Quantification of sBCMA in Human Plasma using a High-Throughput Hybrid IP-MRM Based Mass Spectrometry Workflow

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INTRODUCTION

- B cell maturation antigen (BMCA) is expressed on the surface of normal and malignant plasma cells and is a rational target for treatment of multiple myeloma.
- Multiple emerging therapies including CAR T-cells, antibody drug conjugates (ADC) and bi-specific antibodies are directed at the plasma cell surface BCMA. (1)
- In addition to surface-bound BCMA, a soluble form of the extra-cellular domain (sBCMA) is generated, and released in the blood stream, when cleaved by a γ-secretase (Figure 1).
- sBCMA not only provides information about patient prognosis but can also report on the extent of disease burden following treatment. (2)
- However, measurement of sBCMA with conventional ligand binding methods can present challenges due to potential interferences from endogenous ligands APRIL and BAFF as well as high concentrations of therapeutic antibodies and ADCs.
- Here we present an overview of a high-throughput hybrid immunoaffinity LC-MRM based method for the precise and accurate quantification of sBCMA.

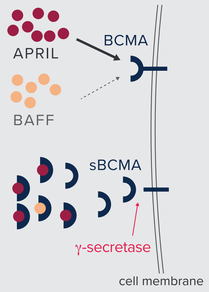


FIGURE 1.
Illustration of membrane bound BCMA cleavage to generate the soluble form and interactions with APRIL and BAFF.(1)

METHODS

ASSAY PROCEDURES

- Samples are processed in a 96 well plate format with an initial 7-fold dilution.
- Soluble BCMA is immunoaffinity enriched and digested to peptides.
- Peptides are then analyzed on a SCIEX QTRAP® 6500 using a microflow setup and 10-minute LC gradient.
- Peptide peak integration is performed using MultiQuant software and a peak area ratio is calculated between the endogenous peptide and the stable isotopically labeled (SIL) peptide standard.

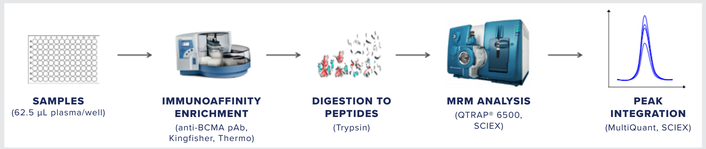


FIGURE 2.
Schematic of the hybrid immunoprecipitation LC-MRM workflow. The assay sensitivity is enhanced with immunoaffinity enrichment and is combined with the specificity of LC-MRM analysis to quantify a specific surrogate peptide of sBCMA.

SAMPLES

EXTERNAL CURVE (N=1)	Surrogate matrix (rat plasma) + sBCMA recombinant protein + SIL peptide (fixed)	STD1 to STDB
QC SAMPLES (N=2)	Surrogate matrix (rat plasma) + sBCMA recombinant protein + SIL peptide (fixed)	QC0 endo QC2 mix QC3 high
STD0 (N=2)	Surrogate matrix (rat plasma) + SIL peptide (fixed)	
BLANKS (N=2)	Rat plasma	

TABLE 1.
Along with 75 study samples, the control samples included in each batch are processed. Rat plasma is used as a surrogate matrix for the calibration curve and QC samples in human plasma matrix are analyzed for each batch of study samples.

RESULTS

QUANTITATIVE DATA ANALYSIS

CONVENTIONAL EXTERNAL CURVE

U.S. Food and Drug Administration
Bioanalytical Method Validation.
Guidance for Industry (2018)

- External calibration curve
- Unknown sample

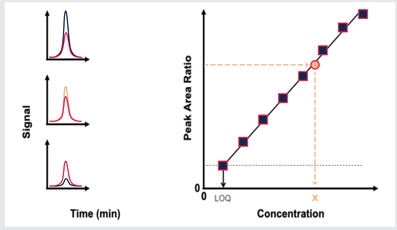


FIGURE 3.
An illustration of the quantitative data analysis. The calibration curve is constructed by plotting the peak area ratio of the light vs. heavy peptides as a function of the recombinant sBCMA protein concentration using a linear regression (weighted $1/x^2$). The measured peak area ratios of the study samples are compared to the calibration curve to determine the sample concentration.

PRECISION AND ACCURACY

	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
NOMINAL	**1.00**	**2.00**	**10.00**	**80.00**	**250.00**	**500.00**	**800.00**	**1000.00**
Run 1	1.04	1.92	8.45	75.60	268.40	544.00	809.00	1041.90
% Bias	3.60%	-4.20%	-15.50%	-5.50%	7.40%	8.80%	1.10%	4.2%
Run 2	1.08	1.69	6.421*	70.80	238.40	565.40	817.40	1084.5
% Bias	7.80%	-15.40%	N/Ap	-11.50%	-4.60%	13.10%	2.20%	8.5%
Run 3	1.05	1.87	8.38	74.90	264.00	530.20	878.20	1027.7
% Bias	4.90%	-6.60%	-16.20%	-6.40%	5.60%	6.00%	9.80%	2.8%
Inter-run % CV	2.00%	6.50%	0.60%	3.50%	6.30%	3.20%	4.50%	2.8%
Inter-run % Bias	5.40%	-8.70%	-15.80%	-7.80%	2.80%	9.30%	4.40%	5.1%

* Sample Rejected From Calibration Curve

TABLE 2.
Data from the intra- and inter-run precision and accuracy experiments. Across all three runs the assay shows high precision on the calibration data with inter-run CV ≤ 6.5% and bias < 20%.

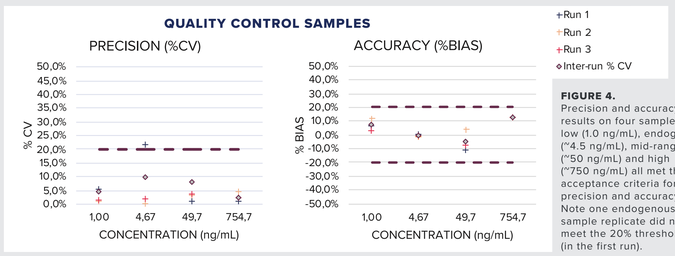


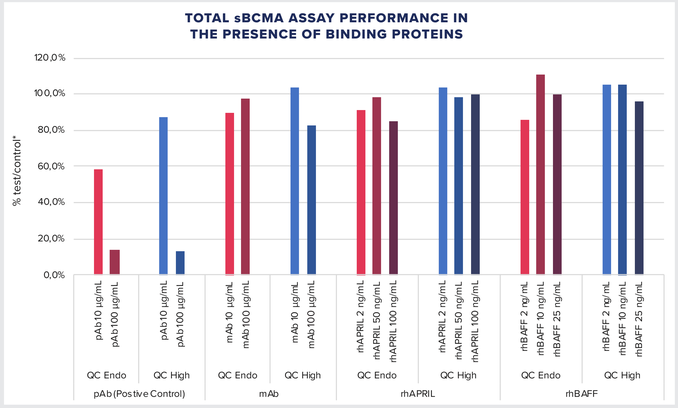
FIGURE 4.
Precision and accuracy results on four samples at low (1.0 ng/mL), endogenous (~4.5 ng/mL), mid-range (~50 ng/mL) and high (~750 ng/mL) all met the acceptance criteria for precision and accuracy. Note one endogenous QC sample replicate did not meet the 20% threshold (in the first run).

SELECTIVITY

	ENDOGENOUS	SPIKE 45 ng/mL			SPIKE 750 ng/mL		
SAMPLE DESCRIPTION	**Concentration (ng/mL)**	**Nominal Concentration (ng/mL)**	**%CV**	**%Bias**	**Nominal Concentration (ng/mL)**	**%CV**	**%Bias**
Plasma Healthy Donor 1	3.61	49.34	6.4%	1.5%	833.61	2.2%	10.6%
Plasma Healthy Donor 2	5.31	49.37	4.0%	-1.9%	804.51	2.8%	6.5%
Plasma Healthy Donor 3	4.30	44.90	11.4%	-8.9%	798.99	4.7%	5.9%
Plasma Healthy Donor 4	4.67	47.14	3.5%	-5.1%	809.02	4.7%	7.2%
Pooled Serum	4.76	48.57	0.5%	-2.4%	816.57	3.7%	8.2%
Visually Lipemic Plasma	3.50	47.75	0.6%	-1.6%	825.99	5.4%	9.6%
Hemolyzed Plasma (1%)	4.61	51.56	0.6%	3.9%	870.74	7.2%	15.4%
Hemolyzed Plasma (2%)	5.87	47.25	1.8%	-7.1%	812.39	1.1%	7.5%

TABLE 3.
Selectivity was tested to determine the role of hemolysis and lipemia on sBCMA spiked at mid- (45 ng/mL) and high- (750 ng/mL) levels. Hemolysis was tested by spiking in artificially hemolyzed blood at 1% and 2%. All samples, at both low and high levels, passed acceptance criteria (%CV and %Bias <20%).

INTERFERENCE TESTING



* Peak area ratio of treated/Peaked area ratio of control x 100

FIGURE 5.
Quantitative analysis of sBCMA binding partner interference. The interference was assessed as the peak area ratio relative to the control sample (*Peak Area Ratio (Treated) / Peak Area Ratio (Control) X 100*%). Acceptance criteria require that samples are within 20% of the control condition.

- Endogenous QC (3-5 ng/mL) and QC high (750 ng/mL) are tested for an assessment of interference by BCMA binding partners.
- As a positive control, the addition of polyclonal immunoprecipitation antibody was evaluated, and interference was shown to occur both at 10 µg/mL and 100 µg/mL. The precipitation was significantly inhibited at 100 µg/mL with <20% of the sBCMA enriched.
- The commercially sourced monoclonal was not a significant source of interference with all measurements within the 20% acceptable measurement bias.
- Similarly, both APRIL and BAFF were tested at three concentrations and neither showed interference with the sBCMA measurement relative to the control sample.

CONCLUSION

- A high-throughput hybrid immunoprecipitation - mass spectrometry (MRM) based assay has been developed for **quantitation of sBCMA in human plasma with ~3-5 day turnaround time**.
- The assay has a **linear range from 1 ng/mL to 1000 ng/mL**, which covers the endogenous levels of sBCMA in plasma from normal individuals and subjects with multiple myeloma.
- The assay is notably robust to interference from both APRIL and BAFF as well as high concentrations of anti-BCMA antibody.
- This assay may be analytically validated and deployed for clinical studies based on the intended use of the data.

REFERENCES.

1. Cho *et al. Front Immunol* 2018; 9:1821.
2. Ghermezi *et al. Haematologica* 2017; 102(4): 785–795.