SPR741 is a novel polymyxin derivative devoid of direct antibacterial activity that is currently being investigated as a partner in combination with antibiotics for the treatment of multi-drug resistant Gram-negative infections. In in vitro tests, MIC of multiple partner agent classes have been dramatically lowered by the concurrent administration of low microgram/mL concentrations of SPR741. Four methods were validated for regulated use in monkey and rat plasma and urine.

**Challenges**

- Significant non-specific binding to plastic is observed in neat aqueous solutions.
- R&D analysis utilizing protein precipitation provided insufficient cleanup and yielded poor accuracy and precision results.
- SPE cleanup required precise pH control for analyte retention and elution.
- Binding to plastic is observed in neat aqueous solutions

**Sample Preparation Method**

- Validated range: 50-90,000 ng/mL in plasma, 1-1000 µg/mL in urine.
- Stocks and spiking solutions are prepared in aqueous 1% formic acid solution (pH 2.2) to eliminate non-specific binding to plastic.
- Isotope labelled SPR741 with 13C and 15N was synthesized to improve assay performance.
- Waters™ Oasis WXC weak cation exchange SPE plate were used for improved sample cleanup.
- LC/MS/MS assay for the determination of SPR741 has been developed and validated in four matrices. A Waters™ Oasis weak cation exchange SPE plate isolates SPR741 and its isotopic internal standard with high recovery and significant removal of matrix interferences. Binding to plastic is mitigated through pH control and non-specific binding losses from urine are controlled by the use of acidified CHAPS. The methods are precise and accurate across a 1000 fold range, and have been successfully employed for regulated analysis of hundreds of preclinical samples.

**Conclusions**

Development of LC-MS/MS Methods in Plasma for SPR741, a Cyclic Nonapeptide Which Potentiates Antibiotic Activity Against Gram-negative Pathogens

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**Overview**

- SPR741 is a novel polymyxin derivative devoid of direct antibacterial activity that is currently being investigated as a partner in combination with antibiotics for the treatment of multi-drug resistant Gram-negative infections.
- In in vitro tests, MIC of multiple partner agent classes have been dramatically lowered by the concurrent administration of low microgram/mL concentrations of SPR741.
- Four methods were validated for regulated use in monkey and rat plasma and urine.
- Accuracy, precision, selectivity, and carryover were significantly improved through development of a solid phase extraction procedure in place of a protein precipitation.

**Instrumental Analysis Method**

- Waters™ Acquity UPLC
- Thermo™ TSQ Vantage MS

**Validation Results Overview**

- 12 core runs across 4 validations met acceptance criteria for calibration standards and QCs.
- Recovery from the WXC plate is 80-85% for both SPR741 and its internal standard.
- Addition of acidified CHAPS reagent to monkey urine samples (2% v/v) is sufficient to reverse non-specific binding of SPR741.
- Calibration curves for all 4 validations are linear across 3 orders of magnitude.
- Excellent sensitivity and S/N at LLOQ through low pH mobile phase favoring +2 ion formation.

**Chromatograms**

- LLQO Calibration Standard (50.0 ng/mL)
- ULOQ Calibration Standard (50,000 ng/mL)