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On-line aptamer affinity solid-phase extraction direct mass spectrometry for the rapid analysis of α-synuclein in blood

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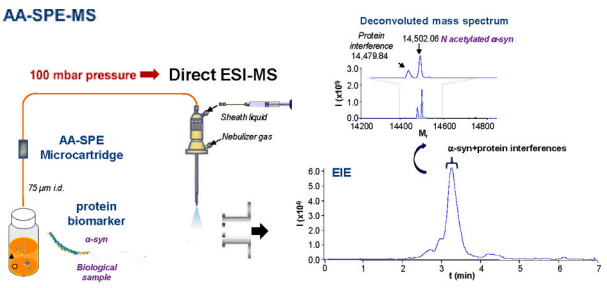
HIGHLIGHTS

- An aptamer affinity sorbent for the protein biomarker α -synuclein is prepared.
- An on-line AA solid-phase extraction direct mass spectrometry method is developed.
- The valve free AA-SPE-MS is a straightforward adaptation from AA-SPE-CE-MS.
- Up to 500-fold sensitivity enhancement compared to direct MS is obtained.
- A rapid and accurate analysis of human α-synuclein in blood samples is achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

On-line aptamer affinity solid-phase extraction direct mass spectrometry (AA-SPE-MS) is presented for the rapid purification, preconcentration, and characterization of α-synuclein (α-syn), which is a protein biomarker related to Parkinson's disease. Valve-free AA-SPE-MS is easily implemented using the typical SPE microcartridges and instrumental set-up necessary for on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry (AA-SPE-CE-MS). The essential requirement is substituting the application of the separation voltage by a pressure of 100 mbar for mobilization of the eluted protein through the capillary towards the mass spectrometer. Under optimized conditions with recombinant α-syn, repeatability is good in terms of migration time and peak area (percent relative standard deviation (%RSD) values (n = 3) are 1.3 and 6.6% at 1 µg mL^{-1}, respectively). The method is satisfactorily linear between 0.025 and 5 µg mL^{-1} ($R^2 > 0.986$), and limit of detection (LOD) is 0.02 µg mL^{-1} (i.e. 1000, 500, and 10 times lower than by CE-MS, direct MS, and AA-SPE-CE-MS, respectively). The established AA-SPE-MS method is further compared with AA-SPE-CE-MS, including for the analysis of α-syn in blood. The comparison discloses the advantages and disadvantages of AA-SPE-MS for the rapid and sensitive targeted analysis of protein biomarkers in biological fluids.

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1. Introduction

Simplifying the analytical procedure while minimizing sample handling, increasing analytical throughput, and enhancing detection sensitivity is a continuing demand in analytical chemistry, especially when analyzing peptides and proteins in minute amounts of biological samples by mass spectrometry (MS)-based techniques. For example, capillary electrophoresis-mass spectrometry (CE-MS) is a powerful microseparation technique for the analysis of polar and ionizable biomolecules such as peptides and proteins [1–6]. CE provides high separation efficiency and resolving power with low sample, solvent, and reagent consumption while electrospray ionization-mass spectrometry (ESI-MS) allows detailed characterization, sensitive detection, and accurate quantification. Today, it is widely accepted for hyphenated MS techniques, that resolving the components of complex samples as narrow peaks before arriving to the mass spectrometer is critical to avoid interferences (e.g. ion suppression), increase signal-to-noise ratio (S/N), and achieve the best limit of detection (LOD) [7,8]. However, with the aim of simplifying the instrumental set-ups and speeding up the analyses, different authors have proposed to disregard the chromatographic or electrophoretic separation, to fully trust in the capabilities of direct MS detection. Even novel ambient ionization methods have been developed, such as, among others, desorption electrospray ionization (DESI) [9,10], direct analysis in real time (DART) [11], paper spray ionization [12,13], and coated blade spray [14,15], which together with the continuous improvement of mass spectrometers, promise a bright great future ahead. Unfortunately, direct MS analysis of complex samples, such as biological fluids, typically results in poor outcomes due to matrix effect, especially with ESI-based methods that are more susceptible to such undesired effect [14,15]. Therefore, an appropriate sample-preparation step is still necessary for sample purification and preconcentration. In this sense, on-line sample preparation approaches are recommended, as they can be automated to minimize sample handling and increase the analytical throughput. Today, solid-phase extraction (SPE) meets all the necessary requirements for the on-line coupling with direct MS [14–26].

In recent years, on-line solid-phase extraction direct mass spectrometry (SPE-MS) has been demonstrated for the analysis of complex biological samples using different ESI-MS interfaces [14–26]. In many of these studies, SPE is referred as solid-phase microextraction (SPME), due to the reduced amount of sorbent needed. Different SPE devices have been described, such as in-tube monolithic microcartridges in instrumental set-ups operated with valves coupled to conventional ESI-MS or DART-MS interfaces [16–18], microfluidic open ESI-MS interfaces for dispersive or fiber-based extraction [19,20], and different coated materials as sheets, tips, or blades ready to use as ESI-MS interfaces [14,15,21–26]. However, all these studies are dealing with the analysis of small molecules, which have been appropriately cleaned-up and preconcentrated with typical chromatographic sorbents [14–16,18–20], as well as with more selective molecular imprinted polymers [17,21–24] or immunoaffinity sorbents [25,26]. Indeed, peptides, but specially proteins, require high-selective sorbents to achieve the best performance in SPE-MS, as they are more difficult to ionize and prone to undesired matrix effects. Aptamers, which are typically single-stranded oligonucleotides, are currently regarded as excellent candidates to prepare SPE sorbents for targeting proteins and other bioactive compounds with high affinity and selectivity [27–34]. In addition to their very convenient properties as ligands, aptamers are very stable, easily derivatizable, reusable, and, once appropriately selected, they can be reproducibly produced at low cost [27,28].

In this study, on-line aptamer affinity solid-phase extraction direct mass spectrometry (AA-SPE-MS) is presented for the rapid purification, preconcentration, and characterization of α-synuclein (α-syn), which is a ~14 kDa protein biomarker with different post-translational modifications related to Parkinson's disease [35–38]. A valve-free AA-SPE-MS method is developed taking as a starting point an on-line aptamer affinity solid-phase extraction capillary electrophoresis-mass spectrometry (AA-SPE-CE-MS) methodology that we previously described for the same protein biomarker [29,30]. Method development is straightforward, as the typical SPE microcartridges and instrumental set-up necessary for valve-free unidirectional AA-SPE-CE-MS are used [29,39]. More specifically, a microcartridge containing the AA sorbent for the target protein is integrated near the inlet of another capillary, which is now only used for transport and connection to the ESI-MS interface. The system allows loading a large volume of sample for selective clean-up and preconcentration of the target protein, before washing and eluting with a much smaller volume of an appropriate solution, which is transported by pressure instead of by voltage to the mass spectrometer. The AA-SPE-MS method is optimized and validated with α-syn standards and applied to the analysis of blood. The performance of the established method is further compared with direct MS, CE-MS, and AA-SPE-CE-MS to disclose the advantages and disadvantages of AA-SPE-MS for the rapid sensitive targeted analysis of protein biomarkers in biological fluids.

2. Experimental section

2.1. Materials and reagents

The separation background electrolyte (BGE) and solutions were prepared with chemicals of analytical reagent grade or better. Acetic acid (HAc, glacial), ammonium hydroxide (NH_4OH) (25%), formic acid (HFor, 99.0%), and sodium hydroxide (≥99.0%) were provided by Merck (Darmstadt, Germany). Propan-2-ol and water of LC-MS grade were purchased from Fisher Scientific (Loughborough, UK).

The DNA aptamer M5-15 [40], modified with a C6 spacer arm terminated by 5'amino (M5-15-5′, 66-mer, relative molecular mass (M_r) = 20690) and purified by HPLC, was supplied by Integrated DNA Technologies (Coralville, IA, USA). MAGicBeads ACT magnetic beads (MBs) of 45–165 μm diameter were provided by MAGic BioProcessing (Uppsala, Sweden).

2.2. Separation background electrolyte and sheath liquids

A 0.1 M HAc (pH 2.9) BGE was used for all the experiments, after filtering through a 0.20 μm nylon filter (Macherey-Nagel, Düren, Germany). The sheath liquid solution for direct MS, CE-MS, and AA-SPE-CE-MS experiments consisted of a mixture of 60:40 (*v/v*) propan-2-ol:water with 0.05% (*v/v*) of HFor. The optimized sheath liquid solution for AA-SPE-MS experiments had the same hydroorganic composition but 1% (*v/v*) of HFor. Both sheath liquids were delivered at a flow rate of 3.3 μL min^{-1} by a KD Scientific 100 series infusion pump (Holliston, MA, USA). All solutions were degassed for 10 min by sonication before use.

2.3. Protein standard and blood samples

Recombinant human α-syn expressed in *Escherichia coli* was supplied by Analytik Jena (Jena, Germany). The commercial stock solution (5000 μg mL^{-1} in phosphate-buffered saline (PBS)) was aliquoted and stored in a freezer (−20 °C). After thawing, the aliquots were diluted in water to prepare working standard solutions of desired concentrations. These solutions were stored in the fridge (4 °C) when not in use.

For direct MS and CE-MS analyses, α-syn standard stock solutions were desalted by passing through 3000 M_r cut-off cellulose acetate filters (Amicon Ultra-0.5, Millipore, Bedford, MA, USA) following the manufacturer's instructions.

Human blood from a healthy volunteer was processed, and research was conducted following standard operating procedures with appropriate approval of the Ethical and Scientific Committees of the University of Barcelona. Thermo-enriched red blood cell (TE RBC) lysates were prepared from fresh blood as described in previous studies [29,30,37].

2.4. Apparatus and instruments

Centrifugal filtration, agitation, and incubation were performed in a Mikro 220 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany), a Vortex Genius 3 (Ika®, Staufen, Germany), and a TS-100 thermoshaker (Biosan, Riga, Latvian Republic), respectively. pH was measured with a Sension + PH3 potentiometer and an electrode 50 14 T (Hach Lange Spain S.L.U., Barcelona, Spain). A neodymium cube magnet (12 mm, N48) was supplied by MAGic BioProcessing.

A 7100 CE system coupled with an orthogonal G1603 sheath-flow interface to a 6220 oa-TOF LC/MS spectrometer equipped with ChemStation and MassHunter softwares (Agilent Technologies, Waldbronn, Germany) was used for all the analyses. The CE instrument autosampler was kept at 13 °C using an external water bath (Minichiller 300, Peter Huber Kaltemaschinenbau AG, Offenburg, Germany). The TOF mass spectrometer was calibrated daily in positive ESI mode following the manufacturer's instructions using ESI-L low concentration tuning mix (Agilent Technologies). It was operated under optimized conditions using the following parameters: capillary voltage 4000 V, drying temperature 300 °C (for CE-MS, direct MS and AA-SPE-CE-MS experiments) and 350 °C (for AA-SPE-MS experiments), drying gas flow rate 4 L min^{-1}, nebulizer gas 7 psig, fragmentor voltage 325 V, skimmer voltage 80 V, and OCT 1 RF Vpp voltage 300 V. Data were collected in profile at 1 spectrum·s^{-1} between 100 and 3200 *m/z*, with the mass range set to high-resolution mode (4 GHz).

Fused silica capillaries were provided by Polymicro Technologies (Phoenix, AZ, USA). New capillaries of the desired length were activated off-line to avoid the unnecessary contamination of the mass spectrometer flushing at 930 mbar with water (15 min), 1 M NaOH (15 min), water (15 min), and BGE (15 min).

2.5. Direct MS and CE-MS

Direct MS and CE-MS analyses were performed at 25 °C in a 72 cm total length (L_T) × 75 μm inner diameter (i.d.) × 365 μm outer diameter (o.d.) capillary. After activation and between analyses, the capillary was conditioned flushing at 930 mbar with water (2 min) and BGE (2 min). Samples were injected at 50 mbar for 10 s. A pressure of 100 mbar was applied in direct MS, and a separation voltage of +25 kV (normal polarity, cathode in the outlet) in CE-MS.

2.6. AA-SPE-MS and AA-SPE-CE-MS

Preparation of aptamer affinity-magnetic beads (AA-MBs), microcartridge construction, and AA-SPE-CE-MS method were described in our recent works [29,30]. Fritless particle-packed microcartridges (0.7 cm long (L_T) × 250 μm i.d. × 365 μm o.d. capillary) [29,39] were completely filled by vacuum with AA-MBs and connected with plastic sleeves to two capillary fragments (7.5 cm long (L_T) × 75 μm i.d. × 365 μm o.d. (inlet) and 64.5 cm long (L_T) × 75 μm i.d. × 365 μm o.d. (outlet)), which were previously activated [29,39].

All AA-SPE-CE-MS experiments were performed at 25 °C and capillary flushes at 930 mbar. Capillaries were conditioned flushing with BGE (2 min). Then, samples were loaded flushing for 5 min (ca 30 μL, estimated using the Hagen-Poiseuille equation [41]), followed by another flush with BGE (2 min) to eliminate non-retained molecules and fill the capillaries before the separation. All these steps were performed with the nebulizer gas and the ESI capillary voltage switched off to prevent the entrance of contaminants into the mass spectrometer. Then, both were switched on, and a small volume of eluent (0.1 M NH_4OH, pH 11.2) was injected at 50 mbar for 20 s (ca 100 nL [41]). For a rapid and repeatable protein elution, the small plug of eluent was pushed with BGE at 50 mbar for 100 s before applying the separation voltage (+25 kV) and a small pressure (25 mbar) to compensate for the microcartridge counter-pressure. In AA-SPE-MS, the voltage-driven separation step was substituted by applying a pressure of 100 mbar. Between consecutive analyses, to prevent carryover, the capillary was flushed with water (1 min), the eluent was injected at 50 mbar for 40 s, and again it was flushed with water (2 min).

2.7. Quality parameters

All quality parameters were calculated from data obtained by measuring migration time (t_m) and peak area from the extracted ion electropherogram (EIE) of α-syn main proteoforms (considering the *m/z* of the most abundant molecular ions). Linearity range was established by analyzing standard solutions of recombinant α-syn at concentrations between 10 and 60 μg mL^{-1} for direct MS and CE-MS, between 0.025 and 5 μg mL^{-1} for AA-SPE-MS, and between 0.5 and 10 μg mL^{-1} for AA-SPE-CE-MS. LODs were obtained by analyzing low-concentration standard solutions of α-syn (close to the LOD level, as determined from a S/N = 3). Repeatability (n = 3) was evaluated as the percent relative standard deviation (%RSD) of t_m and peak areas analyzing 40 μg mL^{-1} and 1 μg mL^{-1} α-syn standard solutions in direct MS and AA-SPE-MS, respectively. The microcartridge lifetime was also investigated at 1 μg mL^{-1}. The microcartridge was discarded when a significant loss of extraction efficiency was detected, which meant that the α-syn peak area decreased more than 25% compared to the mean value of the first three analyses with the microcartridge under consideration [29].

3. Results and discussion

3.1. Direct MS

Direct MS analysis of α-syn was conducted by adapting the CE-MS method developed in our previous study [29], which required a BGE of 0.1 M HAc (pH 2.9) and a sheath liquid of 60:40 (*v/v*) propan-2-ol/water with 0.05% (*v/v*) of HFor. The voltage for the electrophoretic separation was switched off in order to transport only by pressure the injected sample to the mass spectrometer. Different pressures were tested in accordance with the injection and flushing capabilities of the CE instrument internal air pump, including 30, 40, 50, 75, 100, and 930 mbar. Fig. 1-A shows the EIE obtained for a 100 μg mL^{-1} recombinant α-syn standard applying 100 mbar, which presents a split protein peak. Similar results were observed no matter the pressure applied. A careful inspection of the mass spectra for the two protein peaks and the peak valley, indicated that the protein was preferentially detected as the expected protonated molecular ions within the peaks and as sodiated molecular ions within the valley (see the deconvoluted mass spectra for the two protein peaks and the peak valley in Fig. 1-B). This splitting phenomenon did not happen when applying voltage in CE-MS, which allowed stacking the protonated and sodiated protein molecular ions as a single peak. This suggested the necessity for desalting the protein sample through 3000 M_r cut-off cellulose acetate filters, which definitively solved the issue.

Regarding the peak protein intensity, there were no important differences when the applied pressure ranged between 30 and 100 mbar, while t_m decreased when the applied pressure increased, ranging from 8.0 to 3.5 min at 30 and 100 mbar, respectively. Although application of a 930 mbar pressure favored the shortest t_m, the protein peak presented a lower intensity than at 100 mbar and it was split into two partially resolved twin peaks, due to ionization disruption (Fig. S1). Therefore, a pressure of 100 mbar was selected for direct MS because of the good compromise between peak intensity and t_m (Fig. S1). Fig. 2-A and 2-B i) show a comparison between the EIEs obtained for a 40 μg mL^{-1} α-syn standard under the optimized conditions by direct MS and CE-MS [29], respectively. As can be observed, by direct MS, α-syn shows a shorter t_m, but the protein peak was slightly wider and smaller than by CE-MS. In addition, there was no difference at the mass spectra and deconvoluted mass spectra level using desalted samples (Fig. 2 ii) and iii), respectively).

Table 1 shows the quality parameters for direct MS and CE-MS.

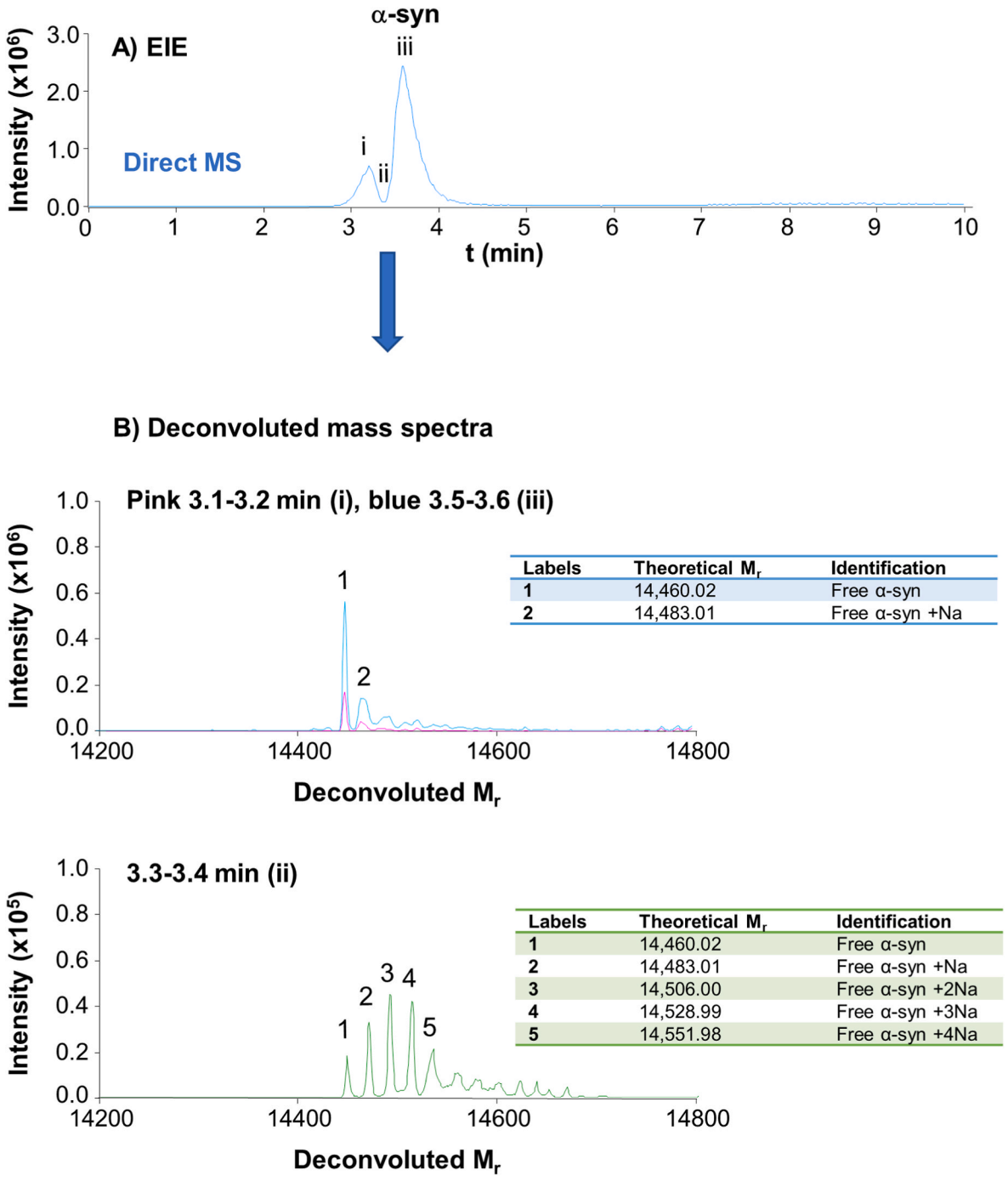

Fig. 1. Direct MS for a 100 μg mL^{-1} α-syn standard applying a mobilization pressure of 100 mbar. (A) Extracted ion electropherogram (EIE) and (B) deconvoluted mass spectra for the two protein peaks and the peak valley observed in the EIE. The standard solution was not desalted before the analysis.

Under the optimized conditions by direct MS, consecutive analyses of an α-syn standard were repeatable in terms of t_m and peak area (%RSD (n = 3) were 0.3 and 4.1% at 40 μg mL^{-1}, respectively) and there were no significant differences with CE-MS (%RSD (n = 3) were 0.2 and 4.3% at 40 μg mL^{-1}, respectively). Linearity was investigated between 10 and 60 μg mL^{-1}, and direct MS was linear over the whole studied range (y = 0.160x-0.953, $R^2 > 0.997$), while 10 μg mL^{-1} could not be detected by CE-MS (y = 0.212x-2.145, $R^2 > 0.990$, 20–60 μg mL^{-1}). As a consequence of the wider linearity range and smaller slope value, the LOD by direct MS was around 10 μg mL^{-1}, which was slightly better than by CE-MS (around 20 μg mL^{-1}).

3.2. AA-SPE-MS

The capability of AA-MBs to clean-up and preconcentrate α-syn from standard solutions and TE RBC lysates by AA-SPE-CE-MS was proved in our previous study [29]. Relying on this recently developed method, a BGE of 0.1 M HAc (pH 2.9), a sheath liquid of 60:40 (*v/v*) propan-2-ol: water with 0.05% (*v/v*) of HFor, and an eluent of 0.1 M NH_4OH (pH 11.2) were first investigated for AA-SPE-MS, applying the 100 mbar pressure optimized by direct MS. As in direct MS, α-syn was detected rapidly (in less than 4 min) but the protein peak was split (Supplementary Figs. S2–A) and the peak intensity was significantly lower than by AA-SPE-CE-MS (data not shown).

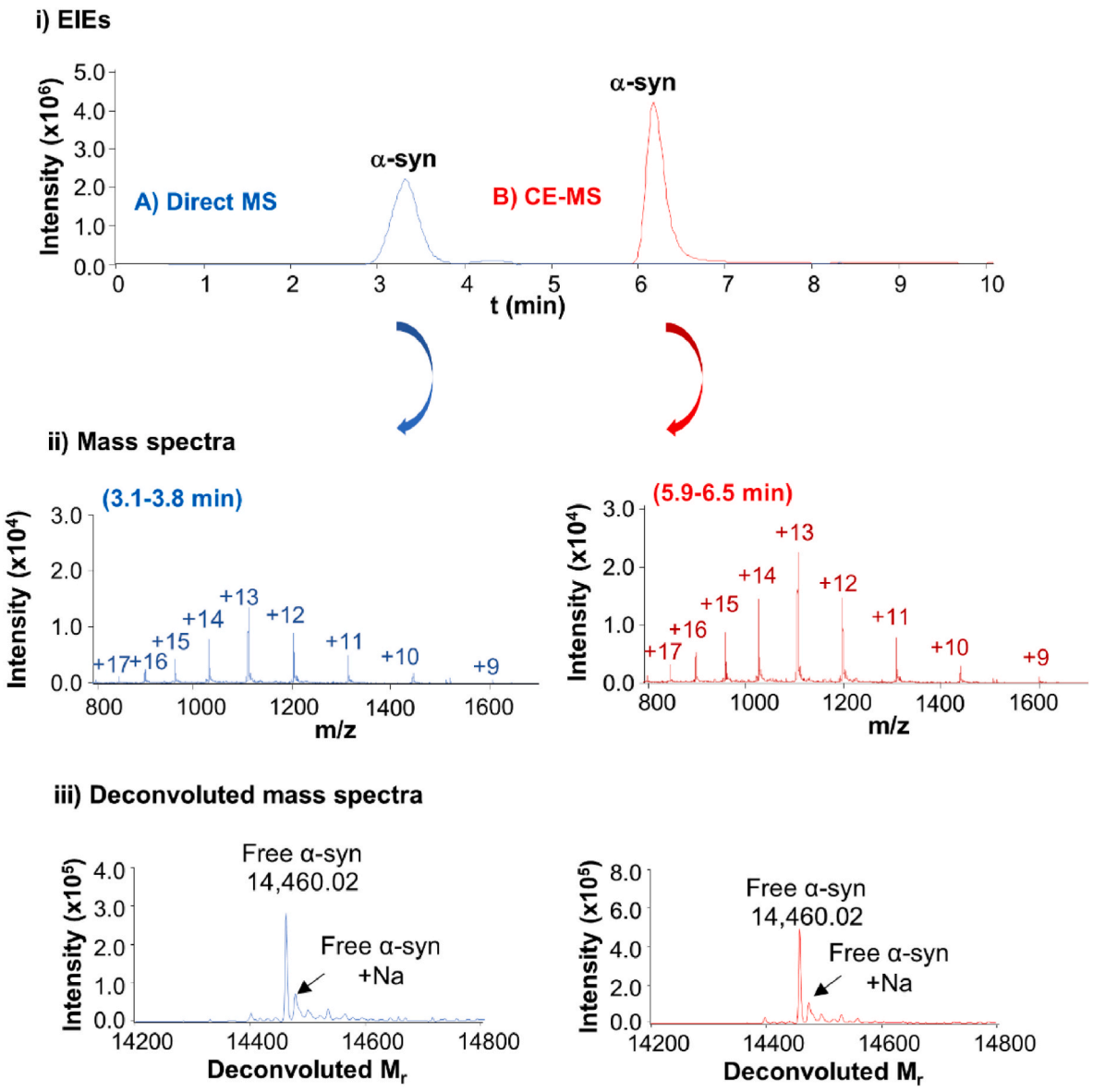

Fig. 2. Extracted ion electropherograms (EIEs) for a 40 μg mL^{-1} α-syn desalted standard by (A) direct MS and (B) CE-MS under the optimized conditions. (i) Extracted ion electropherograms (EIEs), (ii) mass spectra, and (iii) deconvoluted mass spectra.

Table 1
Repeatability, linearity, and LOD for the direct MS, CE-MS, AA-SPE-MS, and AA-SPE-CE-MS methods.

	Repeatability (%RSD (n = 3))[a]		Linearity		LOD (μg·mL^{-1})
	t_m	Peak area	Range (μg·mL^{-1})	R^2	
Direct MS	0.3	4.1	10–60	>0.997	< 10
CE-MS	0.2	4.3	20–60	>0.990	< 20
AA-SPE-MS	1.3	6.6	0.025–5	>0.986	0.02
AA-SPE-CE-MS [29]	2.1	5.4	0.5–10	>0.994	0.2

[a] 40 μg·mL^{-1} or 1 μg·mL^{-1} α-syn standard solutions were analyzed by direct MS and CE-MS or AA-SPE-MS and AA-SPE-CE-MS, respectively.

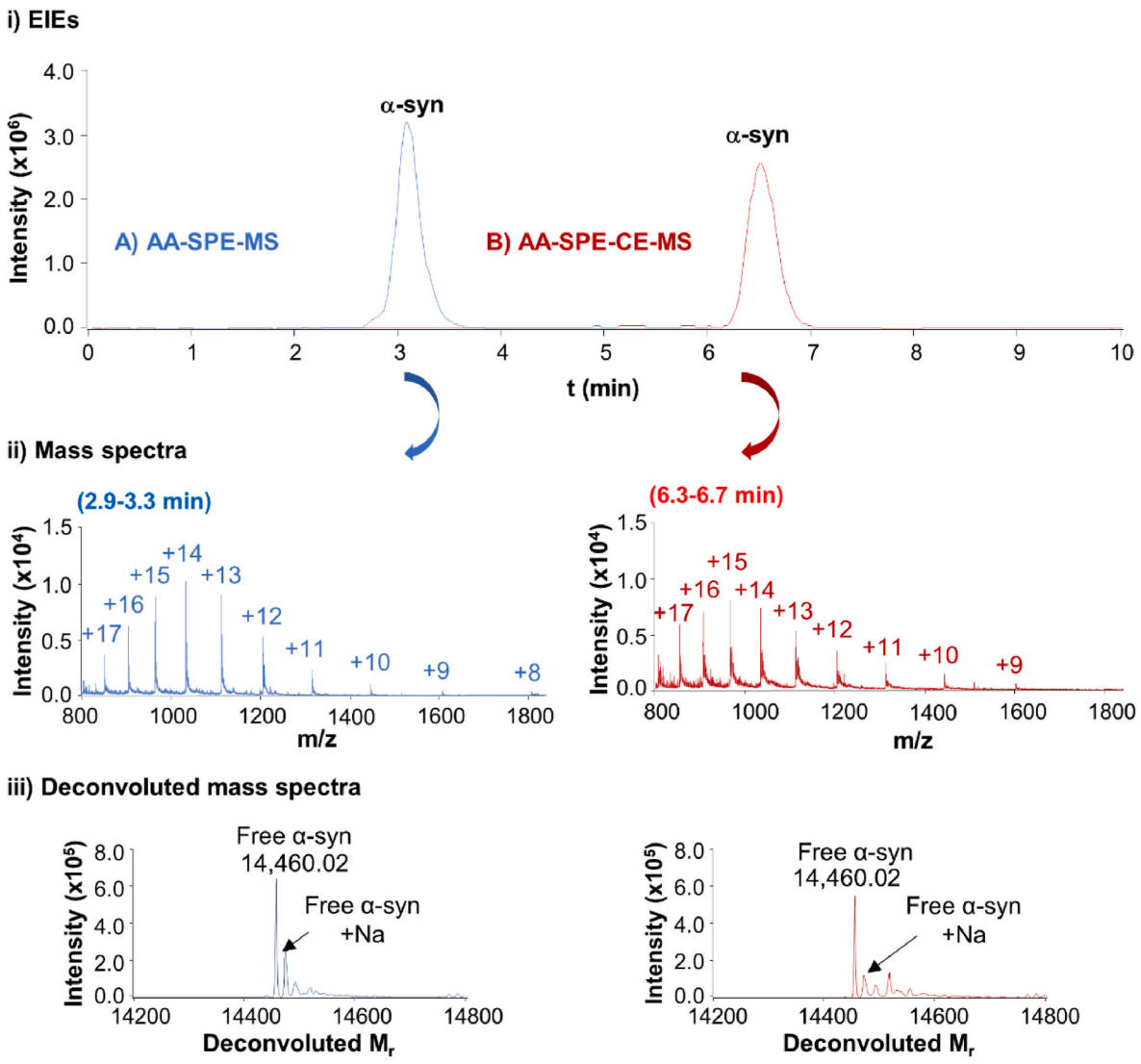

Fig. 3. (A) AA-SPE-MS and (B) AA-SPE-CE-MS for a 2 μg mL^{-1} α-syn standard. (i) Extracted ion electropherograms (EIEs), (ii) mass spectra, and (iii) deconvoluted mass spectra.

Some changes related to the sheath liquid, mass spectrometer parameters, and eluent were studied to prevent peak splitting and enhance sensitivity. First, the HFor concentration in the sheath liquid was increased from 0.05 to 1% (*v/v*), leading to a single but broad protein peak (Supplementary Figs. S2–B). As no further improvements were observed with a 1.25% (*v/v*) of HFor, a sheath liquid with a 1% (*v/v*) of HFor was selected for the rest of experiments. Then, different mass spectrometer parameters were investigated to improve the ionization efficiency, including the nebulizer gas pressure (i.e., 5, 7, and 15 psig), drying gas temperature (i.e., 250, 300, and 350 °C), drying gas flow rate (i.e., 4 and 6 L min^{-1}), and fragmentor voltage (i.e., 275, 325, and 350 V). Only increasing the drying gas temperature from 300 to 350 °C produced an appropriate repeatability and a significant improvement in protein peak width and intensity, probably due to the enhanced spatial focusing and desolvation of the ions (Supplementary Figs. S2–C). Further experiments related with the NH_4OH concentration in the eluent and the eluent volume, did not allow better results. Thus, increasing the NH_4OH concentration from 0.1 M to 0.2 M or 0.5 M decreased protein peak intensity, as well as injecting 40 s instead of 20 s at 50 mbar. This detrimental effect was probably due to the increased ion suppression under these conditions.

Fig. 3-A and 3-B i) show a comparison between the EIEs obtained for a 2 μg mL^{-1} α-syn standard under the optimized conditions by AA-SPE-MS and AA-SPE-CE-MS [29], respectively. As was observed before without preconcentration (Fig. 2), α-syn t_m was shorter by AA-SPE-MS, but protein peak was slightly narrower and higher than by AA-SPE-CE-MS. In addition, no significant differences were observed again between the mass spectra or the deconvoluted mass spectra (Fig. 3 ii) and 3 iii), respectively).

Table 1 shows the quality parameters for AA-SPE-MS and AA-SPE-CE-MS. Under the optimized conditions by AA-SPE-MS, consecutive analyses of an α-syn standard were repeatable in terms of t_m and peak area (%RSD (n = 3) were 1.3 and 6.6% at 1 μg mL^{-1}, respectively). Therefore, repeatabilities were similar to those previously obtained by AA-SPE-CE-MS (%RSD (n = 3) were 2.1 and 5.4% at 1 μg mL^{-1}, respectively) [29]. The AA-SPE-MS method was satisfactorily linear (R^2 > 0.986) in a slightly narrower concentration range (0.025 and 5 μg mL^{-1}) than in our previous study with AA-SPE-CE-MS (0.5 and 10 μg mL^{-1}) [29]. Accordingly, the LOD by AA-SPE-MS was found to be around 0.02 μg mL^{-1}, which was an improvement of 10, 500, and 1000 times compared to AA-SPE-CE-MS, direct MS, and CE-MS, respectively. In addition, no significant differences were observed in the micro-cartridge lifetime by AA-SPE-MS, which could be used for around 20 analyses as by AA-SPE-CE-MS [29].

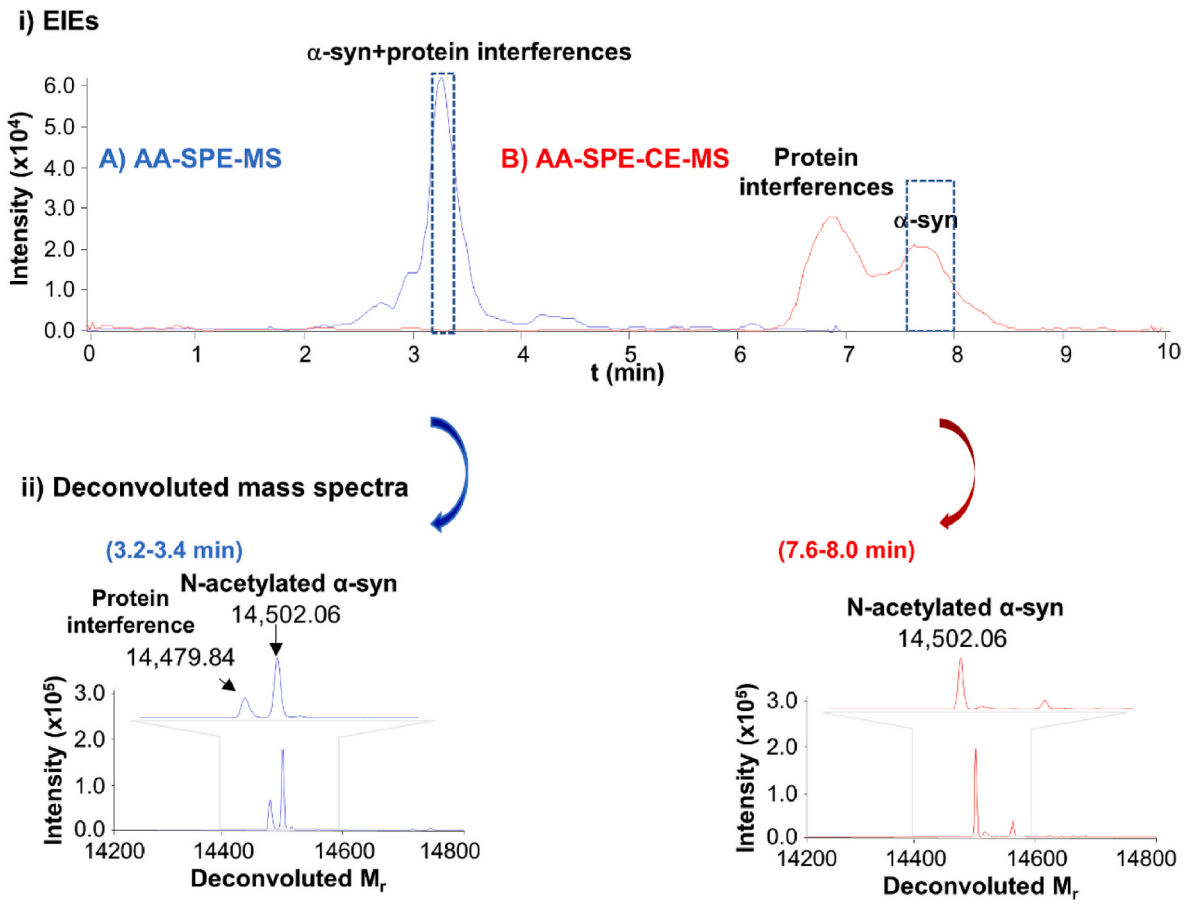

Fig. 4. (A) AA-SPE-MS and (B) AA-SPE-CE-MS for a thermo-enriched red blood cell (TE RBC) lysate sample. (i) Extracted ion electropherograms (EIEs) and (ii) deconvoluted mass spectra. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Analysis of α-syn in blood samples

The applicability of the developed AA-SPE-MS method was evaluated to analyze α-syn from human blood. Blood was selected as sample because the concentration of α-syn is higher than in cerebrospinal fluid, in addition to requiring a less invasive collection. The majority of blood α-syn (>99%) is contained in the red blood cells (RBCs) and the N-acetylated proteoform is the most abundant in both blood and brain cytosol [35–38]. As in our previous studies for the analysis by AA-SPE-CE-MS of blood α-syn at the intact and peptide map levels [29, 30], RBC lysates were prepared from fresh blood and α-syn was thermo-enriched (TE), taking advantage of its thermostability compared to other potentially interfering proteins [37].

Fig. 4-A and 4-B i) show a comparison between the EIEs obtained for N-acetylated α-syn in a TE RBC lysate sample by AA-SPE-MS and AA-SPE-CE-MS, respectively. As expected, the analysis time was shorter by AA-SPE-MS (<4 min), and a single protein peak was detected because there was no electrophoretic separation. In contrast, by AA-SPE-CE-MS, N-acetylated α-syn was separated from the remaining interfering proteins, such as ubiquitin and hemoglobin (subunit alpha and beta), among others [29]. In any case, N-acetylated α-syn could be unambiguously identified due to the excellent mass accuracy and resolving power of the mass spectrometer, as shown in Fig. 4 ii) when the mass spectra corresponding to the α-syn protein peaks were deconvoluted in the M_r range comprised between 14200 and 14800, where N-acetylated α-syn was expected. As can be observed in Fig. 4-A ii) by AA-SPE-MS, the excellent identification capabilities of the mass spectrometer allowed differentiation from N-acetylated α-syn of a protein interference with a very close M_r ($\Delta M_r = -23$). In order to evaluate if disregarding separation in AA-SPE-MS resulted also in ion suppression, TE RBC lysate samples spiked with 0.5, 1, and 2 μg mL^{-1} of recombinant α-syn standard were analyzed by AA-SPE-MS and AA-SPE-CE-MS. As in our previous study [29], recombinant α-syn standard ($\Delta M_r = -42$) was codetected with N-acetylated α-syn at all the spiked concentrations by AA-SPE-CE-MS, but only at 2 μg mL^{-1} by AA-SPE-MS (Supplementary Fig. S3). Supplementary Fig. S3 shows the deconvoluted mass spectra for a TE RBC lysate sample spiked with 1 μg mL^{-1} of α-syn standard, where recombinant free α-syn was only detected by AA-SPE-CE-MS. This confirmed the detrimental effect on detection sensitivity due to ion suppression by AA-SPE-MS, when the electrophoretic separation is discarded after AA extraction for the analysis of complex samples.

4. Concluding remarks

In this study, valve-free AA-SPE-MS was introduced for the rapid purification, preconcentration, and characterization of human α-syn, as a straightforward adaptation from AA-SPE-CE-MS. The adaptation required the typical microcartridges and instrumental set-up necessary for AA-SPE-CE-MS, as well as substituting the separation voltage by a mobilization pressure of 100 mbar and reoptimizing the sheath liquid composition and drying gas temperature in the ESI-MS interface. Analyzing recombinant α-syn standards the method was repeatable, satisfactorily linear between 0.025 and 5 μg mL^{-1}, and α-syn could be detected up to 0.02 μg mL^{-1} in less than 20 min. This LOD was 1000, 500, and 10 times less compared to CE-MS, direct MS, and AA-SPE-CE-MS respectively. When the AA-SPE-MS method was applied to blood samples, ion suppression arose due to sample matrix complexity and other protein interferences were codetected with α-syn. However, the method performance in terms of sensitivity was good enough for the reliable detection and identification of α-syn through accurate and high-

resolution molecular mass measurements. Being aware of this shortcoming, AA-SPE-MS could be also applicable in other cases, with these or others microcartridge designs and instrumental set-ups. However, for some other applications, it would require further and improved off-line sample pretreatments, or the development of novel AA sorbents presenting a larger active surface area, with aptamers of enhanced affinity and selectivity attached to low non-specific binding supports.

CRediT authorship contribution statement

Hiba Salim: Methodology, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Laura Pont:** Methodology, Investigation, Writing – review & editing. **Estela Giménez:** Conceptualization, Supervision, Investigation, Writing – review & editing. **Fernando Benavente:** Conceptualization, Supervision, Investigation, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on reasonable request

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2023.341149.

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On-line aptamer affinity solid-phase extraction direct mass spectrometry for the rapid analysis of α-synuclein in blood

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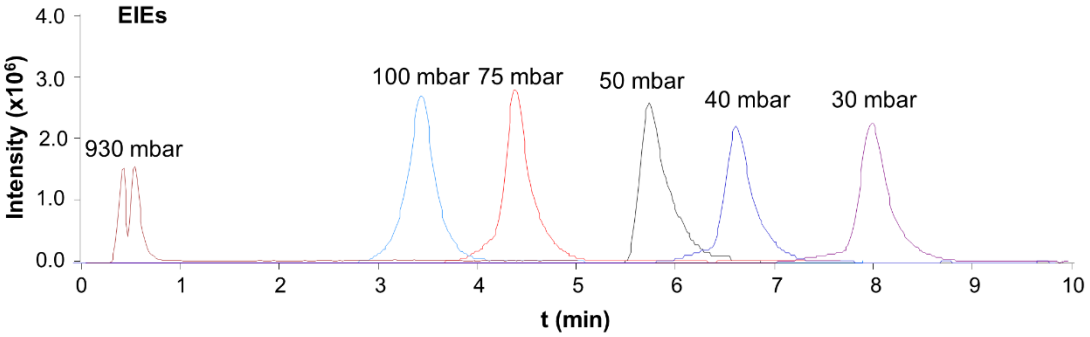
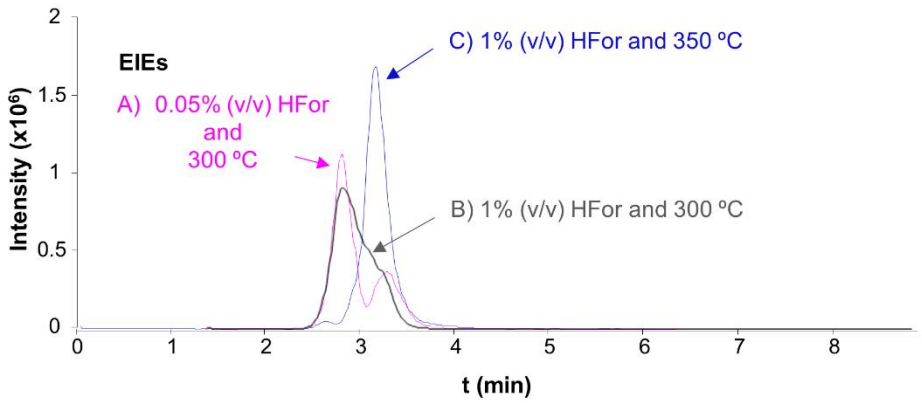
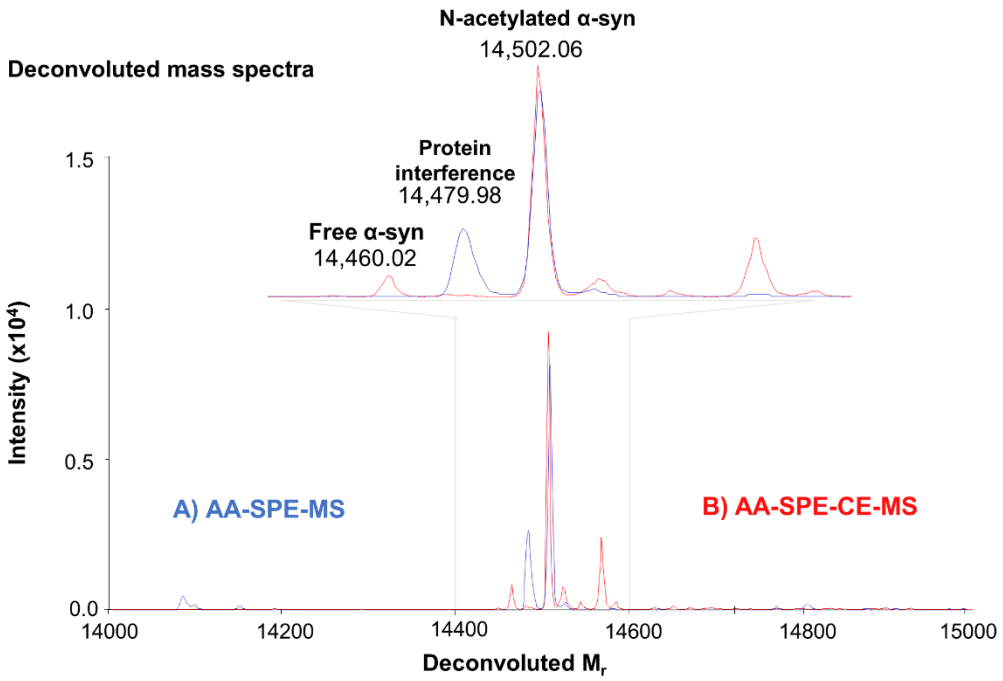


Supplementary Figure S1. Extracted ion electropherograms (EIEs) for a 40 μg·mL^{-1} α-syn desalted standard by direct MS applying different mobilization pressures.



Supplementary Figure S2. Extracted ion electropherograms (EIEs) for a 2 μg·mL^{-1} α-syn standard by AA-SPE-MS with different HFor contents in the sheath liquid and drying gas temperatures, respectively. (A) 0.05% (*v/v*) and 300 ºC, (B) 1% (*v/v*) and 300 ºC, and (C) 1% (*v/v*) and 350 ◦C.



Supplementary Figure S3. Deconvoluted mass spectra for the α-syn protein peak in a TE RBC (thermo-enriched red blood cell) lysate sample spiked with 1 $\mu g \cdot mL^{-1}$ of α-syn standard by (A) AA-SPE-MS and (B) AA-SPE-CE-MS. Note that N-acetylated α-syn was detected by both methods, but the protein interference and free α-syn only by AA-SPE-MS and AA-SPE-CE-MS, respectively.