

Soluble APP β and sAPP α Kinetic Curve Results are improved with utilization of a novel nanoflow LC-MS Platform



Justyna Dobrowolska Zakaria¹; Helena Svobodova²; Gary Valaskovic²; Amanda Berg²; Robert J. Vassar¹
¹Northwestern University Feinberg School of Medicine, Chicago, IL; ²New Objective Inc, Littleton, MA



Background

Stable Isotope Labeling Kinetic (SILK) studies provide a powerful technique in assessing turnover of proteins implicated in disease. Infusion of an amino acid that has nonradioactive isotopes of carbon or nitrogen, will allow for these labeled amino acids to be incorporated in proteins through translation. Quantitation of labeled proteins establishes production & clearance rates of proteins *in vivo* in healthy and diseased states using a combination of immunoprecipitation (IP) and nanoLC/MS approaches. Using this method, we have previously determined that there is a decreased clearance of sAPP β in cerebrospinal fluid (CSF) in Alzheimer's disease (AD).

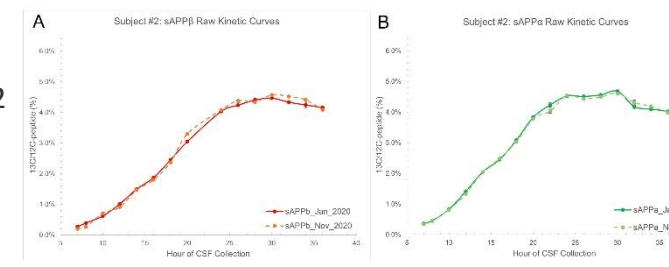
Research Objectives

We intend to measure other AD-relevant (albeit less abundant than sAPP β) proteins' metabolism in CSF; therefore, improved sensitivity and reproducibility of samples run on MS are integral to accurate and precise quantitation. To this end, we evaluated performance of two different version of New Objective PicoChip columns having different chemistries, bed lengths, and operating temperature while monitoring the mid-domain APP peptide VESLEQEAANER.

Results

Proteins' kinetic curves for samples tested on this set-up 10 mo apart using 2 analytical 10 cm columns (15 μ m tip x 75 μ m ID) packed with 3 μ m C18 without application of heat are highly reproducible.

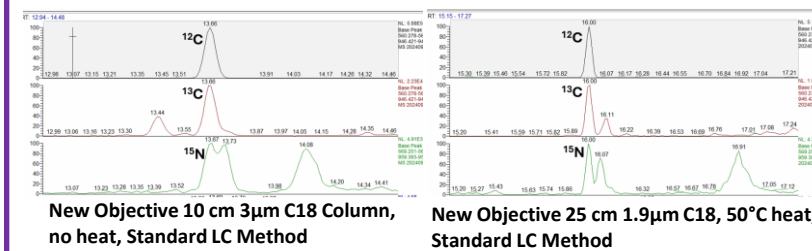
Low mean Kinetic curve CV representative of a robust analytical workflow.



Kinetic Curve Datapoints	Mean CV (%)	Min CV (%)	Max CV (%)	Mean Stdev (%)	Min Stdev (%)	Max Stdev (%)
sAPP β (all hours)	6.0%	0.7%	28.3%	0.07%	0.03%	0.15%
sAPP β (excluding h7&8)	3.1%	0.7%	9.2%	0.08%	0.03%	0.15%
sAPP α (all hours)	1.9%	0.0%	8.0%	0.05%	0.00%	0.14%
sAPP α (excluding h7&8)	1.8%	0.0%	8.0%	0.05%	0.00%	0.14%

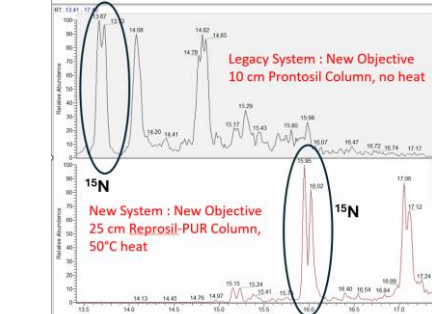
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Comparison of chromatograms depicting 3 monitored peptides



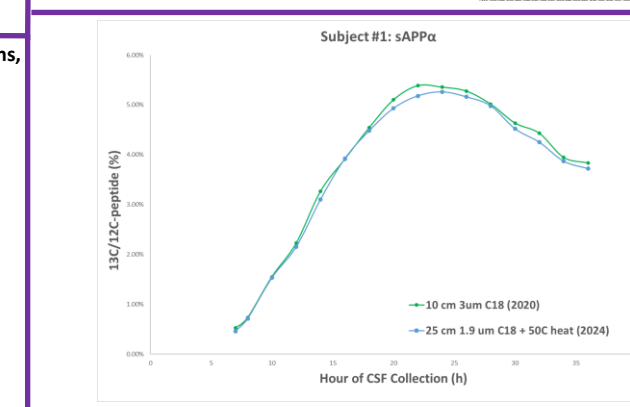
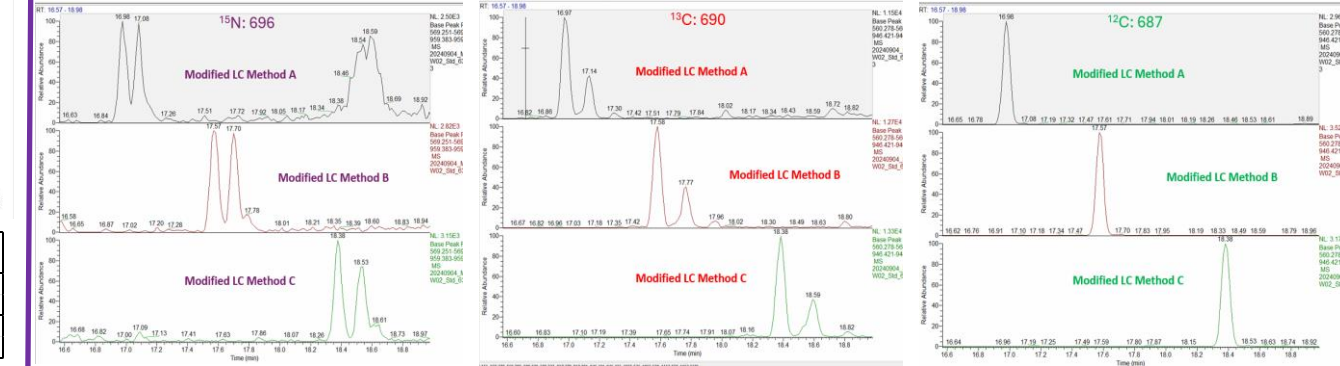
New Objective 10 cm 3 μ m C18 Column, no heat, Standard LC Method
 New Objective 25 cm 1.9 μ m C18, 50°C heat, Standard LC Method

¹⁵N peptide evaluated on 2 New Objective Columns, Standard LC Method



Legacy System : New Objective 10 cm ProntoSil Column, no heat
 New System : New Objective 25 cm Reprasil-PUR Column, 50°C heat

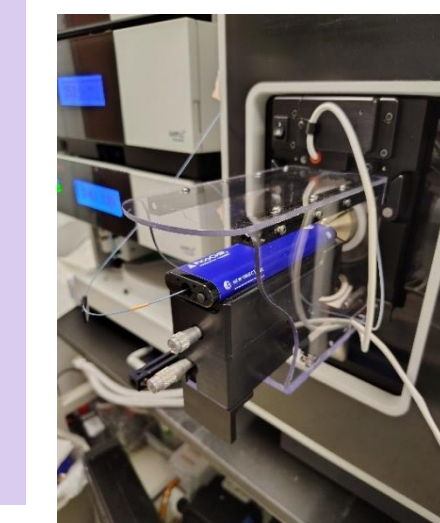
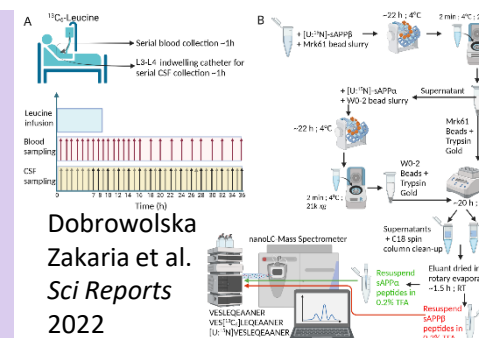
Testing varying LC Methods on the New Objective 25 cm 1.9 μ m C18, 50°C heat



Thirty-six hour kinetic curve of human sAPP α utilizing 10 cm 3 μ m C18 vs. 25 cm 1.9 μ m C18, 50°C heat.

Methods

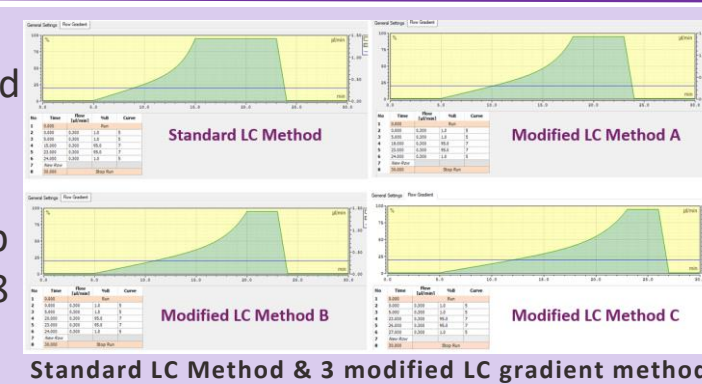
- ¹³C₆-Leucine IV infusion into human subject & CSF/ blood collection over 36h
- Immunoprecipitation of AD-related Proteins, digest & analysis by UltiMate 3000 RS nanoLC-TSQ Altis (Thermo Fisher Scientific) equipped with nanospray ionization (NSI) source.
- New Objective's 10 cm PicoChip analytical column packed with 3 μ m C18 was used at room temperature (75 μ m ID, 15 μ m emitter).



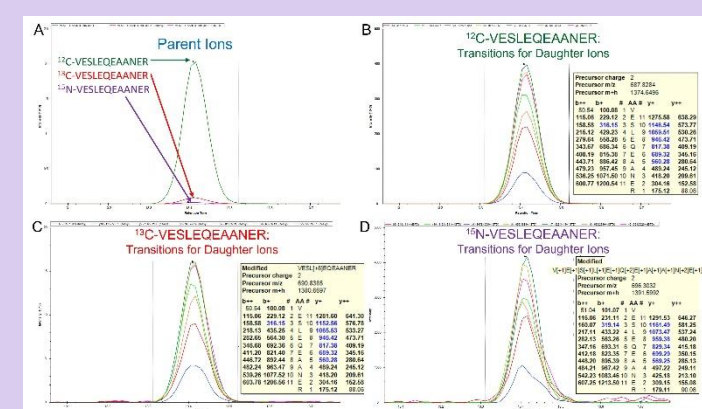
- nanoLC Flow Gradient Method
- Evaluate the performance of New Objective's 25 cm PicoChip column, packed with 1.9 μ m C18 with heat applied at 50°C.

Compound	Start Time (min)	End Time (min)	Retention (min)	Product (m/z)	Collision Energy (eV)
1 VESLEQEAANER-12C	10	18	687.828	376.15	30
2 VESLEQEAANER-12C	10	18	687.828	560.279	25
3 VESLEQEAANER-12C	10	18	687.828	689.321	25
4 VESLEQEAANER-12C	10	18	687.828	817.38	24
5 VESLEQEAANER-12C	10	18	687.828	946.422	24
6 VESLEQEAANER-12C	10	18	687.828	1075.507	25
7 VESLEQEAANER-12C	10	18	687.828	1146.539	23
8 VESLEQEAANER-13C	10	18	690.838	376.15	30
9 VESLEQEAANER-13C	10	18	690.838	560.279	25
10 VESLEQEAANER-13C	10	18	690.838	689.321	25
11 VESLEQEAANER-13C	10	18	690.838	817.38	24
12 VESLEQEAANER-13C	10	18	690.838	946.422	24
13 VESLEQEAANER-13C	10	18	690.838	1065.527	25
14 VESLEQEAANER-13C	10	18	690.838	1152.559	23
15 VESLEQEAANER-15N	10	18	696.303	376.151	30
16 VESLEQEAANER-15N	10	18	696.303	569.252	25
17 VESLEQEAANER-15N	10	18	696.303	699.292	25
18 VESLEQEAANER-15N	10	18	696.303	829.344	24
19 VESLEQEAANER-15N	10	18	696.303	959.384	24
20 VESLEQEAANER-15N	10	18	696.303	1073.465	25
21 VESLEQEAANER-15N	10	18	696.303	1167.494	23

SRM transitions on TSQ Altis



Standard LC Method & 3 modified LC gradient methods



SICs for Representative Peptide SRMs
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Conclusions & Future Directions

- 25 cm 1.9 μ m C18 PicoChip column at 50°C provides better separation of peaks of interest (particularly ¹⁵N and ¹³C peptides) than 10 cm 3 μ m PicoChip column with no heat.
- A more shallow LC gradient further promotes peak separation.
- A 36h human CSF sample time course run 4 years apart under the 2 conditions, shows improvement in the smoothness of the kinetic curve for sAPP α using 25 cm heated PicoChip column, with modified LC method C, because of less noise resulting from minimizing contaminating peaks.
- We plan to repeat our testing of these 2 conditions using additional subjects' 36h time course CSF.
- Evaluate a 10 cm 1.9 μ m C18 PicoChip column at 50°C.

Acknowledgments

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