Method Optimization for Enhanced Quantification of CGRP in Human CSF by IA-LC-MS/MS to Support a Target Engagement Study with Fremanezumab

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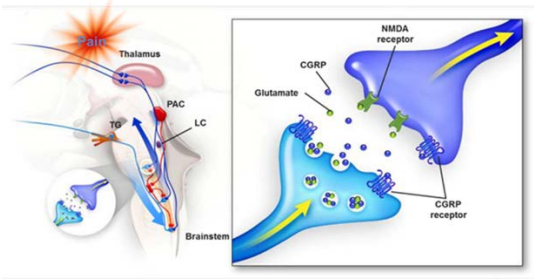
Introduction

Calcitonin gene-related peptide (CGRP) plays an important role in the pathophysiology of migraine. This neuropeptide is a well-validated target for migraine therapy. Fremanezumab (anti-CGRP monoclonal antibody) is effective in the prevention of migraine. Although it is administered peripherally, the question of a potential site of action within the central nervous system remains unresolved. In a study conducted in healthy volunteers, a bioanalytical method for detecting and quantifying CGRP in human cerebrospinal fluid (CSF) was deemed necessary to evaluate the target engagement (TE) of the antibody medicine to CGRP. Considering that the CGRP concentrations are expected to be at trace levels in CSF from healthy subjects, a bioanalytical assay with superior sensitivity was developed and optimized.

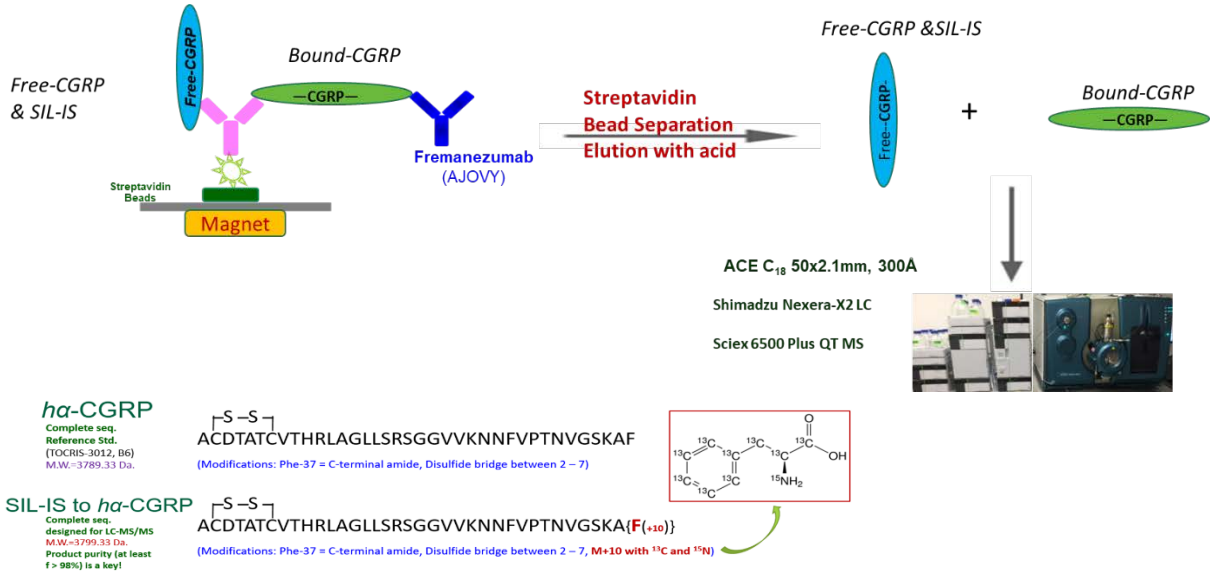
Teva's AJOVY was approved by FDA in Sept. 2018

- Fremanezumab (AJOVY) is humanized monoclonal antibody for the treatment of Migraine
- Potently and selectively binds CGRP to prevent its interaction with the CGRP receptor



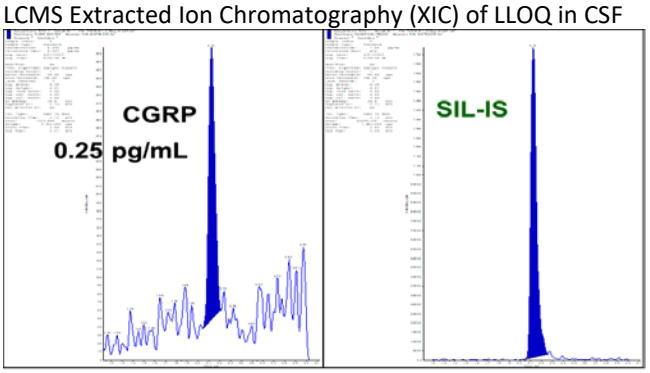
Methods

A 400-µL CSF sample was enriched by immunoaffinity (IA) capture using a biotinylated anti-CGRP antibody-coupled-streptavidin magnetic beads. The beads were then washed several times, followed by elution with acid. The final eluent was separated on an analytical column (ACE C18, 3µ, 300Å, 2.1 x 50 mm) with a gradient mobile phase elution (acetonitrile/water with 0.1% acetic acid). A Sciex 6500 Plus QTrap mass spectrometer (MS) was set to positive electrospray ionization (+ESI) with multiple reaction monitoring (MRM) modes to detect both CGRP analyte and its stable isotope-labeled internal standard (SILIS). Assay performance parameters (including accuracy, precision, matrix selectivity and analyte stability) were evaluated during bioanalytical method qualification.

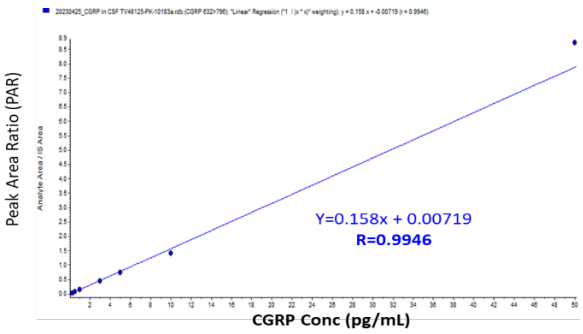


Results

LCMS Extracted Ion Chromatography (XIC) of LLOQ in CSF



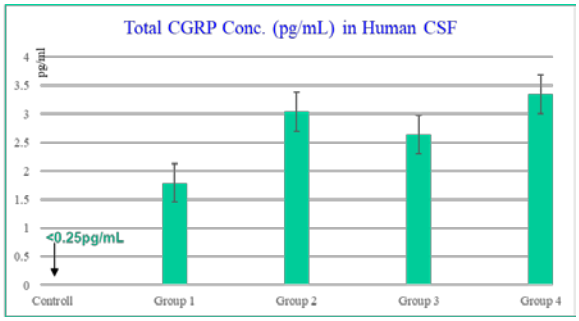
Calibration Curve of Analytical Run in Human CSF

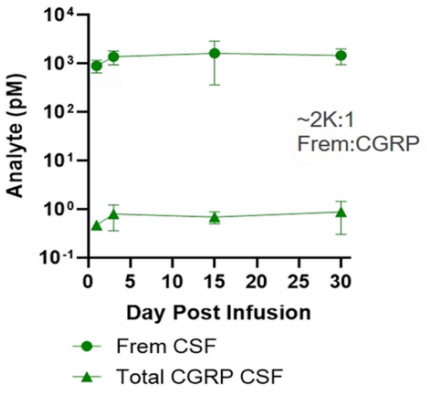


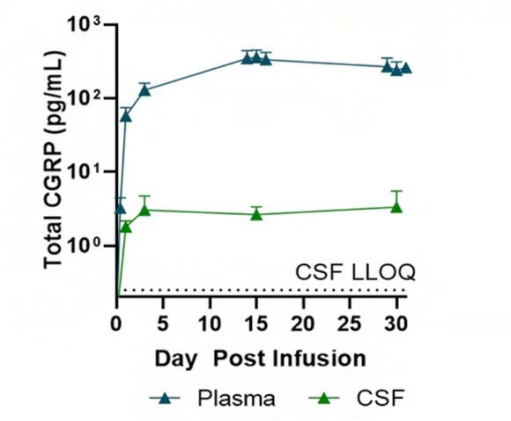
Summary of IA-LCMS Method Qualification of CGRP in CSF

Parameter	Target Acceptance Criteria	Observed Result
Standard Curve	Accuracy: %Bias within ±20%; within ±25% at LLOQ	Accuracy: 86.9 to 119%
	Precision: Inter-assay %Coefficient of Variation (CV) ≤20%; %CV ≤25% at LLOQ	Inter-assay precision: 2.71 to 12.7%
	Curve Range: 0.25 pg/mL ~ 50 pg/mL	LLOQ: 0.25pg/ml and ULOQ: 50 pg/mL in a surogate matrix
Quality Control (QC) Samples	Accuracy: %Bias within ±20%	Inter-assay accuracy: 83.9 to 106%
		Intra-assay accuracy: 88.2 to 99.7%
	Precision: %CV ≤20%	Inter-assay precision: 7.05 to 10.8%
		Intra-assay precision: 3.74 to 13.1%
Sensitivity	Study demand 0.25pg/mL	0.25 pg/mL in CSF

CGRP Mean Conc. in Human CSF







Conclusions

The improved IA-LC-MS/MS assay for determination of α-CGRP in human CSF was developed with a calibration range from 0.25 to 50 pg/mL. The sensitivity of the method was confirmed to be 0.25 pg/mL in CSF matrix. A similar CGRP assay in human plasma was also qualified with a dynamic standard range of 2 to 500 pg/mL.

All human CSF and plasma samples from TV48125-PK-10183 study were analyzed. Levels of α-CGRP were quantifiable in the CSF samples at 24 hr after infusion of fremanezumab and sustained through 30 days, with a concentration range of 1.79 to 3.35 pg/mL. The corresponding α-CGRP levels in plasma samples came out to be from 47.4 to 479 pg/mL.

The results of the TV48125-PK-10183 study demonstrated that fremanezumab can potentially access the CSF and can bind to its target CGRP.