QUANTITATION OF Aβ42

Introduction

Amyloid-Beta peptides are important research tools to further our understanding of Alzheimer's Disease onset and progression. Use of these compounds has enabled mechanistic analysis of the formation of disease plaques as well as characterizations of relative cell toxicity caused by variants of Amyloid-Beta peptides. Please see **AmideBio's research note RN-101** for further discussion.

When using commercially sourced Amyloid-Beta peptides the researcher must be confident that peptide concentrations are accurately defined to ensure that experimental results are correctly interpreted as relating to input peptide concentrations.

For example, if a research product is labeled as containing 50 µg of material and is dissolved in 50 µL of solution buffer, the presumed concentration would be 1 mg/mL. However, if the actual mass of material differs from the stated amount of 50 µg then the calculated concentration will be incorrect and lead to both the inaccurate interpretation of concentration-dependent experimental results and the inability of other researchers to reproduce the results. Further, since Amyloid-Beta peptides have a propensity to aggregate, insoluble peptide aggregates formed during the dissolution process will result in a lower concentration of solubilized material and therefore an inaccurate calculated concentration. This error can be further complicated when using Hexafluoroisopropanol (HFIP) as a solvent as HFIP extracts UV absorbing compounds from plastic tubes.

To illustrate this concept and to serve as a comparative framework for relating experimental results collected using Amyloid-Beta materials sourced from different manufacturers, a set of quantitation procedures was performed on commercially obtained Amyloid-Beta 1-42 (Aβ42) samples as detailed below. These procedures serve as the basis for **AmideBio's SOP-212** that describes the proper handling and quantification of amyloid-Beta peptides prior to experimental studies.

Materials and Methods

Commercially produced Amyloid-Beta 1-42 (Aβ42) was obtained from 4 different manufacturers; these included 2 recombinant products (AmideBio, and Mnfr3) and 2 synthetic products (Mnfr1 and Mnfr2). The samples were store at the manufacturers' suggested conditions prior to dilution.

Initial Sample Preparation

One vial of each Aβ42 sample was dissolved in 1% NH_4OH to a concentration of 1mg/mL, and divided into 50 µg aliquots. These samples were lyophilized and stored at -20°C until use.

Absorbance at 280nm

A second vial of each Aβ42 sample was dissolved in 0.2% NH_4OH to a calculated concentration of 1 mg/mL, then absorbance at 280nm was measured in triplicate (Molecular Devices SpectraMax M5). The calculated concentration was determined using an extinction coefficient at 280nm of 1490 $M^{-1}cm^{-1}$ [1].

HPLC A280

20 µL of Aβ42 samples at 1 mg/mL calculated concentration were analyzed by RP-HPLC (Ascentis Express ES-CN column ; A=H_20+0.05% TFA, B=ACN+0.05% TFA; 60°C). The Aβ42 peak area was integrated to determine the Total Integrated Absorbance (TIA). The TIA for each sample was compared to standard values generated from Amino Acid Analysis to derive the measured mass of input sample.

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HPLC A214

10 µL of each Aβ42 sample at 1 mg/mL calculated concentration was analyzed by RP-HPLC (Ascentis Express ES-CN column; A=H_20+0.05% TFA, B=ACN+0.05% TFA; 60°C) and the Aβ42 peak area was integrated to determine Total Integrated Absorbance. The TIA for each sample was compared to standard values generated using Aβ42 quantified by amino acid analysis. The extinction coefficient at 214nm for Aβ42 is calculated to be 76,848 $M^{-1}cm^{-1}$ [2].

BCA Assay

Triplicate samples of Aβ42 peptides at presumed concentrations of 200 µg/mL and 100 µg/mL were tested for total protein concentration using the Bicinchoninic acid (BCA) assay (Pierce PN23225) according to the manufacturer's instructions. Absorbance values were compared to BSA standard curve to derive total protein concentration. Note that several factors affect the color development of the BCA assay including protein structure, number of peptide bonds and the presence of cysteine, cystine, tryptophan and tyrosine [3] and it is to be expected that equivalent concentration of Amyloid-Beta and BSA will result in different final absorbances, which can be eventually accounted for using carefully determined correction factor.

SDS-PAGE Analysis

Duplicate samples of 4 µg of each Aβ42 sample were analyzed by SDS-PAGE using a 10%-20% gel (Novex EC6135), along with 2, 4, and 8 µg of lysozyme samples that serve as quantitative standards. Integrated densitometry values of Aβ42 sample bands were determined and compared to lysozyme standards to calculate the peptide concentrations.

Measured vs Calculated Quantitation

Each Aβ42 measured value was compared to the presumed input amount to determine the actual amount of material in the samples, according to the measurement method used. Table 1 shows the results of the quantitation measurements and the comparative fraction expressed as a percent of the calculated input of material.

Results

Amyloid-Beta 1-42 samples from 4 commercial sources were analyzed by standard methods and the results were compared to the assumed concentration based on label-specified quantities of each commercial product. Each of the commercial samples showed discrepancy between the measured quantity and the calculated concentration. Generally, measured amounts of Aβ42 were lower than the calculated input amount. Further, the discrepancies were not consistent across analytical methods.

While most of the measured results suggest that the actual concentrations were significantly lower than the calculated amounts, in one instance (Mnfr3; A280nm), the measured amount was almost 40 times more concentrated than the calculated amount, even though other test results using alternate methods to the same sample indicated a lower overall concentration. Given that the SDS-PAGE analysis of that sample showed a smaller band than the other samples, and that the HPLC showed significant impurities, we conclude the high A280nm measurement may be the result of UV absorbing impurities that interfere with an accurate 280nm absorbance measurement.

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Discussion

Amyloid-Beta 1-42 whether produced by recombinant or synthetic methods, presents quantitation challenges for several reasons. First, the small number of aromatic amino acids results in a low extinction coefficient at 280nm, rendering the most commonly used quantitation method impractical (Aβ42 contains 1 Tyrosine, for a calculated extinction coefficient at 280nm of 1490 $M^{-1}cm^{-1}$ [3]). Second, the peptide's inherent propensity to aggregate will cause the actual concentration of solubilized material to decrease with time. Third, the presence of biological or synthetic contaminants as side- or by-products of the manufacturing process can interfere with analytical measurement and result in erroneous values.

Here we have shown that when actual concentration measurements are compared to a presumed concentration based on dilution of a manufacturer's stated mass quantity, large discrepancies in concentration demonstrate the importance of establishing an actual measured concentration of Aβ42 samples prior to performing experiments with Aβ42.

For these reasons, AmideBio recommends that researchers perform a quantitative measurement of the Aβ42 sample prior to experimental use. AmideBio also recommends the BCA assay as the reliable method of peptide quantitative measurement for rapid and accurate quantitation of Aβ42. For a standard protocol, see **AmideBio's SOP-212.**

Table 1 Quantitation of Amyloid-Beta 1-42 by standard analytical methods

Amyloid-Beta 1-42		**AmideBio**	**Mnfr 1**	**Mnfr 2**	**Mnfr 3**
Purity (manufacturer stated)		> 99%	> 95%	> 95%	> 97%
Salt Form		NH4-Formate	TFA	TFA	TFA
Labelled mgs		0.1 mg	0.5 mg	0.5mg	0.5 mg
Measurement	**Theoretical**	**Analytical Result (Measured material)**			
Concentration mg/mL A280nm +	1.0	1.27	2.59	3.54	38.18
HPLC A280nm Integrated Area μg *	20.0	14.6	11.8	13.2	12.9
HPLC A214nm Integrated Area μg *	10.0	7.37	6.49	7.76	9.45
HPLC A214/A280 Ratio	51.6	52.0	58.0	62.0	76.0
BCA Assay Concentration μg/mL ^	100.0	72.2	42.4	33.9	19.4
SDS-PAGE μg #	4.0	2.10	2.17	0.46	0.17
Ratio of Measured to Calculated		**Percent Measured vs Calculated**			
A280		127%	259%	354%	3818%
HPLC A280nm		73%	59%	66%	65%
HPLC A214nm		74%	65%	78%	94%
BCA Assay		72%	42%	34%	19%
SDS-PAGE		53%	54%	12%	4%
+ A280nm measurement used an extinction coefficient of 1490 M-1cm-1 [3]					
* HPLC quantification was derived by comparison to standard analyzed by Amino Acid analysis.					
^ BCA Absorbance compared to BSA Standard Curve					
# Coomasie-Blue stain densitometry compared to Lysozyme Standard					

References

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2. Kuipers, B. J. H., and Gruppen, H., (2007), *J. Agric. Food Chem*., **55**: 5445-5451.
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