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OVERVIEW

Purpose:

A RP-UPLC method was developed on a Waters H-Class Bio instrument to evaluate the major oxidation products present in Filgrastim samples. Agilent's Intelligent System Emulation Technology (ISET) was then used to emulate a H-Class, using an Agilent 1290 Infinity II, and chromatographic equivalence between the two systems was evaluated using typical method transfer criteria.

Methods:

Samples were degraded to generate oxidation products which were then separated and analysed using RP-UPLC-UV.

Results:

Main peak retention time deviation and retention time precision obtained from the H-Class was compared to those obtained using the emulated system.

INTRODUCTION

Background:

Filgrastim was used as a surrogate in this study: it comprises of 175 amino acids, has an average mass of 18,799 Da, is comprised of 1 chain, that has α-helical structure, is non-glycosylated, and is expressed in *Escherichia coli*. There are four methionine's that are liable to oxidation: Met^{1}, Met^{138}, Met^{127}, and Met^{122}.

Sample:

Filgrastim CRS was purchased from the Ph.Eur. and formulated to 0.2 $mg.mL^{-1}$ using an acetate buffer.

Stressed Conditions:

Filgrastim samples were spiked with 3% *v/v* H_2O_2 and incubated for 30 minutes and 3 hours at 37 °C. The reaction was quenched with L-methionine and degraded sample was spiked back into a filgrastim CRS sample.

METHODS

Workflow Schematic:

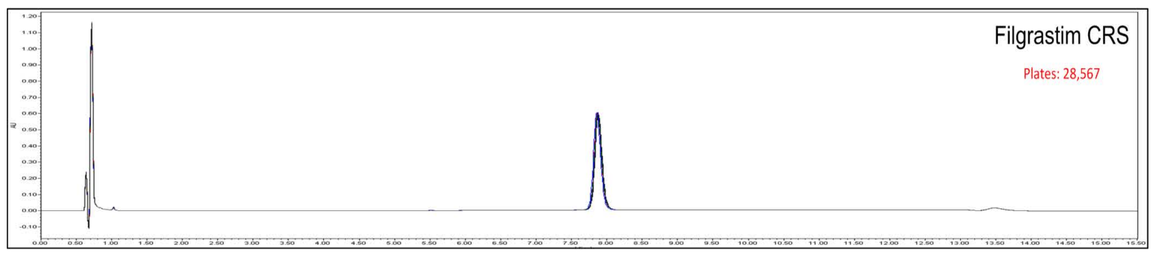
Analyses:

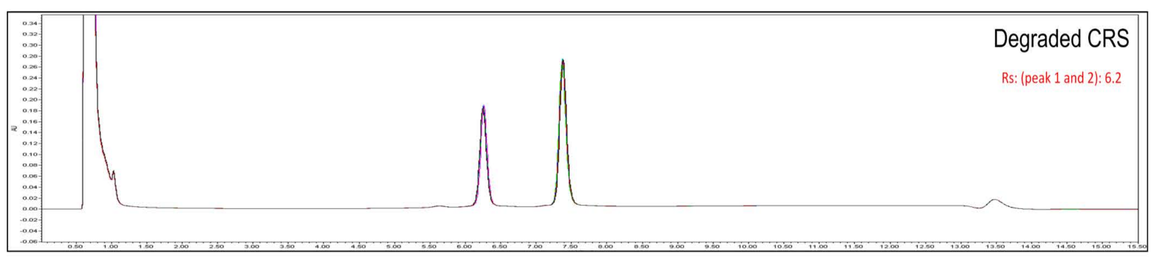
- Waters H-class Bio
- Agilent 1290 Infinity II Native [Mode 1]
- 1290 emulating a H-Class [Mode 2]
- Emulating a H-Class plus fine tuning [Mode 3]

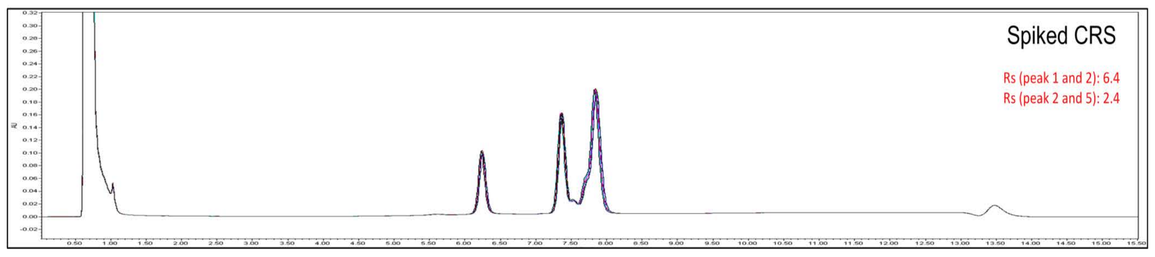
Bioanalytical Method Transfer from a Waters H-Class Bio UPLC to an Agilent UHPLC using ISET

RESULTS

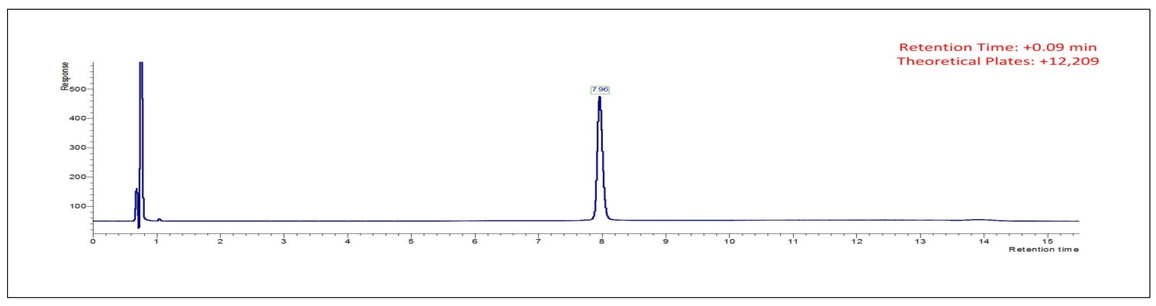
H-Class Bio Chromatography

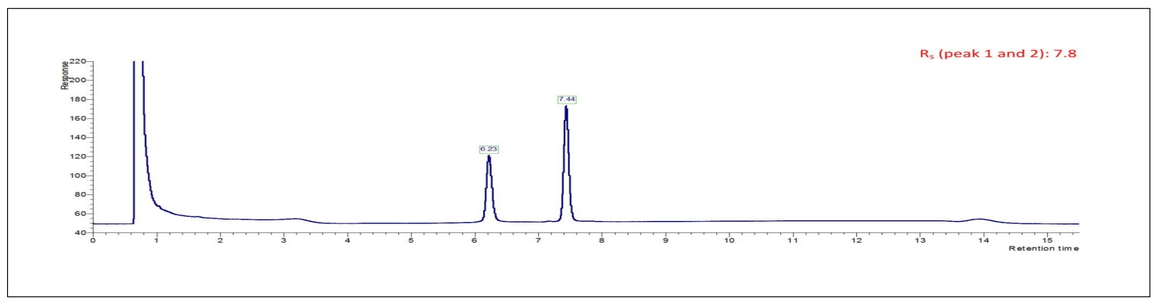


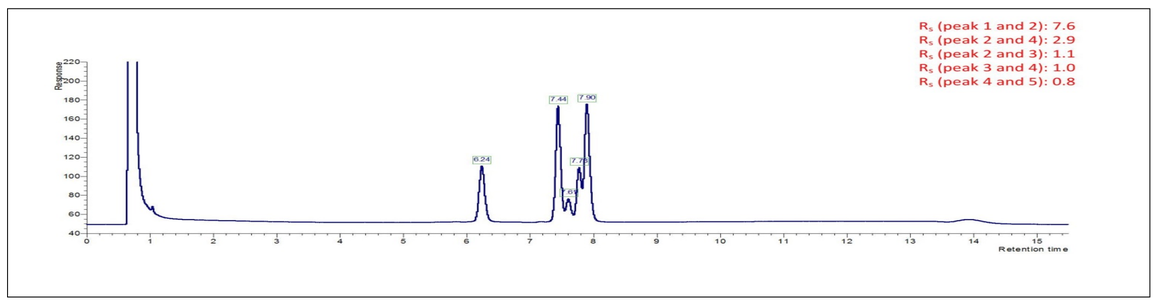




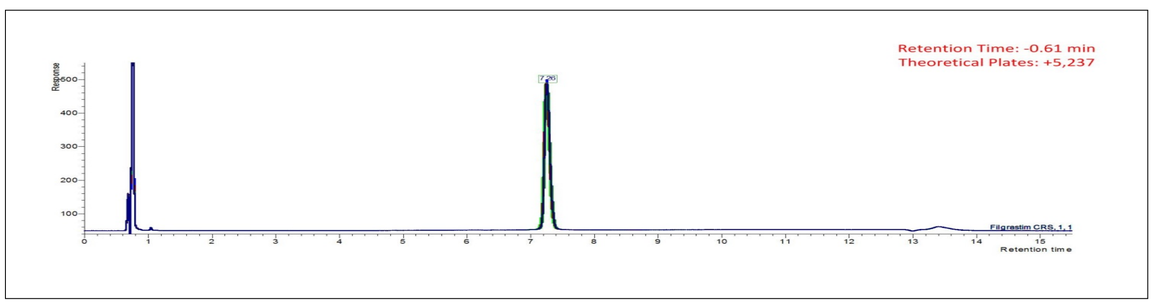
1290 Infinity II Mode 2 Chromatography

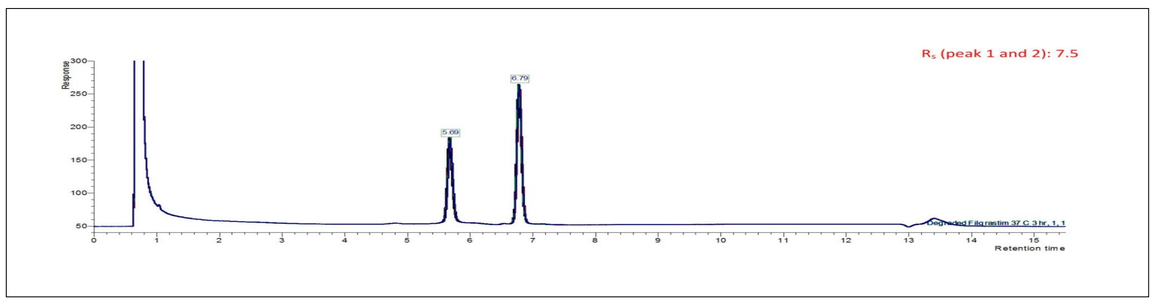


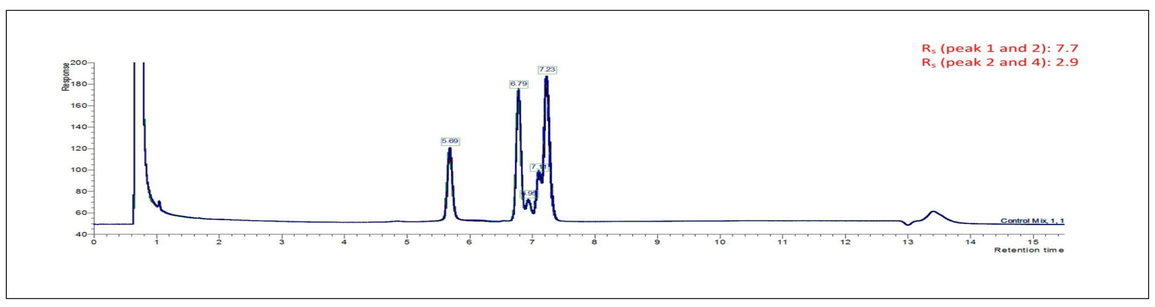




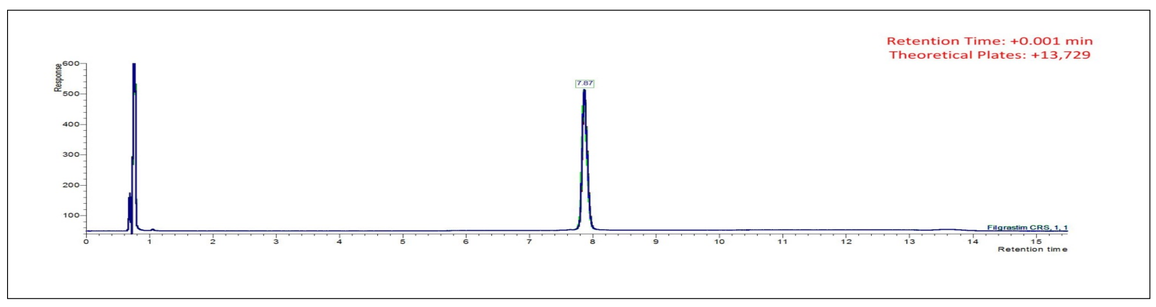
1290 Infinity II Mode 1 Chromatography

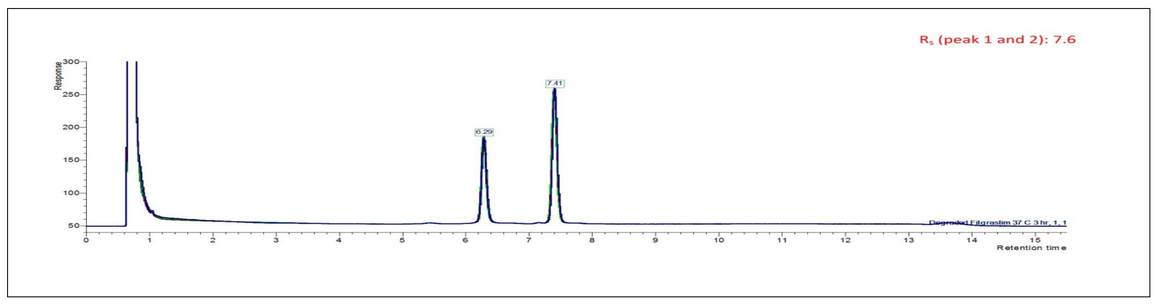


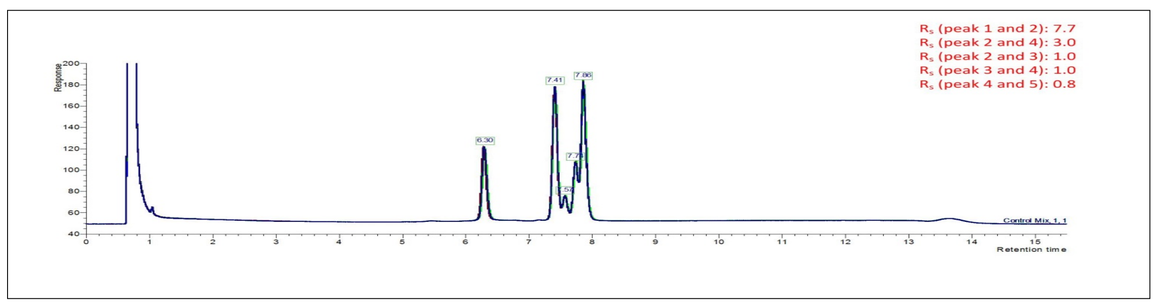




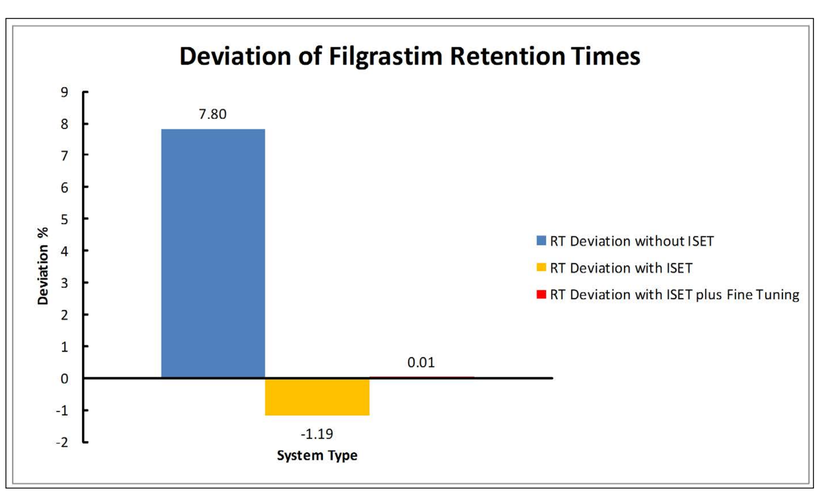
1290 Infinity II Mode 3 Chromatography

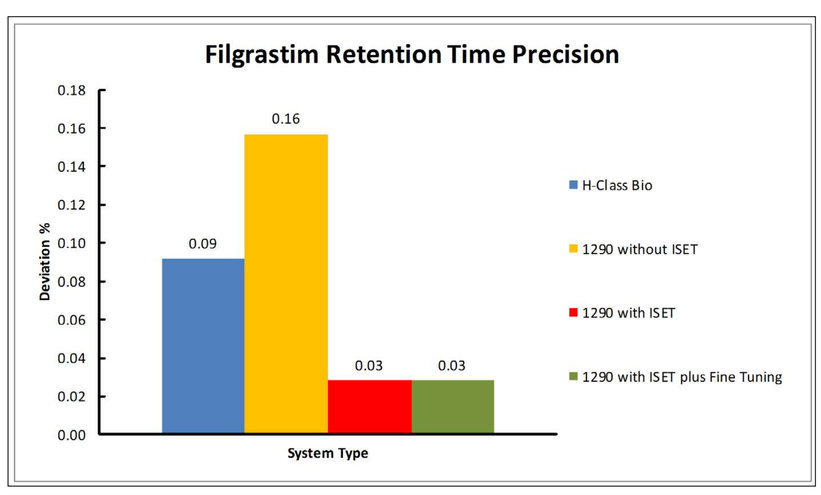


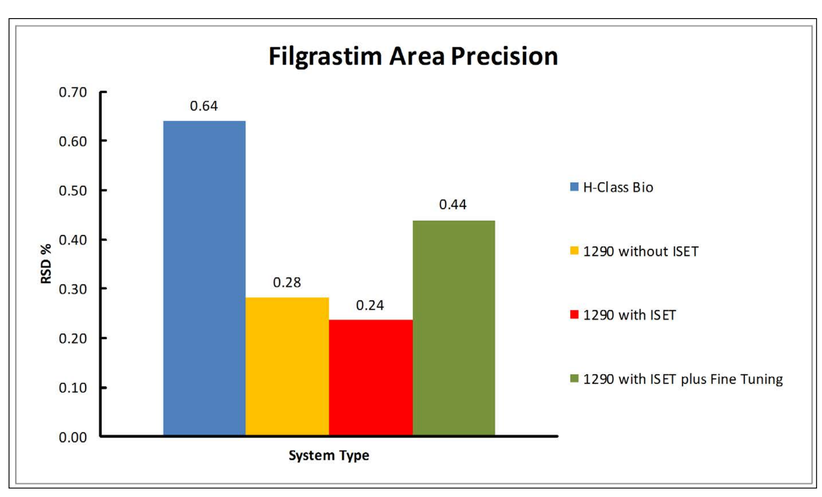


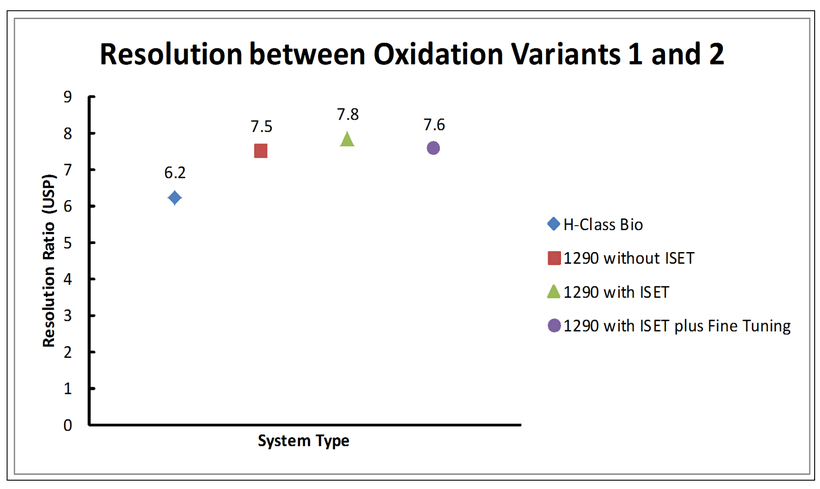


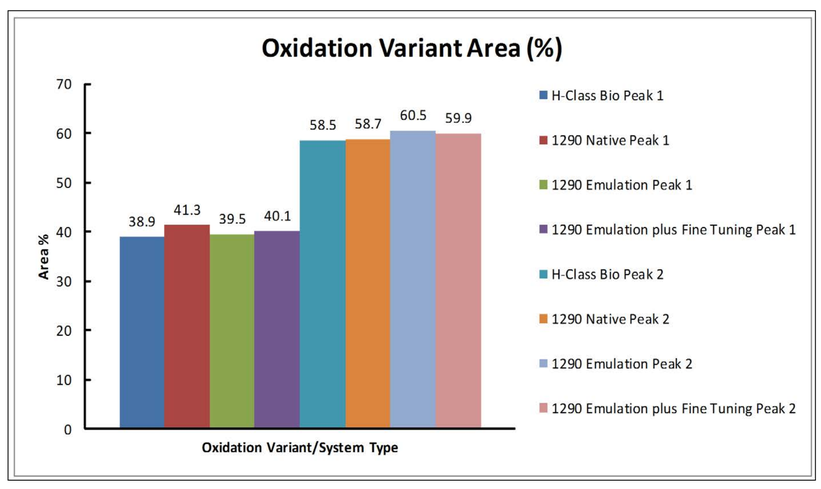
Data Analysis

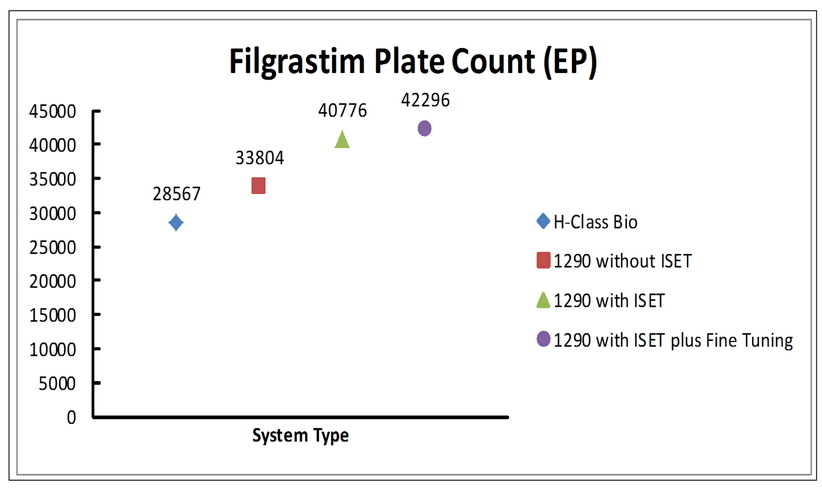












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Method Development :

- RP-UPLC elution order of oxidised species is by relative hydrophobicity.
- Separation achieved in 5 CV, with a gradient change of 1.5% MPB/CV.
- Met^{1}, Met^{127}, and Met^{138} are highly susceptible to oxidation as they are accessible to solvent molecules.
- Met^{122} is located at the hydrophobic core of the protein and is slow to undergo oxidation. Oxidation at Met^{122} results in a conformation change and large shift in hydrophobicity.

Instrument Conditions :

Parameter	Setting			
System	Waters Acquity H-Class Bio			
Column	Waters Xbridge Protein, BEH, C4, 300 Å, 3.5 µm, 2.1 × 100 mm			
Column Temperature	70 °C			
Autosampler Temperature	5 °C			
Injection Volume	10 µL			
Flow Rate	0.4 $mL.min^{-1}$			
Wavelength	216 nm			
Mobile Phase A	Water with 0.1% v/v trifluoroacetic acid			
Mobile Phase B	Acetonitrile with 0.1% v/v trifluoroacetic acid			
Gradient	Time (minutes)	Mobile Phase A (%)	Mobile Phase B (%)	Curve
	Initial	55	45	Initial
	3.0	55	45	6
	9.0	40	60	6
	12.0	40	60	6
	12.1	0	100	1
	12.5	55	45	1
	15.5	55	45	1

CONCLUSIONS

Chromatographic equivalence was evaluated between a Waters H-Class Bio and an Agilent 1290 Infinity II, using typical transfer parameters of retention time precision and deviation, as well as relative area percent and resolution of oxidised species. Using the 1290 in emulation mode plus fine tuning [Mode 3] gave the best performance and the data obtained would be deemed acceptable if used in analytical method transfer.

The utilitarian nature of emulation software when used in combination with a high performance system is of unique benefit to a CRO performing method transfer from a range of sending units employing many different vendor instruments, that may not be immediately available.

Future Work

- Perform MS/MS experiments to confirm oxidation species identity.
- Refine the degradation and quenching steps.
- Improve resolution between peaks 3 and 4, investigating a smaller particle size and alternate gradient shapes.

References

1. Agilent 1290 Infinity II with ISET User Manual, G4220-90310, Edition 09/11
2. Waters Corporation, Acquity H-Class Bio, LITR10167188
3. Mantovani, M., *et al.* Biosimilars Volume 2016:6 pages 45-60 (2016)
4. Holzmann, J., *et al*. Anal Bioanal Chem. (2013)