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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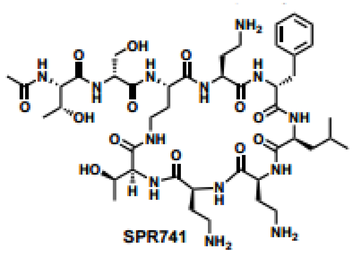
SPERO THERAPEUTICS

Overview



Ac-Thr-Dser-cyclo(Dab-Dab-Dphe-Leu-Dab-Dab-Thr)

M.W. 992.2

- 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's 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.

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 at low concentrations in monkey urine, producing upward sloping quadratic curve

Sample Preparation Method

- Validated range: 50-50,000 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 labeled SPR741 with ^{13}C and ^{15}N was synthesized to improve assay performance.
- Waters™ Oasis WCX weak cation exchange micro-elution SPE plate were used for improved sample cleanup.
- µElution SPE avoids evaporation and thus potential adsorptive losses.
- Neutral pH maintained during loading and washing, keeping –COOH sorbent moiety charged.
- Elution reagents composition is critical for good recovery:
  - Switching from 5% formic acid in methanol to 1% TFA in 25:75 water:acetonitrile doubled recovery.
  - pH <1 using TFA was critical for releasing SPR741 from the WCX plate
  - Adding a small amount of water also boosts recovery.
- The acidic eluent is partially neutralized using ammonium formate solution prior to LC-MS injection.

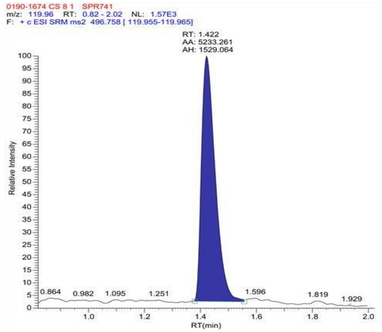
Instrumental Analysis Method

Waters™ Acquity UPLC		**Thermo™ TSQ Vantage MS**	
Run Time:	3.5 min	Ion Source:	HESI
Column Temp.:	30^{0}C	Spray Voltage:	5000
Autosampler Temp.:	7.5^{0}C	Ion Transfer Tube Temp:	300 ^{0}C
Flow Rate:	0.4 mL/min	Vaporizer Temp:	400 ^{0}C
Mobile Phases:	A: 0.5% formic acid in water B: 0.5% formic acid in methanol	Sheath Gas	60
LC Program	Gradient Elution: 15 to 90% MP B over 2.2 minutes	Aux Gas	10
Analytical Column:	Waters™ Acquity UPLC® BEH C8, 1.7 µm, 2.1 x 50 mm	Resolution	Unit/Unit

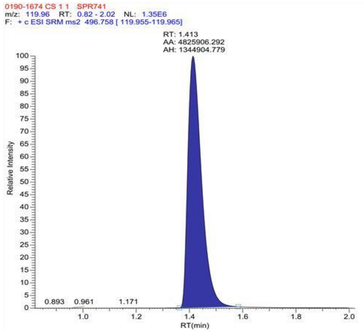
SRM Table

Compound	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	S Lens (V)
SPR741	Positive, +2	496.78	120.02	37	114
SPR00776 (SPR741-$^{13}C_9{}^{15}N_1$)	Positive, +2	501.79	129.02	39	103

Chromatograms



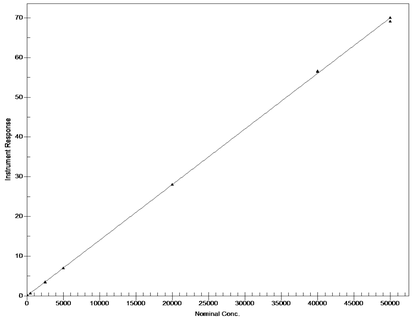
LLOQ Calibration Standard (50.0 ng/mL)



ULOQ Calibration Standard (50,000 ng/mL)

Validation Results Overview

- 12 core runs across 4 validations met acceptance criteria for calibration standards and QCs.
- Recovery from the WCX plate is 80-85% for both SPR741 and its internal standard.
- Addition of acidified CHAPS reagent to monkey urine samples (2% v/v) was 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.



Linear 1/x2 regression of SPR741 from monkey plasma curve

Inter-Assay Performance of QC Samples – Monkey Plasma Core Runs

Summary Statistics	**Run ID**	**LLOQ VS 50.0 ng/mL**	**LOW VS 150 ng/mL**	**MIDDLE VS 3750 ng/mL**	**HIGH VS 37500 ng/mL**
Mean Concentration Found	1, 2 and 5	50.2	149	3780	37900
Inter-run SD		1.82	4.02	43.1	398
Inter-run %CV		3.6	2.7	1.1	1.1
Inter-run %Bias		0.4	-0.7	0.8	1.1
n		18	18	18	18

Conclusions

A sensitive and specific 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.