concentrations of salmon CT were determined by radioimmunoassay (RIA). Individual plasma concentration-time profiles were analyzed using graphical method. The observed values of two important pharmacokinetic parameters, AUC and C_{max}, were used for bioequivalence comparison.

11. The area under the curve (AUC) of both products ranged from 2,548.96 to 4,030.12 pg.min/ml. The mean peak plasma concentration (Cmax) of two products ranged from102.87 to136.84 pg/ml. The average time to peak plasma concentrations (tmax) of both products ranged from10 to 15 min. There were no statistically significant differences in the corresponding pharmacokinetic parameters between the two products ($p > 0.05$, ANOVA). The values of 90% confidence intervals for AUC and Cmax, based on the log transformed data of the test product relative to the innovator product, were 93.80 - 113.01% and 91.32 – 102.61%, respectively. The values of the two parameters were contained within 80.0 –125.0% bioequivalence range. None of the subjects demonstrated any unwanted side effects or withdrew from the study.

12. Based on the results from this study, it can be concluded that the prepared salmon CT nasal spray solutions were both pharmaceutically equivalent and bioequivalent to the innovator product. The data suggested a strong potential for the local manufacture of the more economical version of the salmon CT nasal sprays having acceptable safety and bioavailability. If succeeded, the local production of salmon CT nasal spray is expected to boost the prescription and utilization of this highly expensive medication so beneficial to many osteoporosis and other bone disorder patients.