Development of a Multi-Peptide Immunocapture – LC-MS/MS Assay for the Quantitation of a PEGylated Therapeutic in Rat and Dog Plasma

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OVERVIEW

PURPOSE

To develop a multi-peptide assay based on LC-MS/MS for the quantitative analysis of a PEGylated therapeutic protein to support pre-clinical studies.

METHOD

Pegvisomant was extracted from plasma using direct digestion or immunocapture followed by on-bead tryptic digestion. Two surrogate peptides, N-term LFDNAMLR and C-term VSTFLR peptides were used for quantitation.

RESULTS

Both direct digestion and immunocapture with anti-PEG showed good linearity, precision and accuracy. However, higher sensitivity and specificity were obtained with the immunoaffinity approach. Good agreement of Pegvisomant concentration values between VSTFLR and LFDNAMLR-based quantitation were observed in rat and dog PK samples.

INTRODUCTION

While advances in HRMS have facilitated the analysis of the intact form of proteins, the preferred strategy for the quantitative bioanalysis of large molecules remains the surrogate peptide approach. However, metabolic protein cleavage followed by potentially divergent degradation pathways could introduce a bias in the ultimate determination of intact analyte concentration. To address this challenge, an immunocapture liquid chromatography-mass spectrometry (IC-LC/MS) analysis strategy was developed and optimized for quantitative analysis of a PEGylated therapeutic. The strategy consisted in selecting two surrogate peptides from opposite terms. This dual-peptide quantitation approach was put to test by generating single dose pharmacokinetic profiles of Pegvisomant (1) in both rat and dog models.
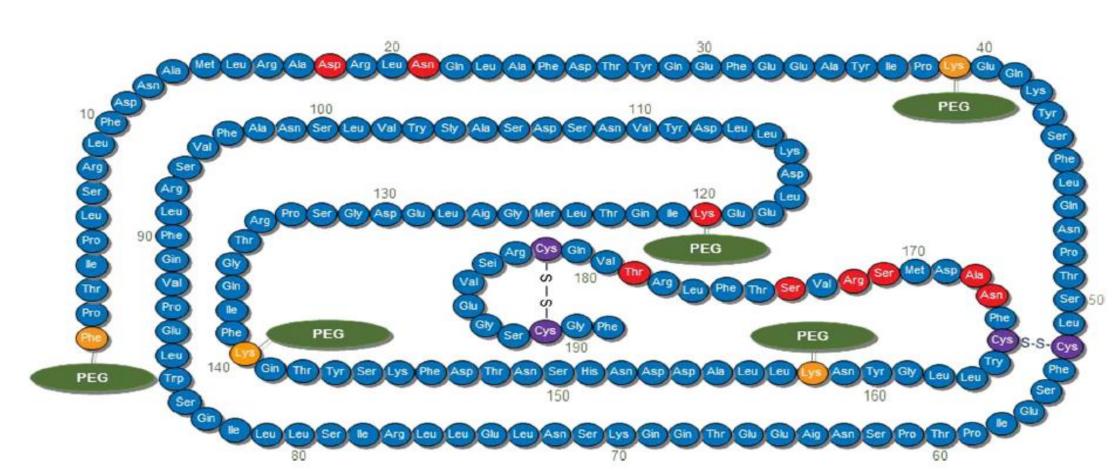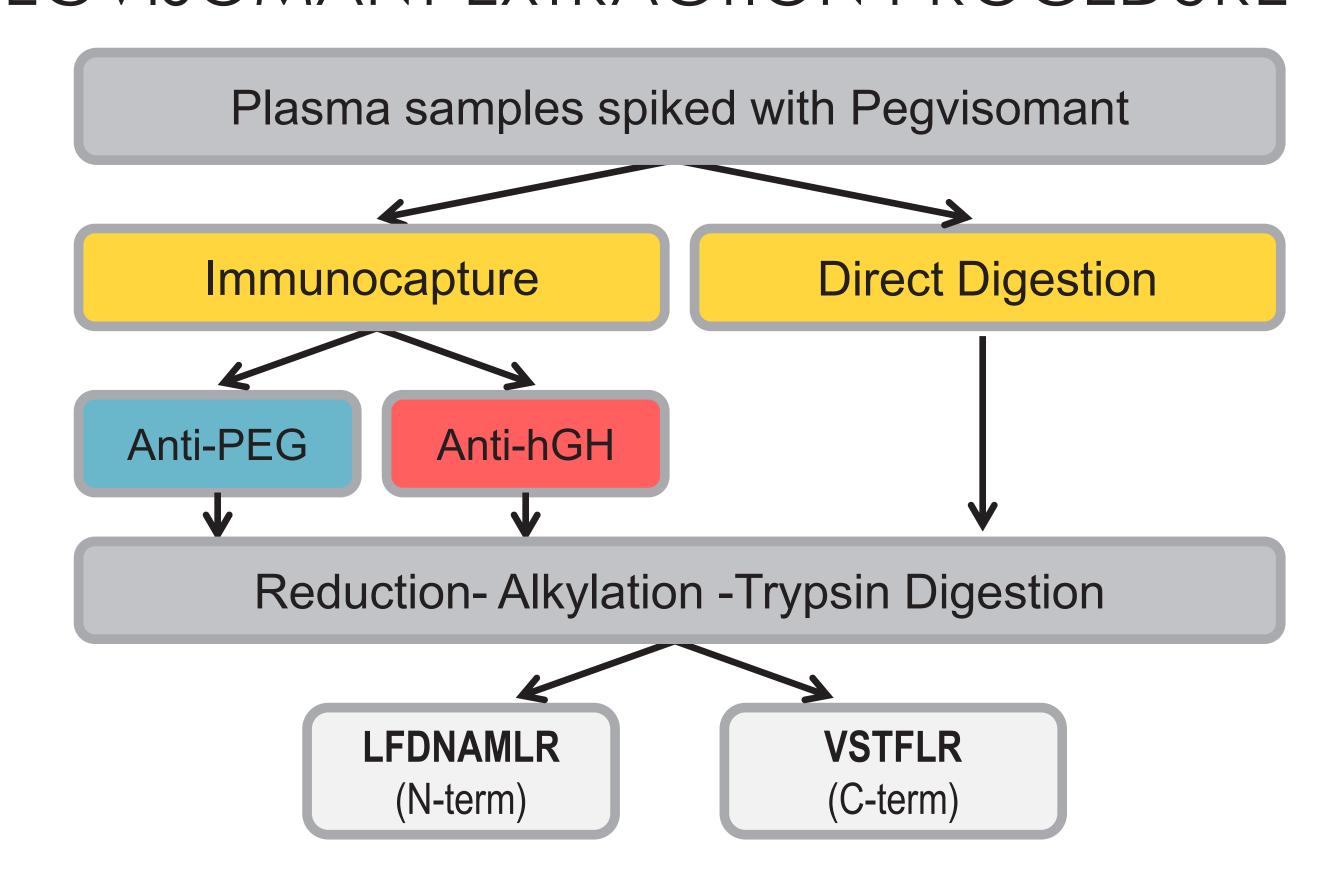

Figure 1. Amino acid sequence of Pegvisomant protein
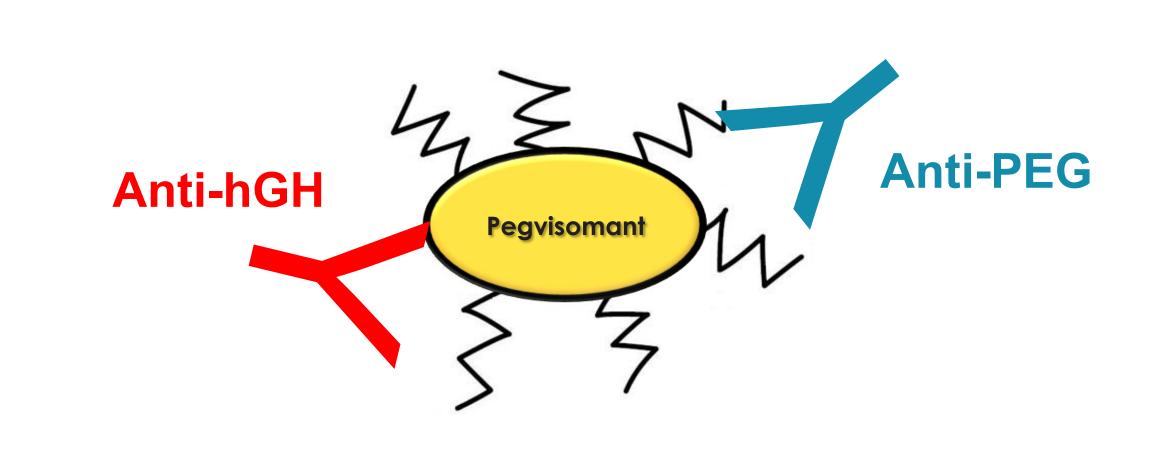
METHODS

PHARMACOKINETIC STUDY

- Four Sprague Dawley rats and one Beagle dog
- Subcutaneous dose at 2 mg/kg
- Collection at 0,1, 8, 24, 48, 72, 96 and 120 hours

PEGVISOMANT EXTRACTION PROCEDURE

IMMUNOCAPTURE

- Biotinylated αPEG and αhGH coupled to Strep magnetic beads
- Magnetic sample processing using KingFisher Flex™

LC-MS/MS DETECTION

- Agilent Zorbax SB300 C_{18} column (50 x 2.1mm, 3.5 μm)
- Gradient elution with 0.1% CH_3COOH in H_2O and ACN
- SCIEX Triple Quad API 5000 operated in (+) ESI-MRM

Table 1. Pegvisomant method MRM transitions.

Peptide	Q1 m/z	Q3 m/z	CE (eV)	Dwell Time (ms)
LFDNAMLR	490.2 (2+)	719.3 (y6)	20	50
VSTFLR	361.7 (2+)	536.3 (y4)	20	50
VSTFLR^ (IS)	366.7 (2+)	546.5 (y4)	20	50

RESULTS

DIRECT DIGESTION vs. IMMUNOCAPTURE LC-MS

Both direct digestion and immunocapture LC-MS approaches were evaluated. Although the direct digestion method is fast and low-cost, it is less sensitive and prone to interferences from the matrix.

Direct digestion of Pegvisomant in plasma after reduction-alkylation shows maximum MS response for both peptides after 20 min incubation with porcine trypsin at 37 ℃ (Figure 3).
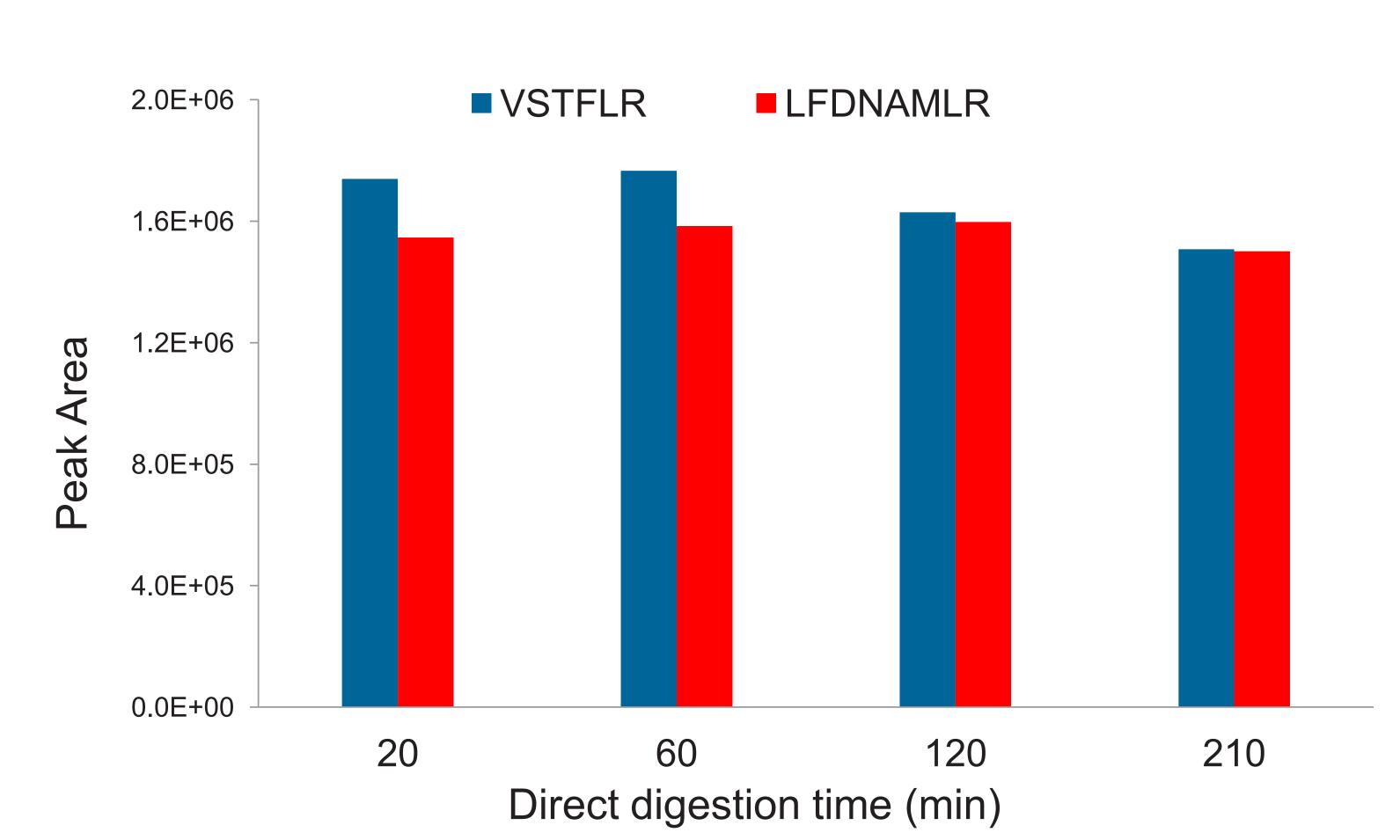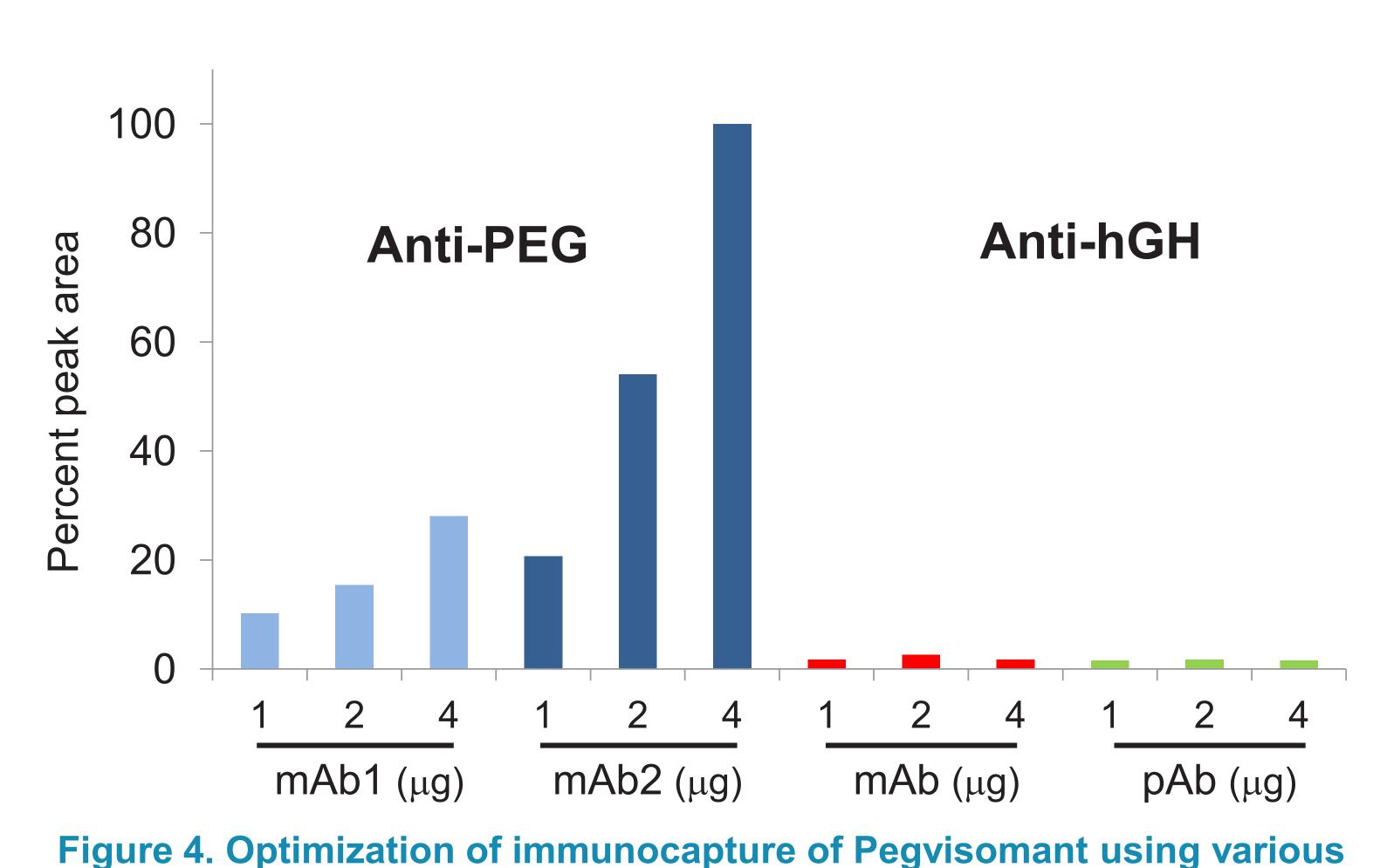

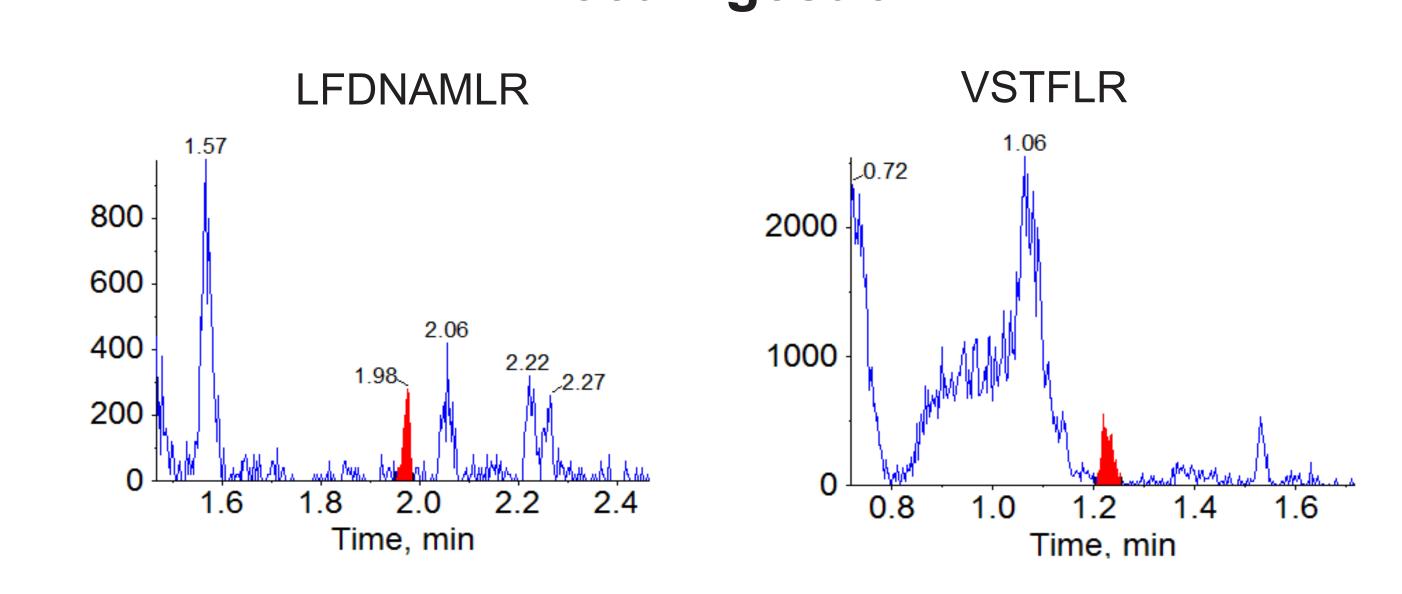Figure 3. Direct digestion kinetics of Pegvisomant using porcine trypsin

For the immunocapture approach, higher extraction efficiency is obtained using anti-PEG antibodies compared to anti-hGH (Figure 4).

Figure 4. Optimization of immunocapture of Pegvisomant using various anti-PEG and anti-hGH antibodies

Significant increase in sensitivity is achieved using the immunocapture approach with anti-PEG mAb for Pegvisomant extraction of only 10 μL of rat plasma compared to 25 μL using the direct digestion approach (Figure 5).
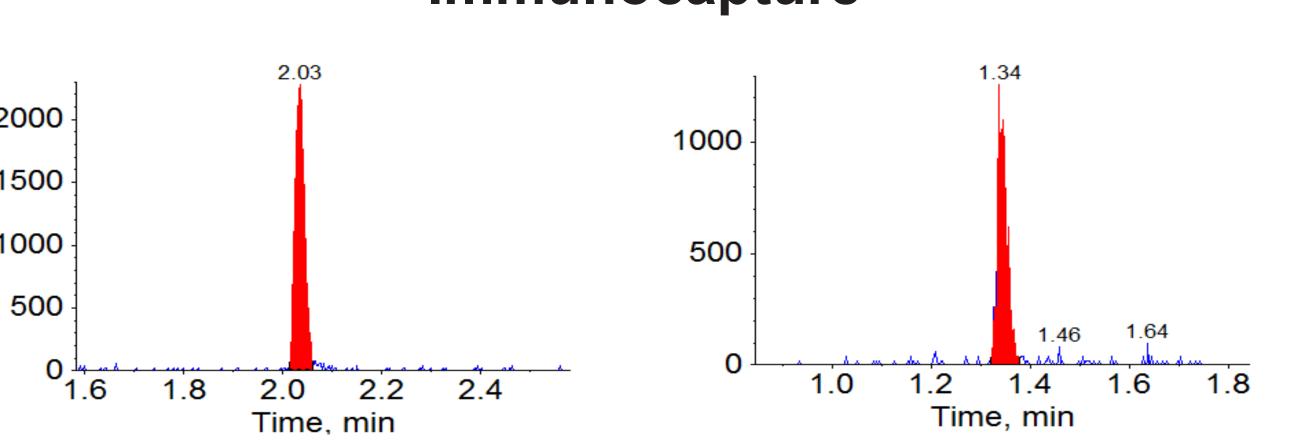

Figure 5. Chromatograms of extracted LLOQ (25 ng/mL) for Pegvisomant in Rat plasma using direct digestion (top) and anti-PEG immunocapture (bottom)

Samples extracted using immunocapture show excellent linearity and precision in the analytical range (25 - 10,000 ng/mL) (Figure 6).
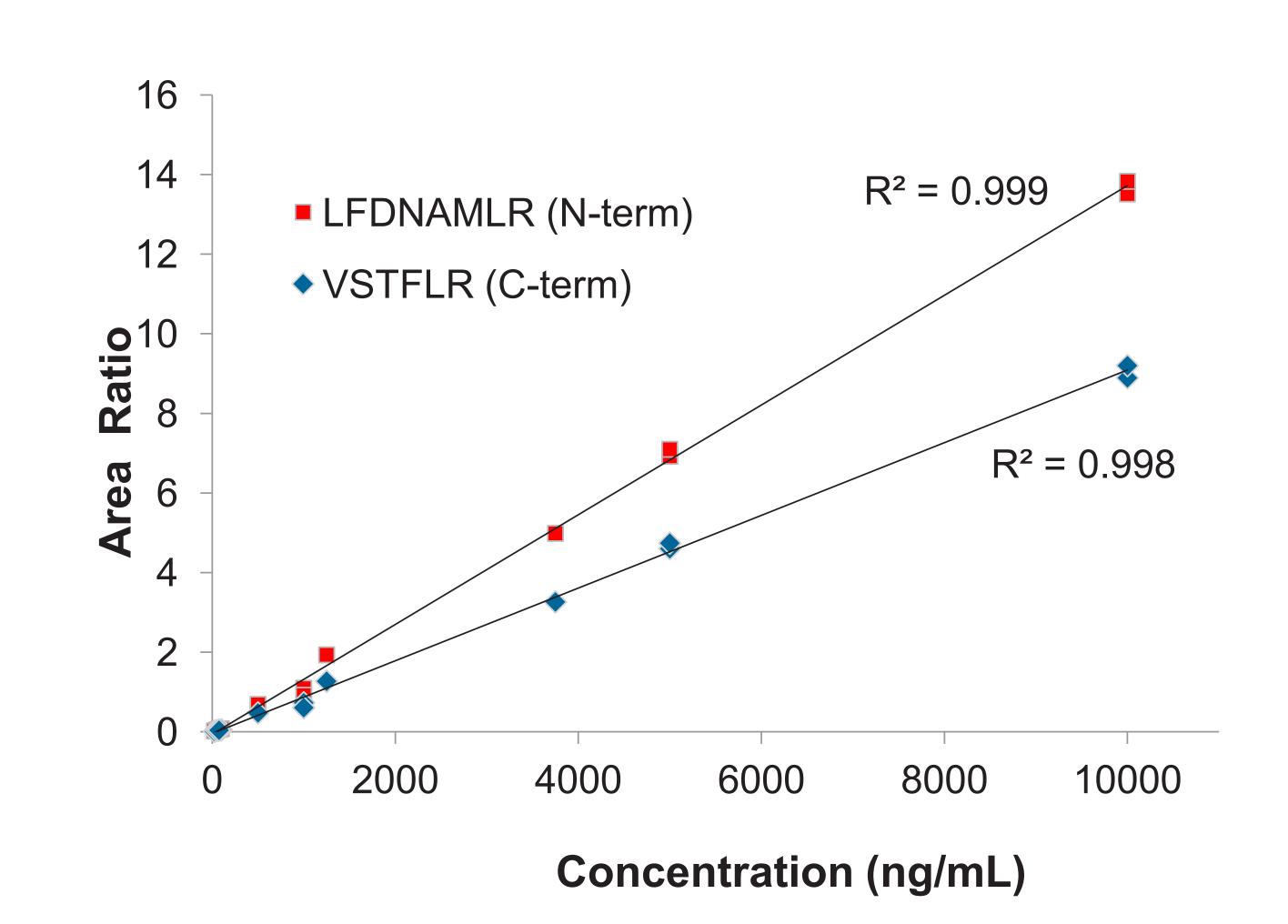

Figure 6. Calibration curves of Pegvisomant from 25 ng/mL to 10,000 ng/mL in rat plasma using the immunocapture approach

PK APPLICATION

Concentrations of Pegvisomant in PK samples (Table 2) generated using either N-term or C-term peptide show good agreement, confirming the structural integrity of the PEGylated protein (Figure 7).
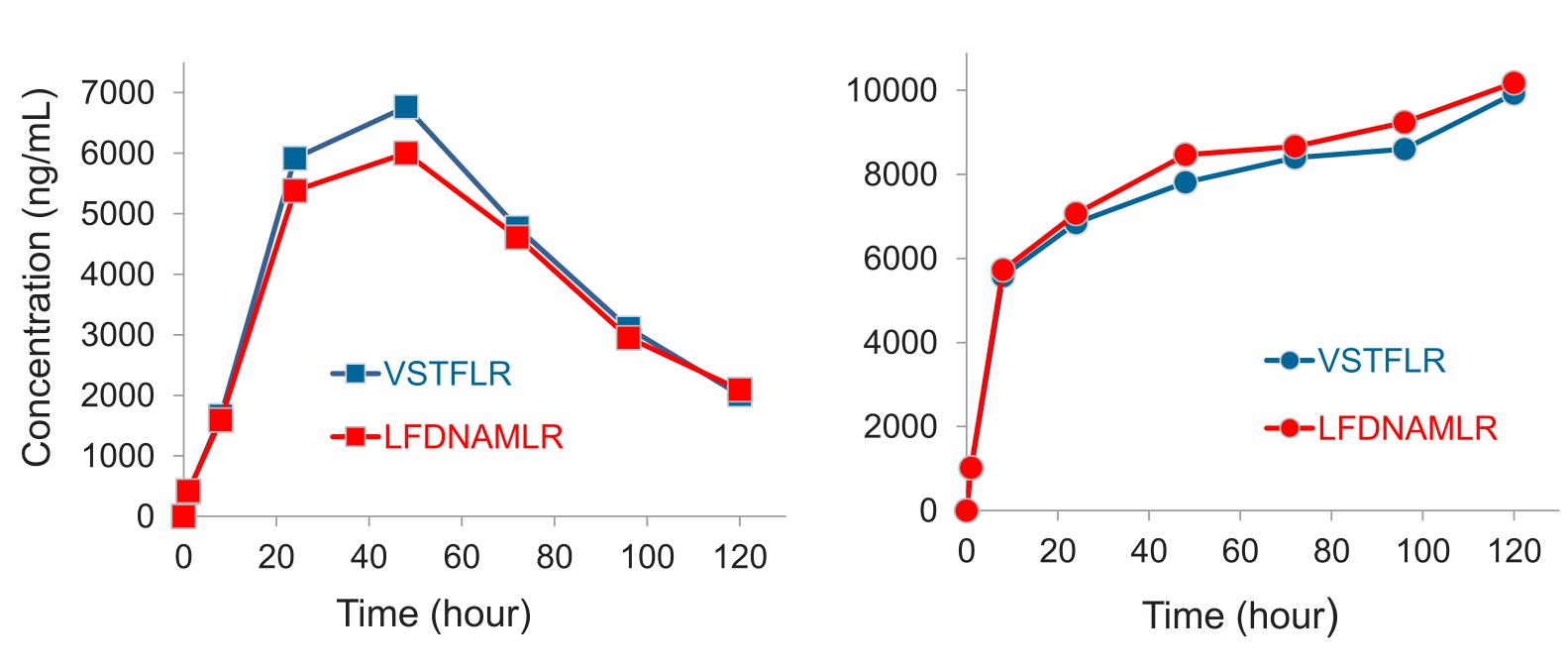

Figure 7. PK profiles of Pegvisomant in Sprague Dawley rat (n=4) (left) and Beagle dog (n=1) (right) following single SC dose at 2.0 mg/kg

Table 2. Pegvisomant Conc. following SC 2 mg/kg dose in rat and dog

Conc. (ng/mL)	Time (hr)						
	1	8	24	48	72	96	120
VSTFLR_Rat	388	1663	5914	6764	4768	3108	2009
LFDNAMLR_Rat	418	1592	5379	5999	4608	2949	2093
Difference (%)	+7.4	-4.4	-9.5	-12.0	-3.4	-5.2	+4.1
VSTFLR_Dog	999	5576	6843	7811	8398	8601	9923
LFDNAMLR_Dog	1019	5726	7067	8466	8665	9238	10177
Difference (%)	+2.0	+2.7	+3.2	+8.0	+3.1	+7.1	+2.5

CONCLUSION

The strategic selection of multiple surrogate peptides for protein quantitation can help address some of the issues facing the bottom-up bioanalysis of proteins by LC-MS and in this case, ensure the structural integrity of Pegvisomant in different biological matrices. Furthermore, this study highlights the advantages of immunocapture LC-MS compared to the direct digestion approach for the quantitation of biotherapeutics.

REFERENCES

Trainer et al. Treatment of acromegaly with the growth hormone-receptor agonist pegvisomant. N Engl J Med 2000; 342:1171-7.