Bioequivalence study of a new sildenafil 100 mg orodispersible film compared to the conventional film-coated 100 mg tablet administered to healthy male volunteers

Appendix 1

Analytical procedures used to determine drug concentrations

A 0.1 mL aliquot of human plasma sample was mixed with internal standard working solution (stable isotope-labeled sildenafil and N-desmethyl-sildenafil at a concentration of 400 ng/mL) and 0.3 mL of sodium chloride solution were added and mixed. The resulting solution was vortexed and extracted with methyl tert-butyl ether (2.5 mL). The upper organic layer was separated, evaporated and the drug was reconstituted in 0.2 mL of the starting LC solvent and injected on a high-performance liquid chromatography system (Shimadzu Nexera LC30; 's Hertogenbosch, The Netherlands) coupled with an AB SCIEX Triple Quad 4000 LC-MS/MS (Nieuwerkerk aan den IJssel, The Netherlands).

Liquid chromatographic separations were achieved using a Waters Xterra MS C18 column (100 × 4.6 mm, 3.5 μm; Waters, Etten-Leur, The Netherlands). The column and auto sampler tray temperature were kept constant at 40°C and 8°C, respectively. The mobile phase consisted of an ammonium formate buffer (0.2 mol/L, pH 4.0) (A) water (B) and methanol (C) and was delivered at a flow-rate of 1.0 ml/min by using a gradient. The buffer (A) was set to 10% and a gradient was set for solvents B and C: starting with 35% B [0–0.5 min] followed by a linear gradient to 15% B [0.5–3.0 min], equilibration for 1.5 min [3.0–4.5 min], followed by a linear gradient from 15% to 35% B [4.5–4.6 min] and finally to equilibration for 1.4% at the starting percentage. The sample injection volume was 20 μL. Quantification was achieved with MS–MS detection in positive ion mode equipped with a Turbo ionspray interface set at 700°C. The ion spray voltage was set at 5000 V. The source parameters: curtain gas ion source gas 1 and 2, and collision gas were set at 30, 80, 80, and 8 psi, respectively. Detection of the ions was carried out in the multiple-reaction monitoring mode, by monitoring the following *m*/*z* transitions: sildenafil 475 → 100, N-desmethyl-sildenafil 461 → 85, stable isotope labeled internal standard of sildenafil 483 → 108 and stable isototope labeled internal standard of N-desmethyl-sildenafil 469 → 93. Quadrupoles Q1 and Q3 were set on unit resolution. The analysis data obtained were processed by Analyst software version 1.5.2.

The LC-MS/MS method for the determination of sildenafil and N-desmethyl sildenafil in human plasma was developed and validated according to the requirements of the FDA Guidance for

Industry,[1] and the EMA guidance on bioanalytical method validation.[2] The methods adhered to the regulatory requirements for selectivity, sensitivity, precision, accuracy, recovery, carryover, matrix effect, and stability. The precision and accuracy (expressed as % bias) of the lower limit of quantification (0.5 ng/mL) was 4.7% and -4.2% for sildenafil and 6.6% and -6.0% for N-desmethyl-sildenafil, respectively. Chromatograms of the lower limit of quantification of sildenafil and N-desmethyl-sildenafil are shown in Figures 1 and 2. The data indicate that the method could be applied to the analysis of clinical samples in bioequivalence studies.

References

1. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). Guidance for Industry: Bioanalytical Method Validation. May 2001.
2. European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009, 21 July 2011.

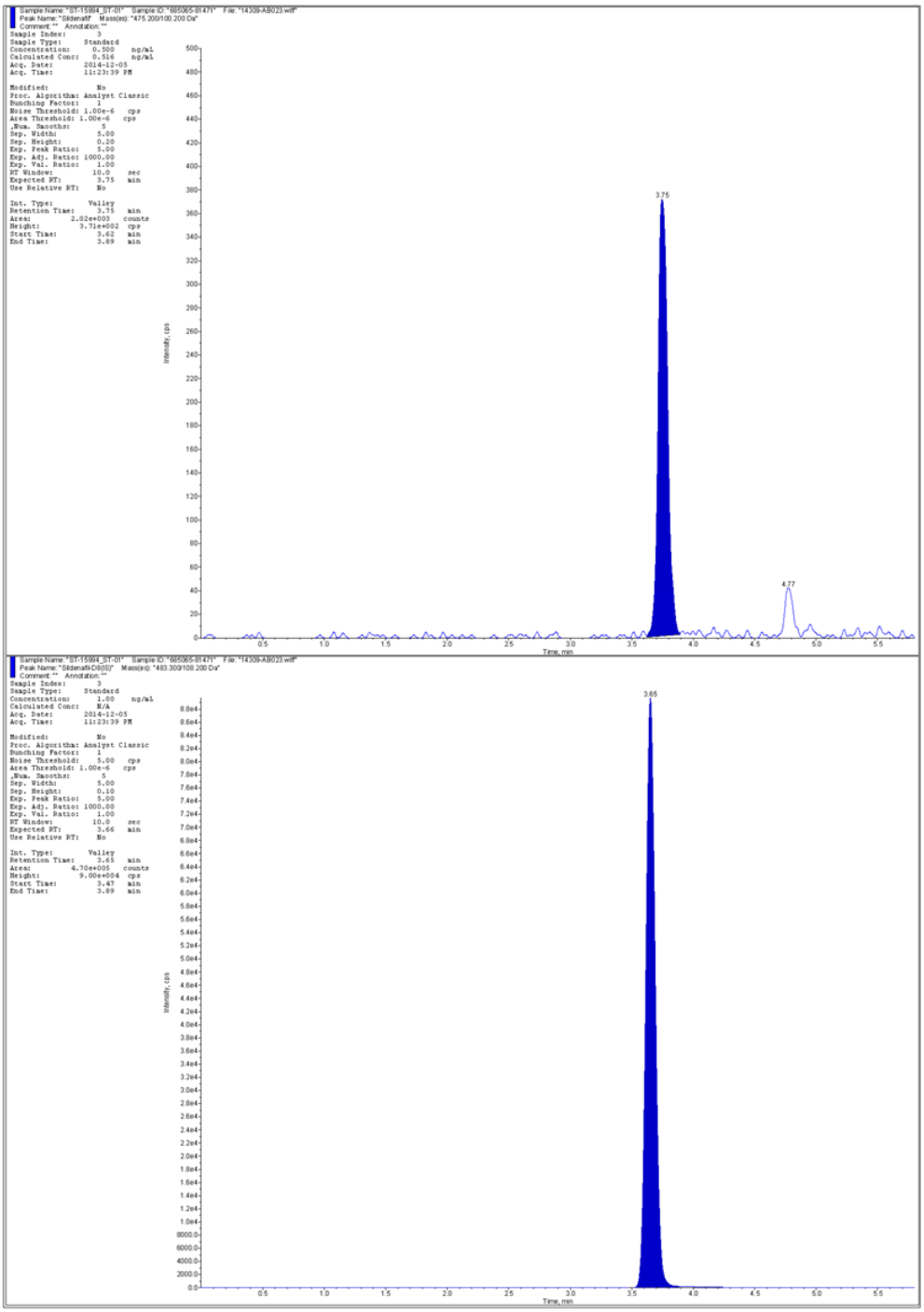

Figure 1 Chromatogram of a LLOQ sample where the upper chromatogram is for sildenafil (MRM transition: 475 → 100) and the lower chromatogram is for its stable isotope labeled internal standard (MRM transition: 483 → 108).

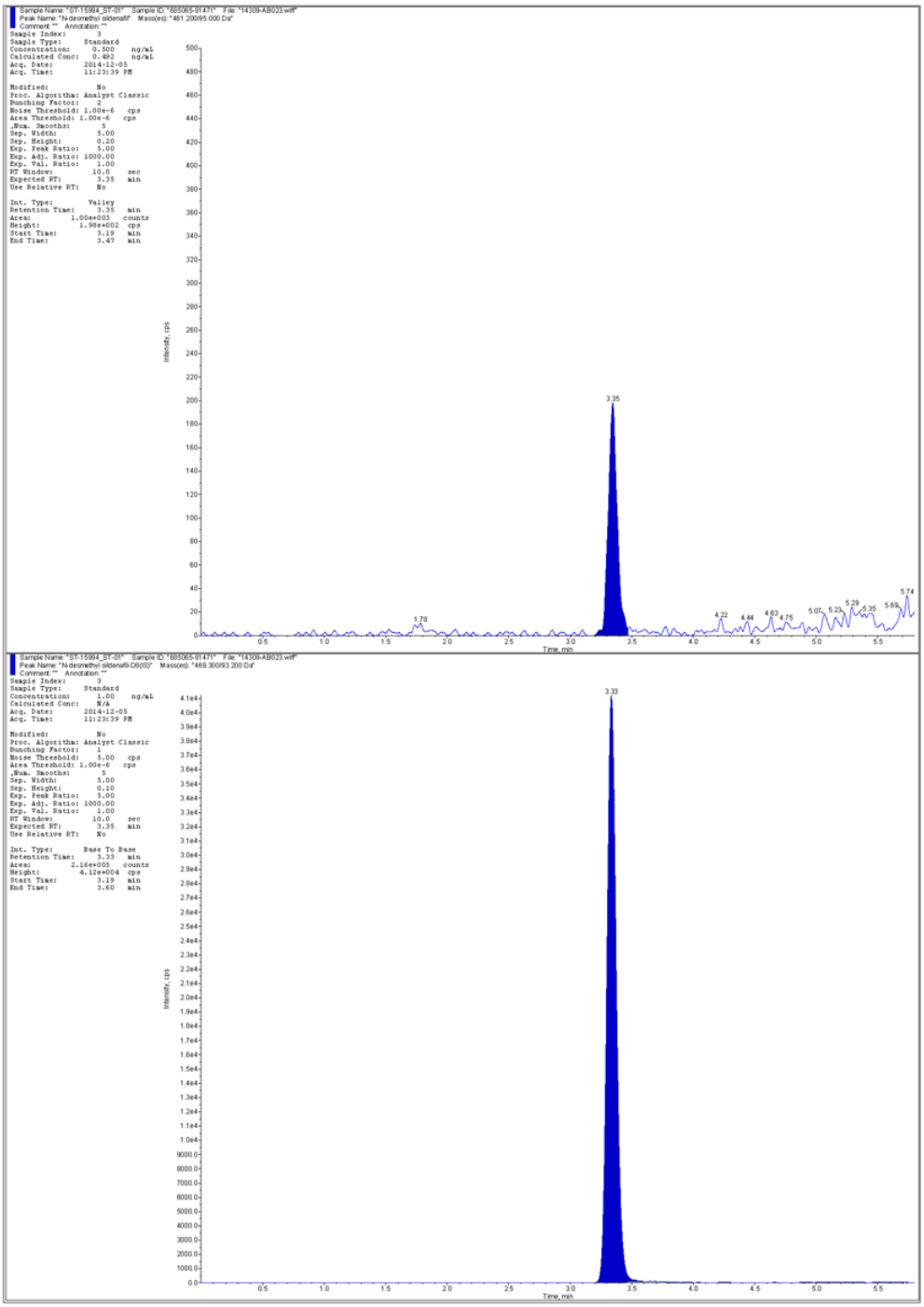

Figure 2 Chromatogram of a LLOQ sample where the upper chromatogram is for N-desmethyl-sildenafil (MRM transition: 461 → 85) and the lower chromatogram is for its stable isotope labeled internal standard (MRM transition: 469 → 93).