Development and Qualification of a Sensitive and Robust Immunoaffinity UPLC-MRM MS Method for Quantitative Measurement of Angiopoietin-1 in Monkey Plasma

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Overview

A high throughput immunoaffinity UPLC-MRM MS assay has been developed to accurately and specifically quantify ANGPT1 in monkey plasma.

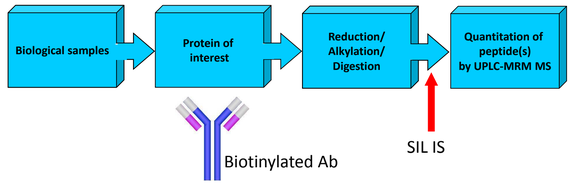
Introduction

Angiopoietin-1 (ANGPT1) belongs to a family of vascular growth factors. As a secreted protein ligand of tyrosine kinase receptor Tie2, it is involved in many biological events, including angiogenesis, endothelial cell survival and proliferation, maintenance of vascular quiescence, and prevention of vascular leakage. Tracking the concentration profile of total ANGPT1 targeted as a biomarker during therapy provides an important contribution to understanding PK/PD relationship both pre-clinically and clinically. However, it is a very challenging task due to its low abundance in animal plasma. In this study, an immunoaffinity UPLC-MRM MS assay was developed and qualified to accurately and specifically quantify the amount of ANGPT1 in monkey plasma.

Methods

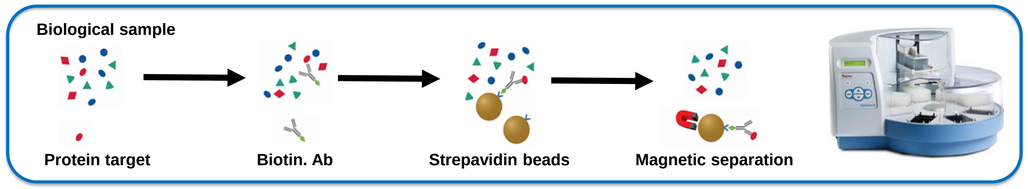
Standards, quality controls, and monkey plasma samples (100 µL) were each aliquoted into a 96-well plate and diluted with BSA-PBST buffer. Immuno-enrichment was performed by incubating the samples with biotinylated ANGPT1 antibody and Dynabeads® (MyOne™ Streptavidin T1). The antigen-antibody-linked Dynabeads® were washed with PBS-Chaps buffer, followed by elution with 150 µL 25mM HCl in water containing stable isotope-labeled peptide internal standards. The samples were then neutralized to pH=8.0 and subject to reduction, alkylation, and tryptic digestion. The resultant digests were acidified and then separated with a Waters BEH C18 2.1x100 mm column using a Waters Acquity UPLC system. The eluted peptides were detected using an AB SCIEX API 5000 mass spectrometer.

Workflow and Instrument



Waters ACQUITY UPLC

Sciex API5000



Results

Table 1. Standard Statistics

Sample Name	Target Conc. (ng/mL)	Number of Values Used	Measured Conc. (ng/mL)	Accuracy
ANGPT1_STD1	0.0651	1 of 1	0.066	101.53
ANGPT1_STD2	0.13	0 of 1	N/A	N/A
ANGPT1_STD3	0.26	1 of 1	0.24	93.19
ANGPT1_STD4	0.52	1 of 1	0.51	97.45
ANGPT1_STD5	1.04	1 of 1	1.12	107.47
ANGPT1_STD6	2.08	1 of 1	2.21	106.43
ANGPT1_STD7	4.17	1 of 1	3.67	87.98
ANGPT1_STD8	8.33	1 of 1	9.30	111.60
ANGPT1_STD9	16.70	1 of 1	15.70	94.03
ANGPT1_STD10	33.30	1 of 1	33.41	100.32
ANGPT1_STD11	66.70	1 of 1	66.86	100.25

Table 2. QC Statistics Results

Sample Name3	Target Conc. (ng/mL)	Measured Conc. (ng/mL)	Accuracy	Standard Deviation	%CV
EB (n=3)	2.52	2.47	98.2	0.16	6.54
LLQC (n=3)	2.65	2.82	106.5	0.032	1.14
LQC (n=3)	3.04	3.42	112.4	0.16	4.78
MQC (n=3)	6.69	7.88	117.9	0.22	2.78
HQC (n=3)	35.90	37.47	104.4	1.33	3.54

Table 3. ANGPT1 Levels in Selected Monkey Plasma

Surrogate Peptide & IS	Q1	Q3	CE
ILEMEGK	410.20	593.26	24
ILEMEG[K(C13, N15)]	414.22	601.30	24

Table 4. Transitions of the Surrogate Peptides of ANGPT1 and ANGPT1-IS on API5000

Sample ID	Measured Conc. (ng/mL)	Comment
Monkey-01	0.319	
Monkey-02	0.0558	BLQ
Monkey-03	0.385	
Monkey-04	0.274	
Monkey-05	0.174	
Monkey-06	0.177	
Monkey-07	0.220	
Monkey-08	0.540	
Monkey-09	0.271	
Monkey-10	0.113	

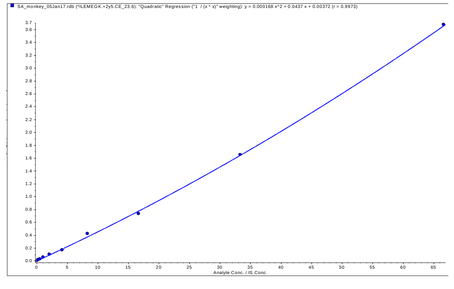


Figure 1. Calibration Curve of ANGPT1 Prepared in Surrogate Matrix (4%BSA in PBST).

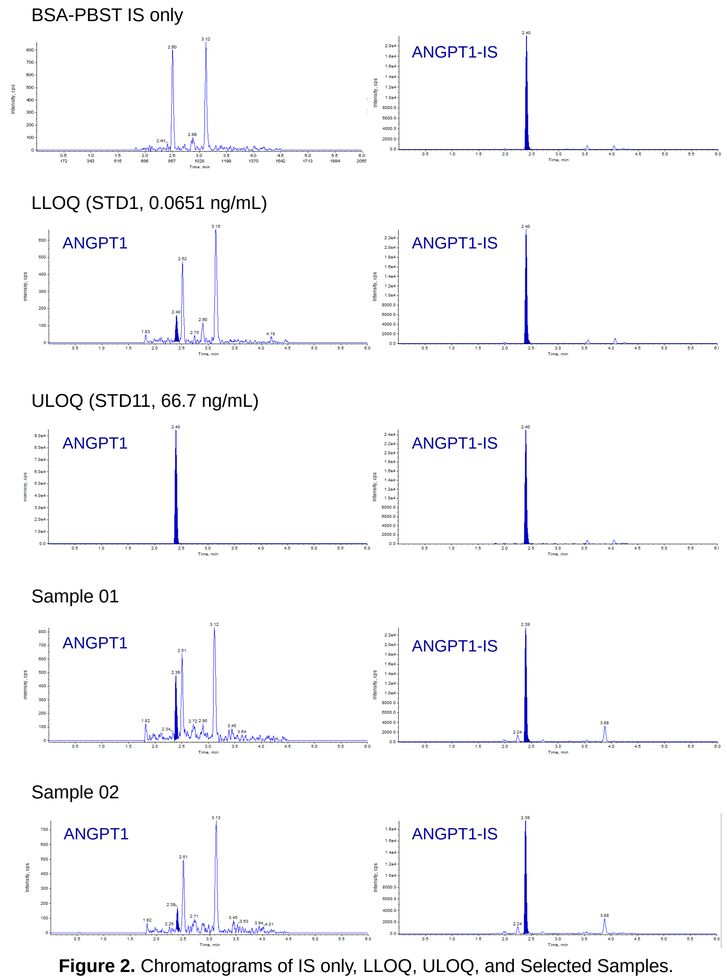


Figure 2. Chromatograms of IS only, LLOQ, ULOQ, and Selected Samples.

Conclusions

- An immunoaffinity UPLC-MRM MS assay with a linear range of 0.0651 ng/mL to 66.7 ng/mL was developed, qualified, and successfully implemented to support pre-clinical studies.
- The immunoaffinity workflow presented here has been used to quantitate more than a dozen protein biomarkers and therapeutics in various matrices at Novabioassays.