ORT_16 - Sustainable compendial grade GMP detergent substitutes for TritonTM X-100 in bioprocessing applications

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Introduction: Triton™ X-100, a detergent widely used in the biopharmaceutical industry degrades to endocrine- disrupting by-products making it an aquatic reproductive toxin. This led to its ban in Europe, requiring biopharmaceutical manufacturers to find alternative detergents that are biodegradable, GMP compliant, and pharmaceutically acceptable for use in manufacturing new cell-derived drug products. Alternatives to Triton™ X- 100 must perform equally regarding viral inactivation (VI), cell lysis, and protein compatibility to ensure global applicability.

Objectives: To identify alternative detergents to Triton™ X-100.

Methodology: Employing XmuLV and the *Feline catus* PG4 cell line as a model lipid-enveloped virus-host system, we identified Virodex™ TXR-1 and TXR-2 as TritonTM X100 replacements for VI after screening 31 detergents belonging to 11 chemical classes. The VI properties of the Virodex™ detergents were assessed at three temperatures (15, 22, and 28°C) to determine VI performance under different conditions. Kinetics of VI, cell lysis capabilities against two cell lines (CHOK1 and HEK293), and protein compatibility using alkaline phosphatase were also determined. Additionally, Virodex™ TXR-1 and TXR-2 were tested for affinity to Protein A resin, alongside LC-CAD and MS analytical methods that quantify both species at low ppm-ppb levels.

Results: Virodex™ detergents showed equivalent or better VI kinetics than TritonTM X-100 after a 15 min exposure. At all temperatures, both detergents achieved an LRF >3 after a 5 min treatment time, and after 60 min, the LRF increased to 6-8, exceeding the industry standard target of 4. TXR-1 and TXR-2 exhibited equivalent or better cell lysis as Triton™ X-100 and did not affect protein stability at concentrations as high as 2.5%. In addition, neither detergent exhibited affinity to Protein A resin. Highly sensitive LC-MS analytical quantification methods achieved an LOQ of <10 ppb.

Conclusion: Virodex™ TXR-1 and TXR-2 are excellent alternatives to Triton™ X-100 which are biodegradable, GMP compliant, and have an established track record of pharmaceutical use and compendial compliance, representing new options for the biopharmaceutical process.

Keywords: Triton™ X-100 replacement; Viral inactivation; Biodegradable