Application of an LC-MS/MS Combined Protein Precipitation and Phospholipid Removal Extraction for the Determination of Endogenous Arginine and Ornithine in Human Plasma

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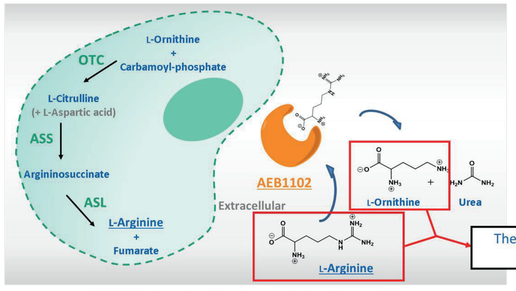
1 Introduction

AEB1102 is a pegylated enzyme derived from human Arginase I with potential therapeutic uses for arginine degradation in people with cancer or Arginase I deficiency. As part of Aeglea Biotherapeutics' clinical development of AEB1102, arginine and ornithine are measured as efficacy and safety biomarkers using a liquid chromatography tandem mass spectrometry (LC-MS/MS) method. The stabilization of arginine and ornithine during sample collection, sample storage, and sample extraction presents a significant challenge to analysis. Additionally, the correction for endogenous amounts of ornithine in matrix complicates quantitation efforts. By overcoming these hurdles it is possible to accurately monitor the amino acid response in patients. This provides the ability to ensure levels are within physiological safety margins.

AEB1102 mechanism of action



AEB1102 degrades L-Arginine to L-Ornithine and Urea

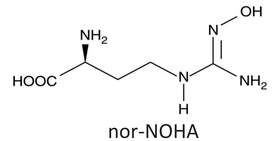
Figure 1. AEB1102 is a modified enzyme from human Arginase I. The modifications improve stability in vivo and degrades arginine to ornithine.

2 Methods

- The analytical method is conducted on wet ice and uses combined protein precipitation extraction with phospholipid removal in a 96-well plate format.
- Requires 25 µL of human plasma containing nor-NOHA and acid for stabilization.

Instrument Parameters

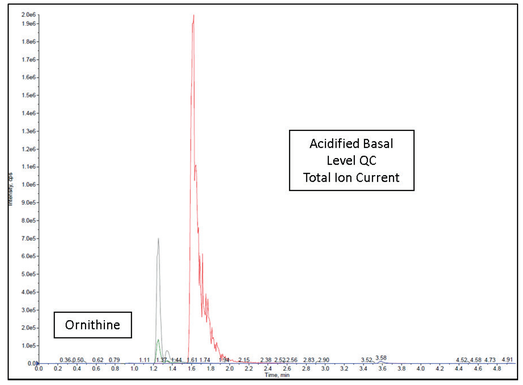
LC Conditions	
Parameter:	
LC System:	Shimadzu Nexera®
Column:	Agilent Zorbax Solvent Saver RRHT SB-CN, 3.0 x 100 mm, 1.8 µm particle size
Mobile Phase A:	10 mM Ammonium Acetate in Milli-Q Water
Mobile Phase B:	5 mM Ammonium Acetate in 10:90 (v/v) Milli-Q Water:Acetonitrile
Sample Temperature:	4°C
Column Temperature:	50°C
MS Conditions	
MS System:	API 5500
Ionization Mode:	ESI Positive Mode
IS Voltage:	5500
Temperature:	600°C



nor-NOHA
(N^{ω}-Hydroxy-nor-L-arginine)

Figure 2. Clinical samples are collected into tubes containing an arginase inhibitor, nor-NOHA (N^{ω}-Hydroxy-nor-L-arginine), to prevent further degradation of arginine as a result of AEB1102 presence in blood after collection.

3 Results



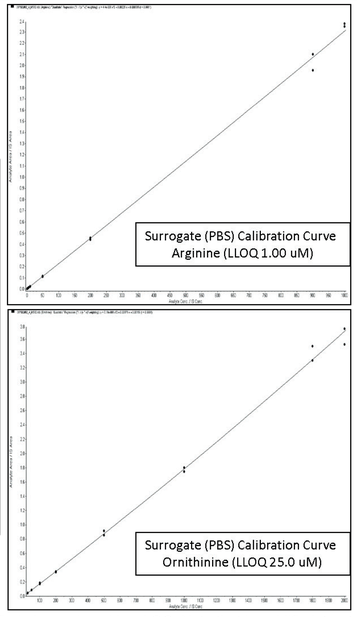


Figure 3. To the left is a TIC of arginine, ornithine, and internal standards in an acidified plasma basal level QC. Note that in acidified, treated plasma there is no endogenous arginine detectable, therefore no correction is needed when preparing QC samples. To the right are surrogate (phosphate-buffered saline) calibration lines for arginine and ornithine from validation.

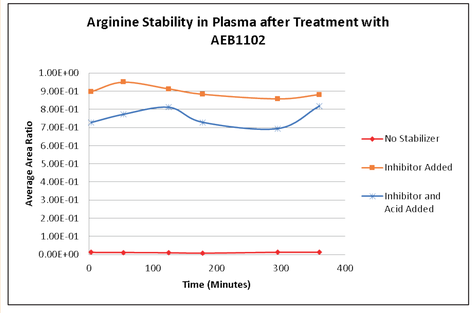


Figure 4. Stability plot of arginine in the presence of AEB1102 with no additives, with nor-NOHA only, and with nor-NOHA and acid. Arginine is unstable in plasma containing AEB1102. After the addition of nor-NOHA it was shown to be stable with and without acid.

Arginine Freeze-Thaw Stability

		Non-acidified Plasma			Acidified Plasma	
Number of FT Cycles		1	2	3	1	2
Nominal Concentration		5.15	5.15	5.15	4.43	4.43
Replicate						
	1	5.64	6.36	6.67	4.40	4.31
	2	6.10	5.79	6.18	4.63	4.47
	3	5.79	5.92	6.41	4.35	4.37
Average		5.84	6.02	6.42	4.46	4.38
% RE		13.46	16.96	24.66	0.68	-1.05
% RSD		4.01	4.96	3.82	3.35	1.84

Acidification of Samples Upon Thawing is required to achieve Freeze-Thaw Stability

Figure 5. Stability in non-acidified plasma was only achieved at -70°C and a single freeze-thaw cycle. Glacial acetic acid is added to plasma samples prior to thaw and is mixed into the sample at point-of-thaw, to stabilize arginine and ornithine for analysis, subsequent freeze-thaw cycles, and long-term storage at -70°C.

LC-MS/MS Assay and Performance Summary

	Arginine	Ornithine
LLOQ (µM)	1.00	25.0
Range (µM)	1.00 – 1,000	25.0 – 2,000
Intra-day % RE (%CV) at LLOQ (Unique QC Pools)		0.7 – 5.8 (2.6 – 19.6)
Inter-day % RE (%CV) at LLOQ (n=22)	+18.0 (19.9)	
Inter-day % RE (%CV) Range for Surrogate Calibration Standards	-2.4 – 4.0 (4.4 – 8.4)	-1.2 – 1.0 (2.1 – 5.9)

Figure 6. The method for arginine and ornithine was validated according to guidelines for bioanalytical method validation (FDA 2001 and EMA BMV 2011). All testing results demonstrated passing criteria, allowing the method's use in supporting clinical development of AEB1102. Nominal concentrations of QCs were corrected for endogenous amino acids when applicable (measured separately for each QC pool).

4 Clinical Results

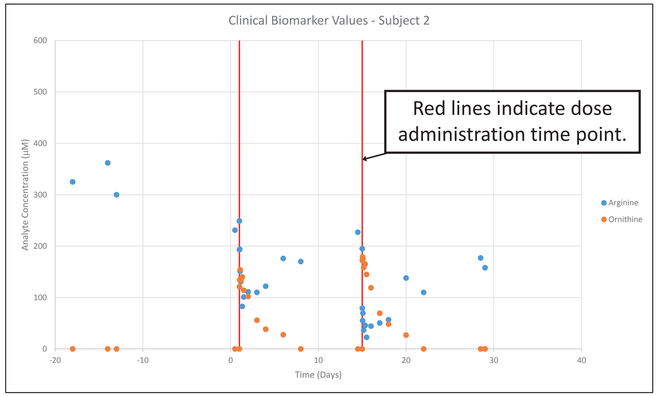


Figure 7. The resulting assay accurately quantitates arginine and ornithine levels in human plasma samples from clinical trials. Proof of concept was observed in clinical patients wherein arginine and ornithine responded in a dose dependent manner to AEB1102 treatment. Prior to dose the average arginine levels are >400 µM and ornithine levels are below the limit of quantitation (BLQ) of 25.0 µM. After administration the concentrations of arginine decrease and the ornithine levels increase due to arginine degrading to ornithine.

5 Conclusions

A method for arginine and ornithine was applied for analysis of clinical study samples in Phase I studies as part of clinical development of AEB1102 (work is ongoing). Acidification at the analysis site prior to thaw and analysis eliminates the need for acidification at the clinical trial site, simplifying plasma processing at hospitals. Bulk quality control preparation prior to analysis, including endogenous correction, aids fast sample throughput providing hospitals information quickly for interpretation of safety assessment data.