#### Review

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# Quantitative detection of amyloid- $\beta$ peptides by mass spectrometry: state of the art and clinical applications

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**Abstract:** Alzheimer's disease (AD) is the most common form of dementia in humans, and a major public health concern with 35 million of patients worldwide. Cerebrospinal fluid (CSF) biomarkers being early diagnostic indicators of AD, it is essential to use the most efficient analytical methods to detect and quantify them accurately. These biomarkers, and more specifically amyloid- $\beta$  (A $\beta$ ) peptides, are measured in routine clinical practice using immunoassays. However, there are several limits to this immunodetection in terms of specificity and multiplexing of the multiple isoforms of the A $\beta$  peptides. To overcome these issues, the quantification of these analytes by mass spectrometry (MS) represents an interesting alternative, and several assays have been described over the past years. This article reviews the different AB peptides quantitative MS-based approaches published so far, compares their pre-analytical phase, and the different quantitative

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**Keywords:** Alzheimer; amyloid- $\beta$ ; biomarkers; clinical chemistry; mass spectrometry; quantification.

#### Introduction

Alzheimer's disease (AD) is the most common form of dementia, a general term encompassing loss of memory and cognitive functions interfering therefore with activities of daily living. With 35 million of patients worldwide, AD represents 50%-80% of cases of dementia and is reaching epidemic proportions in industrialized countries mainly because of the aging of the population. By 2050, the estimates are guite pessimistic with more than 115.4 million people expected to develop AD [1]. Two main forms of AD coexist, sporadic AD which affects around 5% of the population after the age of 65, and familial AD, which is rare (<1% of the patients), often appears before the age of 65, and is linked to mutations in AD relevant genes (PSEN1-2, APP) [2]. Regardless of its form, AD is characterized by behavioral changes, memory impairments, praxis and gnosis disorders leading to a progressive loss of autonomy in patients [3]. As for today, there are no curative treatments available, but patients can still benefit form symptomatic treatments which improve their quality of life [4].

On a neuro-pathological level, AD is characterized by the presence in the brain parenchyma of hyperphosphorylated tau (p-tau) proteins aggregated into neurofibrillary tangles, and amyloid plaques [5]. Amyloid plaques are formed by aggregates of amyloid- $\beta$  (A $\beta$ ) peptides; the A $\beta$  1-42 isoform being the most represented in the plaques of autopsied brains in AD patients [6]. A $\beta$  peptides derive from the amyloid precursor protein (APP), a

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87-kDa transmembrane protein with a large extracellular domain. This protein is metabolically cleaved by  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretases, leading to the formation of several peptide fragments including A $\beta$  1-42 and 1-40 (Figure 1). The decrease of A $\beta$  peptide concentrations in the cerebrospinal fluid (CSF) of AD patients is correlated with the presence of amyloid plaques [7]. This is especially true for A $\beta$  1-42 which has a higher tendency to aggregation than A $\beta$  1-40 [8]. A $\beta$  1-40 is therefore rather involved in advanced stages of the disease [9].

The diagnosis of AD is based on neuropsychological tests, functional and morphologic imaging (MRI, hippocampal volumetry), and the levels of the following biomarkers present in the CSF:  $A\beta$  peptides, tau and p-tau(181) proteins [10–13]. These biomarkers which are now routinely measured in specialized laboratories [12, 14] are useful to predict and diagnose AD. In practice, two A $\beta$  peptides (A $\beta$  1-40 and A $\beta$  1-42) are quantified by enzyme-linked immuno-sorbent assay (ELISA) [13, 14]. This quantification is based the recognition of the epitopes corresponding to the C-terminus amino acids of AB 1-40 and AB 1-42 using, respectively, the 2G3 and 21F12 antibodies. Recently, new multiplex immunoassays detecting multiples isoforms of AB peptides (1-38, 1-40 and 1-42) have been developed using Meso Scale Discovery (MSD) and Luminex platforms [15–19].

Importantly, other A $\beta$  peptides like A $\beta$  2-14, 1-17, 1-18, 1-33, 1-34 or 1-37 have been described [20, 21] and their role and diagnostic interest still need to be explored in human CSF. Developing multiplexed ELISA in this context would be lengthy and costly. Mass spectrometry (MS) with its high specificity and multiplexing capacity appears as a good alternative to immunoassay techniques, and could represent an interesting new diagnostic tool for AD. Incidentally, it is interesting to note that the number of publications related to the analysis of A $\beta$  peptides by MS has increased 12-fold in the past 20 years.

## Physicochemical properties of A $\beta$ peptides

The origin of amyloid plaques is in relation with the tendency of A $\beta$  peptides to aggregate, especially A $\beta$  1-42 [22]. A $\beta$  peptide aggregation is mainly triggered by conformational changes [23]. These peptides which are initially part of the APP transmembrane domain acquire after proteolytic cleavage a  $\beta$ -sheet structure. Then, they aggregate in an antiparallel arrangement to form filaments. Several filaments constitute fibrils which eventually form amyloid plaques [23]. While A $\beta$  peptides auto-aggregate, they also





Graphic representation of the generation of A $\beta$  peptide isoforms from the amyloid precursor protein (APP). APP can be metabolized in two main ways: the amyloid pathway and non-amyloid pathway. In the amyloid pathway, the action of a  $\beta$ -secretase followed by a  $\gamma$ -secretase generates the soluble APP- $\beta$ , the classical A $\beta$  peptides (1-42, 1-40, 1-38) and the amyloid intra cellular domain (AICD). The non-amyloid pathway via the  $\alpha$ -secretase and the  $\gamma$ -secretase cleaves the APP in three fragments: the soluble APP- $\alpha$ , p3 and the AICD. Short truncated isoforms (A $\beta$  1-14, 1-16, 1-17) could result from an additional pathway combining the action of  $\beta$ -and  $\alpha$ -secretases.

tend to bind to other proteins in vivo [24] and they also adsorb in a non-specific manner to the plastic of collection and assay tubes [25] rendering more complex their accurate quantification. The particular physicochemical properties of A $\beta$  peptides require therefore to control and standardize pre-analytical and analytical phases to obtain reliable measurements with both MS and ELISA, and to eventually deliver reliable results to patients [26–28].

#### **Pre-analytical conditions**

#### **Time of CSF collection**

It is known that the concentration of some analytes in the CSF, like melatonin or dopamine, is influenced by circadian rhythms [29]. In 2007, Bateman et al. reported a significant daytime variation (1.5–6-fold) of A $\beta$  1-40 and A $\beta$  1-42 levels in CSF over a 36-h period [30]. However, several other studies did not observe any significant daytime variations [27, 31]. To be on the safe side, it is however recommended to always perform CSF collection at the same time [26].

#### **CSF** collection tubes

Composition of tubes used to collect CSF during lumbar puncture has a strong influence on AB peptide concentrations [32]. This is in relation with the tendency of  $A\beta$ peptides to adsorb to the walls of the tube. Hence, it has been shown that glass and polystyrene tubes led to lower measured concentrations of Aβ peptides [25, 31]. Polypropylene tubes were believed to reduce this phenomenon [32]. However, even polypropylene tubes which are not made entirely of polypropylene, and have various surface treatments, have a major impact on AB peptide measured concentrations [25]. The consequences on AD biological diagnosis could be very important, as demonstrated in a multisite study conducted in four French Centers for Memory Research and Resources (CMRR) (Montpellier, Lille, Lyon and Paris) [33]. Standardization of the collection tubes (using, e.g., the tube Sarstedt 62.610.201) could improve multicenter comparison and decrease the risk of wrong diagnosis [34].

#### CSF sample storage

Storage at -20 °C or -80 °C has apparently little influence on CSF A $\beta$  peptides [31]. It is however recommended to

freeze and store A $\beta$  standards and CSF at -80 °C to avoid stability issues [35–37]. Addition of protease inhibitors could improve protein stability upon storage but add an additional complexity to samples that could affect their commutability [37]. As for many analytes, more than two or three freeze/thaw cycles have also to be avoided to ensure the reproducibility of the results [27].

## Analytical strategies for the detection and the quantification of $A\beta$ peptides by MS

As a result of their low abundance and the wide dynamic range of protein concentrations in CSF ( $10^{10}$ ) [38], A $\beta$  peptides are difficult to detect by MS. A sample preparation step is therefore generally necessary in order to extract and concentrate them before analysis [39]. After thawing CSF, in order to recover monomeric A $\beta$  peptides that otherwise spontaneously auto-aggregate, aggregate to other proteins or get adsorbed to the walls of tubes, the most common approach is to denature samples with guanidine hydrochloride (Gnd HCl 5M). This can also help minimizing enzymatic activities and thus improving intermediate precision of measurements by reducing sample degradation [40]. After denaturation, the samples can be analyzed by various MS techniques as described below and Table 1.

#### Immuno-precipitation – matrix-assisted laser desorption ionization-time of flight-MS (IP-MALDI-ToF-MS)

MALDI-ToF-MS is a sensitive and fast technique for the detection and relative quantification of AB peptides, yielding a relatively high resolution and high mass accuracy. MALDI is based on the use of a laser beam for desorption, sublimation and ionization of AB peptides co-crystalized with a chemical matrix ( $\alpha$ -cyano-4-hydroxycinnamic acid) on a stainless steel target [41] (Figure 2A). However, due to the complex nature of CSF, extraction and purification steps, like immuno-precipitation (IP), are necessary to reduce the matrix complexity in order to measure AB peptides with sufficient sensitivity [43]. Immuno-capture techniques, like those described by Portelius et al., are based on magnetic beads (Dynabeads M-280 streptavidin) functionalized with anti-A $\beta$  peptide antibodies. The most commonly used antibodies are 6E10 and 4G8 which are directed against epitopes 4-9 and 18-22, respectively [41,

**Table 1:** Detection of amyloid-β peptides.

	ELISA	MALDI	SELDI	LC-MS/MS
1-38, 1-40, 1-42	Х	Х	Х	х
1-13, 1-14, 1-15,1-16, 1-20, 1-28, 1-30, 1-37 ox, 1-38 ox, 1-39		х		Х
ox, 1-40 ox, 1-42 ox, 1-43, 2-42, 3-40, 3-42, 5-40				
1-17, 1-18, 1-19, 1-33, 1-34, 1-39		х	х	х
1-29, 2-15,2-16,2-17, 2-19, 2-38, 4-13, 4-14, 4-15, 4-16, 4-19				х
1-35, 1-36, 1-37, 1-40 x-x, 3-44, 3-47, 10-40, 11-40			х	
2-14			х	х
4-40, 4-42, 5-42, 7-42, 8-42, 9-40, 9-42, 10-42, pGlu 11-42,		х		
pGlu 3-40, pGlu 3-42				
11-42		х	х	



#### Figure 2: Illustration of MS detection approaches and profile of Aβ peptides.

(A) MALDI-ToF-MS is a very sensitive technique to determine the mass of proteins and peptides. It is relatively fast and easy to use. It relies on a soft ionization technique in which a short laser pulse is used to ionize molecules. A protein or peptide sample is placed on a target plate where it is mixed with an appropriate chemical matrix. The co-crystallized mixture of samples and matrix is irradiated with a short laser pulse and entered in a gas phase. The ions are accelerated in a time of flight (ToF) analyzer which measures the time it takes for the molecules to travel a set distance. The mass to charge ratio (m/z) of the ions can then be calculated. (B) selected reaction monitoring (SRM) is a very specific and sensitive targeted MS technique used to quantify preselected relevant ions. It is based on the use of triplequadrupole-type mass spectrometers coupled to HPLC. After peptide ionization in the source, one or several peptides with a known mass are selected in the first quadrupole. These "precursor" ions are then fragmented in the second quadrupole. The third quadrupole can monitor one or several ion fragments called "product" ions which are specific to the precursor ion. The monitoring of a precursor ion and one of its product ions is called a "transition" which is specific to the selected peptide and can be quantified. (C) Chromatogram obtained after SPE CSF fractionation according to the Pannee et al. protocol [42] and nanoLC-triple quadrupole analysis with and alkaline mobile phase.

44, 45]. As the 6E10 epitope is located in the N-terminus of the A $\beta$  peptide, it captures mainly different truncated C-terminal isoforms. In 2006 and 2007, Portelius et al. detected 18 A $\beta$  peptides truncated at the N-terminus after

6E10 IP and analysis on a Bruker Autoflex mass spectrometer [41, 46]. Interestingly, the authors showed that A $\beta$ 1-16, A $\beta$  1-33, A $\beta$  1-39, and A $\beta$  1-42 expression profile in CSF (Table 1) could help differentiating patients with sporadic AD from non-demented controls with a global accuracy of 86% [41].

To take into account sample preparation variability, as well as differences in desorption/ionization, the area of each MS peak obtained for the AB peptides was normalized by the sum of peak areas of the most abundant A $\beta$  peptides [41]. However, this approach, called internal normalization, does not yield information on the absolute concentration of  $A\beta$  peptides. It needs also to perform several successive MS acquisitions in order to obtain an interpretable profile for each sample. To overcome these issues, a quantitative analysis by isotope dilution has been developed. The previously described IP-MALDI-ToF-MS technique was modified by adding two isotopically-labeled Aβ peptides as internal standards, Aβ 1-15 Arg<sup>13</sup>C<sup>15</sup>N and A $\beta$  1-34 Arg<sup>13</sup>C<sup>15</sup>N [46]. These labeled peptides triggered a 10 Da shift of the nominal mass and thus did not interfere with MS peaks of endogenous peptides to be quantified. This resulted in an improvement in results accuracy for A $\beta$  1-17 and A $\beta$  1-40 peptides.

Of note, IP-MALDI-ToF MS was used to follow the effect of a  $\gamma$ -secretase inhibitor [Semagacestat (LY450139)] [47]. This compound resulted in an increased production of A $\beta$  1-14, 1-15, and 1-16, a decrease in A $\beta$  1-17 while A $\beta$  1-42 and 1-40 remained unaffected. This illustrates the interest of following additional A $\beta$  isoforms in AD research [39, 48–51].

Of note, SELDI-ToF-MS which is based on an initial capture of A $\beta$  peptides using antibodies covalently immobilized on a proteinchip prior to MALDI-ToF has been used in the past to detect different isoforms of these analytes [52]. This straightforward approach allowed the discovery of several new AD biomarkers [20, 53] and in particular two new A $\beta$  peptides (A $\beta$  3-44, A $\beta$  3-47) [53]. Combining A $\beta$  capture with the N-and C-terminus specific antibodies 6E10 and 4G8 [54], 15 A $\beta$  peptides were detected, including A $\beta$  1-38 and 1-40 peptides, as well as new peptides truncated in the N-terminus: A $\beta$  11-40, A $\beta$  11-42 and A $\beta$  10-40 [20] (Table 1).

#### Immuno-precipitation-liquid chromatography-MS (IP-LC-MS/MS) and solid phase extraction-liquid chromatography-tandem MS (SPE-LC-MS/MS)

Tandem MS (MS/MS) is an approach employing two stages of mass analysis in order to selectively fragment a specific ion in a mixture of precursor ions. For proteomics application, MS/MS is usually coupled with a separation method, such as reverse phase liquid chromatography (LC) (see the paragraphs "Mobile phases" and "Analytical columns" below). Performed with a triple quadrupole mass spectrometer, this approach is considered as the gold standard for proteins and peptides quantitation (Table 2). In this context, the most commonly used MS acquisition mode is the selected reaction monitoring (SRM) (Figure 2B). Briefly, peptides are ionized in the electrospray source (ESI) (see the paragraph "Ionisation mode" below). Ions with a given mass (precursor ions) are then selected in the first quadrupole of the mass spectrometer, fragmented in the second quadrupole, and specific ions produced by the fragmentation reaction (product ions) are selected in the third quadrupole for quantification. The precursor ion/ product ion couple is called a "transition".

Recently, candidate reference methods [reference measurement procedure (RMP)] have been proposed for the absolute quantification of A $\beta$  1-42 by LC-MS/MS [55, 56]. If selected as higher order reference method, they could be used to certify a reference material [certified reference material (CRM)] that would become the "gold standard" with which all in vitro diagnostic kits should be calibrated with to fulfill requirements of the European Commission in vitro diagnostic device directive (IVD Directive 98/79/EC) [57]. This CRM would allow standardizing the different routine methods, thus improving results comparability and reliability.

To set up a SRM method for  $A\beta$  peptides, these analytes were initially concentrated by IP (6E10 antibodies) [58]. IP was progressively replaced by solid phase extraction (SPE) which resulted in a very efficient detection of A $\beta$  peptides [40, 42] (Figure 2C). The first SPEs of A $\beta$ peptides used hydrophilic-lipophilic balanced (HLB) [59] and SepPak C18 [60] stationary phases. Recent advances on mixed-mode of SPE with Mild Cation eXchange (MCX), improved the selectivity and recovery of A $\beta$  peptides [40, 42]. Of note, regardless of the prefractionation approach, CSF is generally dried in a lyophilizer or a vacuum centrifuge concentrator (speedvac) in order to concentrate, freeze-dry and store them before analysis. The dry residues are then resolubilized in an alkaline solution (e.g., 20% ACN, 1% NH, OH), in order to avoid aggregation of A $\beta$ peptides before analysis [61].

#### **Mobile phases**

The use of reversed phase high pressure LC (HPLC) columns associated with an acid mobile phase is the usual strategy for peptide separation in a wide variety of biological matrices [62, 63]. Quantification of  $A\beta$  peptides

	Analytical precision	Possible quantification	Clinical implementation	Complexity of the system and its set-up	Cost of the system
MALDI	+++	++	+	+	++
SELDI	+	+	-	+	+
LC-MS	+++	+++	+++	++	+++

Table 2:	Comparison	of the different	mass spectrometry	y technologies.
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is however mainly done with an alkaline mobile phase which is more adapted to the physical chemistry of  $A\beta$ peptides. As a matter of fact, right from 1992, Burdick et al. showed that aggregation of  $A\beta$  peptides increased when pH ranged between 2 and 9 [61]. To avoid aggregation, the chromatographic separation of  $A\beta$  1-40 and 1-42 is therefore better performed under alkaline conditions. Furthermore, the signals obtained in MS under alkaline conditions are about 10-fold higher than those obtained using an acid mobile phase [58]. Several mobile phases have been tested (ammonium acetate, methanol, acetonitrile, hexa-fluoro-isopropanol) and the mix of acetonitrile and ammonium hydroxide was giving the best results in terms of intensity and chromatographic separation of  $A\beta$ peptides [58].

#### Analytical columns

Due to their hydrophobic nature,  $A\beta$  peptides can be separated on a C8 [64, 65] or C18-type non-polar column [42, 58]. Separation of  $A\beta$  peptides being improved under alkaline conditions, the columns must be resistant to alkaline pH (Table 3). Recently, in order to reduce the contamination of the column by proteins or other hydrophobic molecules, Pannee et al. used a reversed-phase monolith column [42].

#### **Ionization mode**

ESI can be done in negative or positive mode, meaning that either negatively charged or positively charged species can be obtained and monitored. In the literature,  $A\beta$  peptides are often detected in positive mode but according to Oe et al. [58, 66] sensitivity is improved in the negative mode. In 2011, Lame et al. however demonstrated that working in positive mode, but using alkaline instead of acid mobile phases, led to a significant improvement of the ion signal stability over a 24-h period and a lower detection limit for  $A\beta$  peptides.

#### Quantitative detection of Aβ peptides by LC-MS/MS in a clinical context

For the past 15 years, LC coupled to tandem mass spectrometry (LC-MS/MS) has been increasingly used in clinical laboratories [67], and it has been recently adapted to  $A\beta$  peptides detection [64]. Compared to immunological techniques, LC-MS/MS shows a very high specificity, multiplexing capacities and absolute quantification which relies on different standards and calibration strategies as illustrated for  $A\beta$  peptides in the next paragraphs.

Table 3:	Columns used	for alkaline	mobile phases.
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Columns	pH range	Mobile phases	References
Waters C8 Xterra MS	rs C8 Xterra MS 1–12 Mobile phase A: 5% ACN 5% Isopropanol 10 mM		[64]
(3.0×150 mm, 3.5 μm)		ammonium acetate	
		Mobile phase B: 45% ACN 45% Isopropanol	
		10 mM ammonium acetate	
Waters XBridgeC18 column	1-12	Mobile phase A: 0.1% NH, OH	[66]
(150×2.1 mm id, 3.5 μm, 125 Å)		Mobile phase B: 0.1% NH, OH ACN (1:200 v/v)	
Waters Acquity UPLC BEH300 C18	1-12	Mobile phase A: 0.3% NH OH (v/v)	[40]
(2.1×150 mm, 1.7 μm)		Mobile phase B: 90:10 (v/v) ACN/Mobile phase A	
Thermo Scientific ProSwiftRP-4H1	1-14	Mobile phase A: 0.1% NH, OH 5% ACN	[42]
		Mobile phase B: 0.03% NH <sub>4</sub> OH 95% ACN	

#### Use of internal standards

In order to control the variability of the different steps of the protocol (i.e., extraction of A $\beta$  peptides, LC separation, MS ionization and fragmentation), an internal standard can be introduced in the sample at the beginning of the process. Ideally, an internal peptide standard corresponds to a peptide synthetized with the incorporation of stable isotopes such as <sup>13</sup>C or <sup>15</sup>N. These results in a known mass increment, explaining the term often used of "heavy" standard [68]. To quantify A $\beta$  peptides via LC-MS/MS, the ratio between the MS signal intensity of the endogenous peptide and that of its heavy counterpart present at a known concentration, is generally measured in samples and a set of calibrators [69].

#### Preparation of Aβ standard solutions

A $\beta$  standards are commercially available in freeze-dried forms rather than in solution to avoid problems linked to the high aggregation propensity of these peptides, especially A $\beta$  1-42. During their re-suspension, A $\beta$  peptides might however go back to the beta sheet conformation they had prior to freeze-drying and thus could rapidly form new aggregates [70]. To overcome this hurdle, standards are often pretreated with hexafluoroisopropanol (HFIP) [71] in order to denature beta sheets and favor  $\alpha$ helix conformations. A $\beta$  standards need eventually to be re-suspended in solvents compatible with MS analysis, i.e., acetonitrile/ammonium hydroxide (20%:1%, v/v) [42, 66] or dimethylsulfoxide (DMSO) [40].

## Calibration curves and model matrices

Several peptide quantification strategies exist (see below and Figure 3). The choice depends of many issues including: the expected values and dynamic range of the analytes, the availability and the cost of light and heavy standards or the nature and the accessibility of a model matrix in which the calibrators will be prepared. Regarding the matrix, it has to be as close as possible as that of the samples to avoid matrix-related effects, and it should not contain any endogenous analytes. For AB peptides, it is also important to verify that the model matrix does not induce any aggregation or non-specific adsorption. For quantification of A<sup>β</sup> peptides, several model matrices have been used, including human CSF depleted of Aß peptides, rat CSF, artificial CSF (Na 150; K 3.0; Ca 1.4; Mg 0.8; P 1.0; Cl 155 in mM, Tocris Bioscience), artificial CSF supplement with 5% of rat plasma or with 4 mg/mL of bovine serum albumin [40, 56, 66, 72].

#### Normal curve

The "normal curve" approach is commonly used to generate calibration curves (Figure 3A). The procedure is to add to the model matrix a variable amount of light standard and a known amount of heavy standard, which will be also added to each sample. A calibration curve is then established by plotting the ratio between the concentrations



#### Figure 3: Different MS-based quantification strategies.

(A) The "normal curve" consists in adding a variable quantity of light standard and a constant quantity of heavy standard in a model matrix that mimics the biological fluid of interest. The calibration model is obtained from light standard/heavy standard concentration ratios and light standard/heavy standard peak area ratios. (B) The "reverse curve" is the opposite of the "normal curve". The light standard is added in a constant quantity in the relevant biological fluid (CSF, model matrix) while the quantity of heavy standard varies. The calibration model is obtained from the areas ratio of the MS signal of the heavy standard over the light standard according to the concentration ratio of the heavy standard over the light standard.

of the light and heavy standard as function of the ratio between the peak areas of the transitions used to monitor the light and heavy analytes. The concentration of the endogenous analyte in a clinical sample is then computed from the ratio between the peak areas of the endogenous compound and that of the heavy standard that was added to it.

#### **Reverse curve**

On the opposite of the "normal curve", the procedure is to add a variable amount of the heavy standard to the matrix (which could be a pool of samples) in presence of a known amount of light standard (or endogenous analytes) (Figure 3B). This approach has been sometimes chosen for A $\beta$  peptides since, on one hand, commutability studies suggested that only human CSF was a suitable matrix to mimic clinical samples [55], and on the other hand, it was very difficult to fully deplete CSF of A $\beta$  peptides. The use of this type of calibration curve, with CSF pools as a matrix, may help to more accurately determine limits of detection and quantification of A $\beta$ peptides [42, 73].

### Comparison between ELISA and LC-MS/MS

When developing a new analytical strategy, it is important to compare it with the existing ones. Several studies have analyzed samples with both ELISA and LC-MS/MS. In 2006, Oe et al. [66] generated the first results for  $A\beta$ peptides quantification after IP and SRM and compared them to those obtained by ELISA. Correlation coefficients obtained were respectively of 0.64 and 0.91 for A $\beta$ 1-40 and A $\beta$  1-42. In 2013, Pannee et al. [42] developed a MS-based method and obtained lower correlation coefficients ranging from 0.35 to 0.77 and higher AB 1-42 concentration values with the MS method. Such discrepancy between ELISA and LC-MS/MS methods are likely related to differences in standard materials (i.e., purity and aggregation level of the light and heavy standards used to prepare the calibrators), as well as differences in the nature of the two methods: while ELISA relies on the immunological recognition of nearby epitopes, LC-MS/ MS measures transitions that are generated from peptides which could be located in different regions of the biomarker of interest.

#### Conclusions

Clinical laboratories measuring AB 1-40 and 1-42 CSF levels are mostly using simplex ELISA kits from different companies [4, 14]. Immunodetection approaches are straightforward, but they present some drawbacks such as difficulties to develop good multiplex panels, to detect additional forms of A $\beta$  peptides, or to reach a low variability from batch to batch, and between providers. MS could help resolve these issues with the development of LC-MS/ MS reference methods [55, 56] along with the forthcoming availability of a CRM. In addition, MS will be useful to qualify, control and improve comparability of results from existing immunoassays by providing calibrators with target values traceable to a reference method and/or CRM. However, the MS quantification of  $A\beta$  peptides will likely stay restricted to expert laboratories since it requires costly mass spectrometers, and highly qualified staff. In addition, the clinical added value of detecting in routine other A $\beta$  peptides than A $\beta$  1-40 and 1-42 CSF has yet to be demonstrated. Diffusion of the technology in specialized laboratories involved in AD clinical research will anyway benefit from the future development of kits which would include standardized protocols, consumables and reagents (peptide standards).

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