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PII: S0003-2670(23)00263-5

DOI: https://doi.org/10.1016/j.aca.2023.341042

Reference: ACA 341042

To appear in: *Analytica Chimica Acta*

Received Date: 24 November 2022

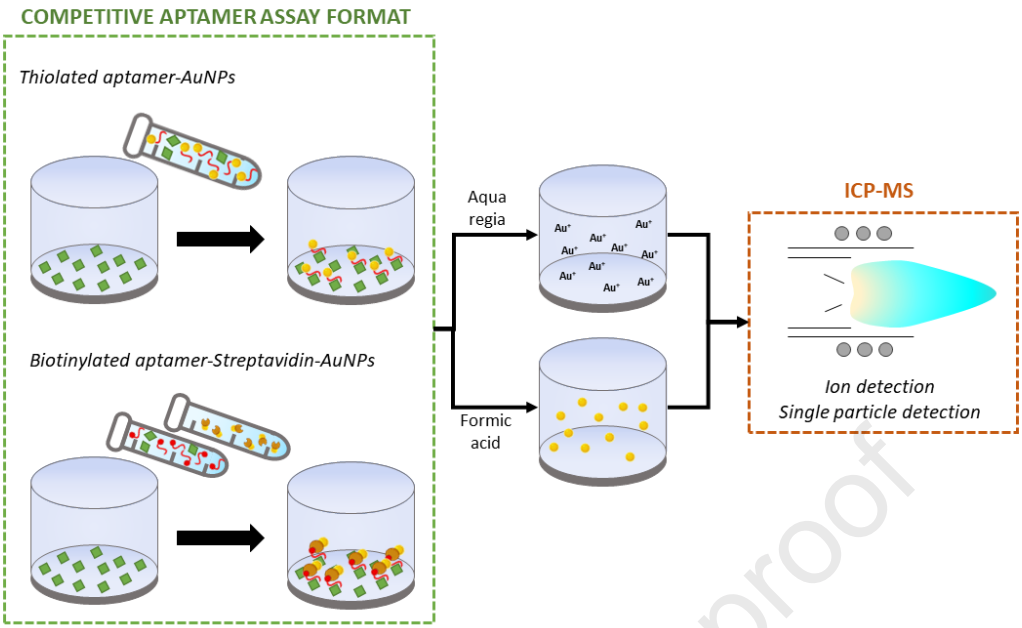
Revised Date: 1 March 2023

Accepted Date: 2 March 2023

Please cite this article as: D. Torregrosa, M. Jauset-Rubio, R. Serrano, M. Svobodová, G. Grindlay, C.K. O'Sullivan, J. Mora, Ultrasensitive determination of β-conglutin food allergen by means an aptamer assay based on inductively coupled plasma mass spectrometry detection, *Analytica Chimica Acta* (2023), doi: https://doi.org/10.1016/j.aca.2023.341042.

CRediT authorship contribution statement

Daniel Torregrosa: Data acquisition, Formal analysis, Writing – original draft, Writing – review & editing. **Miriam Jauset-Rubio:** Methodology, Data acquisition, Formal analysis, Writing – review & editing. **Raquel Serrano:** Methodology, Data acquisition. **Marketa Svobodová:** Methodology, Data acquisition. **Guillermo Grindlay:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Project administration. **Ciara K. O'Sullivan:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision. **Juan Mora:** Formal analysis, Writing – review & editing. All authors: Review the manuscript draft, approved the final version for submission.



Ultrasensitive determination of β-conglutin food allergen by means an aptamer assay based on inductively coupled plasma mass spectrometry detection

Daniel Torregrosa[a], Miriam Jauset-Rubio[b], Raquel Serrano[a], Marketa Svobodová[b], Guillermo Grindlay[a], Ciara K. O'Sullivan[b], Juan Mora[a]

[a]*University of Alicante, Department of Analytical Chemistry, Nutrition and Food Sciences, PO Box 99, 03080 Alicante, Spain.*

[b]*INTERFIBIO Consolidated Research Group, Department of Chemical Engineering, Universitat Rovira i Virgili, 43007 Tarragona, Spain.*

E-mail: guillermo.grindlay@ua.es

Abstract

The overall objective of this work is the evaluation of different competitive aptamer assays based on inductively coupled plasma mass spectrometry (ICP-MS) detection for the determination of β-conglutin (food protein allergen from lupin) in flour samples. To this end, two competitive aptamer assay schemes were developed using either thiolated aptamers chemisorbed onto gold nanoparticles (AuNPs) or biotinylated aptamers linked to streptavidin-AuNPs. The influence of ICP-MS detection mode (i.e., conventional vs single particle) on assay performance was explored. In the case of the thiolated aptamer, the limit of detection (LoD) obtained using the single particle mode was improved 2-fold as compared to the LoD provided by the conventional mode. With regards to the biotinylated aptamer, the use of the conventional mode provided a 5-fold

improvement of LoD as compared to that obtained for the single particle one. Using the optimized conditions, the best LoD of 2 pM was obtained with the biotinylated aptamer operating with conventional ICP-MS detection. When compared to previous reports using the same aptamer in a competitive assay, the developed method significantly improved the LoD by at least an order of magnitude. Different flour samples containing lupin were successfully analyzed according to European Conformity guidelines for the analysis of food contaminants.

Keywords

β-conglutin, aptamer assay, nanoparticles, single particle, inductively coupled plasma mass spectrometry

1. Introduction

Lupin is a leguminous plant employed in the food industry as an alternative to gluten containing wheat (e.g., breads, cookies, pasta, etc.) [1,2], and there have been increasing reports of allergic reactions following ingestion of lupin [3,4]. Lupin-based allergenicity is related to the globulin protein fraction, particularly β-conglutin, and this protein has thus been designated as the Lup an 1 allergen by the International Union of Immunological Societies (IUIS) [5]. Additionally, lupin has been added to the list of substances requiring mandatory advisory labelling on foodstuffs sold in the European Union [6].

Several analytical methodologies have been developed for the determination of β-conglutin in food [4,7-9] including commercially available immunoassays. As an alternative to these techniques, aptamer assays based on different methods detection are attractive due to their simplicity, specificity, and potential high

sample throughput [4,10-17]. An example of this was reported by Svobodová et al. [14] who proposed an ultrasensitive competitive aptamer assay for the determination of β-conglutin in lupin flour based on a combination of aptamer detection with the polymerase chain reaction (PCR), achieving a LoD as low as 85 pM.

Inductively coupled plasma mass spectrometry (ICP-MS) is a well-established technique for elemental analysis at ultratrace levels in many different fields due to its outstanding analytical capabilities (i.e., limits of detection in the ng-pg L^{-1} level, large dynamic range, multi-elemental and multi-isotopic capabilities and high robustness, among others). Furthermore, this technique also allows the analysis of organic molecules provided that an element detectable by means of ICP-MS is naturally present or is intentionally attached to analyte structure through the formation of chemical bond or non-covalent interactions [18]. Thus, ICP-MS has been extensively used as a highly sensitive detector for immunoassays using element-tagged antibodies [19]. Basically, after the immunoassay is completed, the immunocomplex is decomposed with acids and, then, the resulting mixture is directly introduced into the plasma where the element tag is ionized providing a signal that is proportional to analyte levels. Over the years, different element tags have been proposed but metallic nanoparticles (NPs) are usually preferred since they show a great potential for improving sensitivity and LoDs due to the significant number of atoms present in each NP [18,19]. Moreover, this type of tags allows the use of single particle mode for analyte detection (spICP-MS) [20]. In this case, a diluted organic/acid solution is employed to elute NPs without damage and the suspension is introduced into the plasma. By operating microsecond dwell times, each NP

atomized and ionized within the plasma generates a single pulse of intensity. The number of pulses can be correlated to the concentration of the NPs in suspension and, hence, analyte levels. Several reports have highlighted that, for non-competitive bioassays, this mode can improve the LoD, and sample throughput as compared to conventional ICP-MS detection [21-23]. So far, immunoassays based on ICP-MS detection have extensively been employed in clinical analysis for ultrasensitive determination of proteins, hormones, enzymes and even cells [18,19,24], and have also been successfully employed in food security applications for monitoring food allergens [25], antibiotics [26] and toxins [27,28].

Despite methodological similarities with immunoassays, there has been much fewer reports detailing the use of aptamer assays based on ICP-MS detection [29-32], particularly for food security applications. The goal of this work is to develop a new procedure for ultrasensitive β-conglutin determination in foods by the combined use of an aptamer-based assay and ICP-MS detection. To this end, two competitive aptamer assay schemes have been developed using either thiolated aptamers chemisorbed onto gold nanoparticles (AuNPs) or biotinylated aptamers linked with streptavidin-AuNPs (SA-AuNPs). For both approaches, the influence of ICP-MS detection mode (i.e., conventional vs single particle) on assay performance was evaluated. Finally, the analytical methodologies developed have been applied to the determination of β-conglutin in chickpea and soy flour samples spiked with lupin.

2. Materials and methods

2.1. Reagents and materials

All solutions were prepared using ultrapure water (Milli-Q water purification

system, Millipore Inc., Paris, France).

Polyethylene glycol sorbitan monolaurate (Tween 20), chloroauric acid, trisodium citrate and streptavidin from *Streptomyces avidinii* were purchased from Sigma-Aldrich (Steinheim, Germany). Bovine serum albumin (BSA) was obtained from Biowest (Nuaillé, France) whereas phosphate buffer saline (PBS) tablets and F16 maxisorp polystyrene microtiter plates were obtained from Thermo Fisher Scientific (Roskilde, Denmark). Iridium 1000 mg L^{-1} and Au 1000 mg L^{-1} stock solutions were provided by Merck (Darmstadt, Germany). Thiourea, 69% w w^{-1} nitric acid, 35% w w^{-1} hydrochloric acid and 85% w w^{-1} formic acid were purchased from Panreac (Barcelona, Spain). Gold nanoparticles (nominal diameter 40 nm) were obtained from BBi solutions (Cardiff, United Kingdom).

2.2. Oligonucleotides

A β-conglutin aptamer (5'-agc tcc aga aga taa att aca ggg gcc ggg gtg gct cag gca agg ggt tga cct gtc gta ggg att gtt tta aca act agg ata cta tga ccc c-3') previously selected by Jauset-Rubio et al. [15,16,33] and employed in diverse assay formats [34], was used in this work. This aptamer is highly specific against β-conglutin and it shows a limited affinity against other β-conglutin fractions (i.e., α- and γ-) and proteins present in flour samples [33]. Two different versions of this aptamer containing either a thiol or a biotin at 5' were supplied by Biomers (Ulm, Germany).

2.3. β-conglutin standards preparation

Globulin proteins from *Lupinus albus* seeds were extracted with dilute salt solutions, followed by isolation of different globulin fractions by anion-exchange

chromatography and size exclusion chromatography by Sephacryl-200 HR linked to a peristaltic pump [8], as previously reported but with some minor modifications. During the globulin proteins extraction, the pellet was left to air dry overnight instead of 72h and the dialysis step was replaced by the use of a Microcon centrifugal filter to change the buffer and concentrate the obtained extract. Diethylaminoethanol fast flow sepharose resin was used for the anion-exchange chromatography following the program detailed in Table S1. Each fraction was collected after 4 min.

2.4. Solutions and buffers

β-conglutin detection by means of ICP-MS was carried out using two different element tagging schemes based on the use of 40 nm AuNPs. The first one was based on the direct chemisorption of a thiolated aptamer onto AuNPs, carried out following the method described by Jauset-Rubio et al. [15] The second approach was based on linking a biotinylated aptamer with SA-AuNPs. This latter compound was prepared combining citrate-stabilized AuNPs (optical density (OD) of 1) with 12.5 μg mL^{-1} streptavidin followed by a blocking step with BSA 1% w V^{-1}. The suspension was then centrifuged at 15000 rpm (Eppendorf Centrifuge 5417R, Spain) for 30 min, washed three times with 10% w w^{-1} sucrose, 5 mM borate buffer pH 8.8 and 1% w V^{-1} BSA, and resuspended with the same mixture (final OD 10).

The following media were employed for the incubation steps: (i) phosphate-buffered saline (PBS; 10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4); (ii) carbonate buffer solution (15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6); (iii) washing medium (PBS-Tween; PBS + 0.05% V V^{-1} Tween 20); (iv) binding buffer

(PBS + 1.5 mM $MgCl_2$) – this is the buffer that was used in the selection of the aptamer; and (v) blocking medium (2% w V^{-1} BSA in PBS-Tween).

Finally, two different elution media were employed according to the ICP-MS detection mode. A diluted aqua regia (4% V V^{-1} nitric acid and 12% V V^{-1} hydrochloric acid) solution was employed to digest AuNPs for conventional ICP-MS measurements. For spICP-MS, NPs were released from the microtiter plate using a 1 M formic acid solution.

2.5. Aptamer assay procedures

The analysis of β-conglutin by ICP-MS is based on the competitive aptamer assay described by Jauset-Rubio et al. [16] in which varying amounts of free β-conglutin inhibit the binding of specific modified aptamers to the solid phase coated with β-conglutin. Different aptamer assay procedures (i.e., thiolated vs biotinylated aptamers) and ICP-MS detection modes were tested using AuNPs as element tags (see Fig. 1).

2.5.1. Thiolated aptamer assay

For this assay (Fig. 1.A), samples or β-conglutin standards were mixed with the thiolated aptamer-AuNPs suspension and incubated in binding buffer for 15 min at room temperature (step 1a). In parallel, the polystyrene microtiter plate wells were coated with 50 µL of the appropriate β-conglutin concentration in 50 mM carbonate buffer. After a 30 min incubation at room temperature, wells were washed three times with PBS-Tween and blocked with blocking medium for 30 min at room temperature. Subsequently, 50 µL of the β-conglutin/thiolated aptamer-AuNPs mixture were transferred to the plate wells for another incubation

step of 15 min at room temperature (step 2a). Finally, the plate was washed five times with PBS-Tween in order to eliminate β-conglutin-thiolated aptamer-AuNPs complexes present in the solution as well as the excess of the thiolated aptamer-AuNPs.

2.5.2. Streptavidin-AuNPs/biotinylated aptamer assay

In this approach (Fig. 1.B), samples or β-conglutin standards were mixed with the biotinylated-aptamer solution and incubated in binding buffer for 15 min at room temperature (step 1b). In parallel, the polystyrene microtiter plate wells were coated, washed, and blocked as described in Section 2.5.1. In the following step, 50 µL of the β-conglutin/biotinylated aptamer mixture was added to the coated wells and incubated for 15 min at room temperature (step 2b). After washing three times with PBS-Tween, the microtiter plates were sequentially incubated with 50 µL of a 5 µg mL^{-1} SA-AuNPs suspension (OD 0.1) prepared in PBS-Tween for 15 min at room temperature (step 3b).

Irrespective of the aptamer assay, two different ICP-MS detection strategies were evaluated for the determination of β-conglutin: (i) conventional ICP-MS mode (Au^+ signal detection). For this approach, AuNPs were digested for 3h with 150 µL of the acidic digestion mixture and spiked with 50 µL of a 1.0% w V^{-1} thiourea solution containing 10 µg L^{-1} Ir as internal standard; and (ii) spICP-MS mode. In this case, AuNPs were released from the plate surface with 300 µL of 1M formic acid, assisted with 5 min sonication in an ultrasonic bath. These suspensions were diluted 500 times with Milli-Q water prior to spICP-MS measurements. Operating this way, the probability of two or more NPs reaching the ICP-MS detector at the same time is reduced and, hence, pulse signals are directly

proportional to the analyte concentration. All the β-conglutin standards and samples were analyzed in triplicate.

2.6. ICP-MS instrumentation

All ICP-MS measurements were performed by means of an 8900 triple-quadrupole-ICP-MS system (Agilent, Santa Clara, USA) operating either in conventional or spICP-MS modes. Table 1 summarizes the ICP-MS experimental conditions for both approaches. For spICP-MS measurements, dwell time was kept at 100 µs to reduce background and avoid the occurrence of double events [20]. The determination of β-conglutin via conventional ICP-MS detection was performed by means of a calibration curve by using the $^{197}Au^{+}$ ICP-MS signal response against the concentration of β-conglutin. To improve accuracy and precision, the Ir signal ($^{193}Ir^{+}$) was employed as internal standard [27,28]. For the spICP-MS detection mode, the β-conglutin calibration curve was constructed from the number of detected signal pulses (i.e., number of events) derived by NPs atomization/ionization, as previously reported by different authors [21,23]. For both calibration strategies, standards were prepared with concentrations ranging from 0.0001 to 10 nM.

2.7. Analysis of flour samples

Different pure and mixed legume flour samples (i.e., lupin, chickpea and soy) were analyzed following the procedure described by Svobodová et al. [14], with slight modifications. Briefly, 125 mg of dried sample was first defatted with 8 mL of n-hexane and then centrifuged at 6000g for 10 min at room temperature, and the pellet was dried under vacuum. The albumin fraction was then extracted from

the dried pellet using 8 mL of 10 mM $CaCl_2$ and 10 mM $MgCl_2$ (pH 8.0) and then centrifuged twice at 6000g for 30 min at room temperature. To extract the conglutin fraction from the pellet, 8 mL of a solution of 100 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (pH 7.5), 10% w w^{-1} NaCl and 20 mM ethylenediaminetetraacetic acid was used, and then centrifuged at 6000g for 10 min at room temperature. The remaining pellet was removed, and the supernatant was diluted 100 times in binding buffer prior to analysis and used for the determination of the β-conglutin content using the developed competitive aptamer assay.

3. Results and discussion

To date, aptamer assays based on ICP-MS detection have not been widely reported. In this work, for the first time, the influence of the exogenous tag/aptamer conjugation scheme (i.e., AuNPs/thiolated aptamer and streptavidin-AuNPs/biotinylated aptamer) and ICP-MS detection mode (i.e., conventional and spICP-MS) on assay performance have been systematically investigated. Reagent concentrations (coating protein, reporter aptamer, etc.) were optimized using a type of checkerboard titration as previously described [14,27,28].

3.1. Aptamer assay based on AuNPs/thiolated aptamer

The following variables were selected for aptamer assay optimization: (i) concentration of β-conglutin for plate coating (0-180 nM); (ii) AuNPs/thiolated aptamer concentration (0.08-1.25 nM); and (iii) ICP-MS detection mode (conventional/single particle).

First, the optimization of β-conglutin concentration for plate coating was

performed via a direct aptamer assay. To this end, varying concentrations of the protein in carbonate buffer were added to the wells of a microtiter plate and allowed to incubate for 30 min at room temperature. The wells were then washed three times with PBS-Tween, and an excess of the thiolated aptamer solution (5 nM) with AuNPs prepared in binding buffer was added and incubated for 15 min at room temperature. Finally, AuNPs were digested or released as described in Section 2.5.2 for detection. Fig. 2 shows the signal intensity obtained for each β-conglutin concentration used for plate coating. Irrespective of the ICP-MS detection mode (i.e., conventional or spICP-MS), the signal increased with β-conglutin concentration until a plateau was reached at 90 nM. These results were expected considering that the concentration of the coating β-conglutin used is in excess to maximize aptamer capture on the microtiter plate. Consequently, the coating β-conglutin concentration was fixed at 90 nM for further experiments.

The AuNPs/thiolated aptamer concentration was then optimized for a competitive assay (Section 2.5.1). In this case, the goal was to maximize the signal-to-background ratio (SBR) between the blank and the most concentrated β-conglutin standard (i.e., 10 nM). Because of the nature of the competitive aptamer assay, the signal achieved using ICP-MS should be maximum for a blank solution since, in the absence of β-conglutin, all the free aptamer is retained on the plate. Conversely, the signal should be minimum for standards containing a high concentration of β-conglutin in solution as minimal amounts of the aptamer would be left free for binding to the β-conglutin coated on the microtiter plate. Table 2 compiles the signal values obtained for both blank (i.e., maximum aptamer retenction) and 10 nM β-conglutin standards (i.e. minimum aptamer retention) as well as the SBR values. As can be observed, the signals for the blank and 10

nM β-conglutin standards increased with aptamer concentration, regardless of the size of the AuNPs and the ICP-MS detection mode. The signal increase observed for the 10 nM β-conglutin standard suggests the presence of non-specific interaction between AuNPs/thiolated aptamers and the microtiter plate surface [19]. Irrespective of ICP-MS detection mode, the optimum SBR was achieved using an aptamer concentration of 0.3 nM.

Using optimum operating conditions (Table 3), calibration curves for conventional and spICP-MS detection were obtained using β-conglutin standards ranging from 0.0001 to 10 nM (Fig. 3). For the sake of comparison, analytical response in Fig. 3 is expressed as the inhibition factor, defined as $(S_0\text{-}S)\cdot 100/S_0$ where S_0 is the maximal signal obtained with no inhibition (i.e., no β-conglutin was added) and S is the signal observed for each standard. From the corresponding calibration curves, some relevant analytical parameters were calculated for each approach, namely: (i) limit of detection (LoD), calculated as three times the standard deviation of the signal of 15 blank replicates [27]; (ii) half maximal effective concentration (EC_{50}); and (iii) linear range. In general, analytical figures of merit for conventional and spICP-MS detection were rather similar. The LoD obtained using the conventional mode was 200 pM. Single particle ICP-MS was observed to result in a marginal improvement in the β-conglutin detection capability (i.e., 150 pM) as compared with the conventional mode. These results were unexpected since it has previously been observed that, for non-competitive bioassays, spICP-MS improves LoDs between 5- and 10-fold with respect to the conventional mode [21-23]. To explain these findings, it should be considered that, for non-competitive bioassays, the signal is directly proportional to the analyte concentration, and it is easier to distinguish signal pulses for low analyte

standards from the blank solution. For competitive bioassays, however, it is more complex to differentiate signals between the blank and the lowest β-conglutin standards. It must be considered that, because the high number of events detected for these solutions (Fig S1), the uncertainty of measuring pulse frequency is significant (5-10%) thus deteriorating LoDs.

3.2. Aptamer assay based on streptavidin-AuNPs/biotinylated aptamers

The optimization of this assay was carried out in the same manner as the previous procedure but modifying the SA-AuNPs concentration (1.25 to 10 μg mL^{-1}). The optimum β-conglutin coating (Fig S2) and biotinylated aptamer concentration (Table S2) for this assay were identical to that previously observed for the AuNPs/thiolated aptamer assay and no influence of the ICP-MS detection mode was found (Table 3). Fig. 4 shows the influence of the concentration of SA-AuNPs on SBR for both conventional and spICP-MS detection modes, with 5.0 μg mL^{-1} being optimum for both detection approaches. It is interesting to note that background signals for this approach were higher than those previously observed for the thiolated aptamer assay (Tables 2 and S2), thus suggesting higher non-specific adsorption by SA-AuNPs. Nevertheless, sensitivity is enhanced due to the higher number of AuNPs conjugated with the biotinylated aptamer thus giving rise to better SBR values. Fig. 5 shows the corresponding calibration curves for both strategies using these optimized operating conditions. As can be observed, β-conglutin LoD using conventional ICP-MS was lower (5-fold improvement) than that achieved using spICP-MS. Moreover, it was noticed that the dynamic linear range for the latter approach was smaller (Table 3). From these results and considering previous findings for the thiolated aptamer assay, there is no clear

advantage of using spICP-MS over the conventional detection mode for the competitive aptamer assays studied in this work.

Irrespective of the ICP-MS detection mode, the biotinylated aptamer assay performance was better than the thiolated one (Table 3), which can be attributed to the higher number of NPs per aptamer. The LoDs obtained with the biotinylated aptamer and conventional detection were 100-fold better as compared to those obtained with the thiolated aptamer, but this improvement is limited when operating in spICP-MS mode (i.e., 15-fold). Taking these results into account, the biotinylated aptamer assay with conventional ICP-MS detection was selected for further studies.

3.3. Comparison of different methodologies for β-conglutin determination.

As there are no previous reports detailing the determination of β-conglutin via a combination of aptamers and ICP-MS detection, the analytical parameters achieved, such as limit of detection, EC_{50} and linear range, were compared with those reported for other aptamer assays reported in the literature (Table 4). Many of previous reports are based on the same aptamer employed in this work and, hence, it is easy to directly evaluate the benefits derived from using ICP-MS over other detection approaches (e.g., spectrophotometry UV-Vis (UV-Vis); lateral flow assay (LFA); fluorescence resonance energy transfer (FRET); real time polymerase chain reaction (rtPCR); and real time recombinase polymerase amplification (rtRPA)) [14-16]. As it can be observed, when operating with the same aptamer used in this work, the use of ICP-MS results in a 10 to 100-fold improvement in the LoD obtained. These results highlight the potential of using ICP-MS as a detector for aptamer assays. The LoDs obtained were significantly

higher than those achieved by the aptamer assays presented by Jauset-Rubio et al. [15] and Wu et al. [17], but these methodologies included an additional aptamer amplification/preconcentration step. It is expected that by implementing such amplification approaches as well as by increasing AuNPs size [28] or using a more sensitive spectrometer (sector field instruments) [19,35], the LoD could be further improved.

Several works have proposed the use of HPLC-MS methods for lupin determination in foods [4] but few of them have specifically focused on β-conglutin determination. Mattarozzi et al. [36] reported a β-conglutin detection method based on HPLC-ESI-MS/MS which afforded a LoD of 156 pM and a linear range between 0.5 and 170 nM. According to these data, our method affords better LoD (68-fold improvement) but a lower dynamic range (4-fold decrease). Nevertheless, the main benefit of using the aptamer assay with ICP-MS detection lies in sample throughput (10 s $sample^{-1}$), since HPLC-MS is more time consuming (>20 min $sample^{-1}$).

3.4. Method validation for β-conglutin in flour

The biotinylated aptamer assay based on conventional ICP-MS detection has been validated for β-conglutin determination in different flour samples according to the European Conformity guidelines for the analysis of food contaminants [37]. As there is no β-conglutin certified material, accuracy and precision was evaluated by means of a recovery assay. To this end, chickpea and soy flour samples were spiked with known amounts of lupin flour to achieve final β-conglutin concentration levels of 100 and 600 mg kg^{-1} (i.e., approximately 0.05 and 0.3 nM in solution). A total of six replicates were analyzed in each case and

recoveries ranged in all cases from 85% to 115%. These values were within the limits established by the EU for analyte concentrations below 1 mg kg^{-1} (-50%/20%) and demonstrate method trueness. In agreement with previous works [14], the methodology developed was highly specific since no signal change was measured for unspiked chickpea and soy flour samples.

Aptamer assay repeatability (intra-day precision) was evaluated by analyzing six replicates of each sample in the same day. The relative standard deviation (RSD %) of the β-conglutin concentration was within 4-7%. As regards aptamer assay reproducibility (inter-day precision), it was determined by analyzing six replicate samples, which were spiked with the same amount of β-conglutin, on five consecutive days. Reproducibility values range from 5 to 10%. These values agree with previous works combining immuno- or aptamer assays with ICP-MS [27-31].

4. Conclusions

This work demonstrates the ultrasensitive detection of β-conglutin in flour samples using a competitive aptamer assay based on ICP-MS detection. The analytical parameters achieved were dependent on the aptamer assay scheme and ICP-MS detection mode. A better LoD was achieved using biotinylated aptamers linked with streptavidin-AuNPs. According to the results obtained, conventional ICP-MS detection is more advantageous than spICP-MS detection in terms of limit of detection and linear range. Unlike previous findings for non-competitive bioassays, when operating a competitive aptamer assay based on spICP-MS, signals pulses obtained from AuNPs for less concentrated β-conglutin and blank solutions are not easily discernible thus compromising achievable

detection limits. The proposed method improves β-conglutin LoD up to two orders of magnitude as compared to other procedures previously described in the literature. A priori, the β-conglutin LoD could be further enhanced by using more sensitive element tags and mass spectrometer. Similarly, due to the ICP-MS multielement capabilities, it could be feasible to increase the number of food allergens simultaneously measured and, hence, sample throughput. Operating in this way, the use of ICP-MS has clear potential as compared to current detection approaches.

Acknowledgements

The authors would like to thank the Generalitat Valenciana (Project GV/2014/138) and the Vice-Presidency for Research and Knowledge Transfer of the University of Alicante for the financial support of this work (Projects GRE12-19 and VIGROB-050). D. Torregrosa thanks the Spanish Ministerio de Ciencia, Innovación y Universidades for the given fellowship FPU17/02853.

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Table 1. ICP-MS operating conditions for conventional and single particle modes.

	Conventional mode	Single particle mode
Plasma forward power (W)	1550	
Argon flow rate (L min^{-1})		
Plasma	15	
Auxiliary	0.90	0.90
Nebulizer	1.05	0.90
Sample introduction system		
Nebulizer	MicroMist® nebulizer	
Spray chamber	Scott double pass	
Sample uptake rate (µL min^{-1})	340	
Dwell time (ms)	100	0.1
Measuring time (s)	10	60
Nuclides	^{197}Au, ^{193}Ir	^{197}Au

Table 2. Influence of the thiolated aptamer concentration on analyte signal for blank and 10 nM β-conglutin standards. Conventional detection signals are expressed as Au^+ signal in cps. Single particle ICP-MS detection signals are expressed as AuNPs number of events. Values are expressed as mean ± standard deviation, n = 3.

	ICP-MS (Au^+ signal·10^5, cps)			spICP-MS (number of events)		
Standard	Blank	10 nM	SBR	Blank	10 nM	SBR
Aptamer concentration (nM)						
0.08	0.34 ± 0.04	0.10 ± 0.02	3.4	30.4 ± 0.7	8.5 ± 1.4	3.6
0.15	0.52 ± 0.06	0.12 ± 0.04	4.3	66.6 ± 1.0	12 ± 2	5.6
0.30	1.66 ± 0.04	0.20 ± 0.08	8.3	138.7 ± 1.2	20 ± 2	6.9
0.60	3.88 ± 0.14	0.64 ± 0.12	6.1	152 ± 2	30.8 ± 1.4	5.0
1.25	5.1 ± 0.4	0.92 ± 0.06	5.5	160.0 ± 1.5	41 ± 3	3.9

Table 3. Optimum experimental conditions, limit of detection, half maximal effective concentration and linear range for the different aptamer assay strategies developed.

	Thiolated aptamer		Biotinylated aptamer	
	ICP-MS	**spICP-MS**	**ICP-MS**	**spICP-MS**
β-conglutin plate coating concentration (nM)	90	90	90	90
Aptamer concentration (nM)	0.3	0.3	0.3	0.3
Streptavidin-AuNPs concentration (μg mL^{-1})	-	-	5	5
LoD (pM)	200	150	2	10
EC_{50} (nM)	0.9	0.4	0.03	0.1
Linear range (nM)	0.2-2.0	0.1-1.2	0.001-0.08	0.015-0.9

Table 4. Comparison of different β-conglutin detection strategies based on the use of aptamers.

Aptamer	Exogenous tag	Detection technique	LoD (pM)	Linear range (nM)	Reference
Biotinylated[1]	40 nm AuNPs	ICP-MS	2	0.001 – 0.08	This work
Thiolated[1]	13 nm AuNPs	LFA	55	-	15
		LFA-RPA	0.009	-	
Biotinylated[1]	Enzyme (HRP‡)	UV-Vis	34000	-	
Unmodifed[1]	-	rtPCR	20	-	16
		rtRPA	18	-	
		rtRPA with magnetic beads	35	-	
Biotinylated (primary)[1] Thiolated (complementary)[2]	Enzyme (HRP‡) on AuNPs	UV-Vis	0.008	-	17
Biotinylated[3]	Enzyme (HRP‡)	UV-Vis	153000	-	11
Unmodifed[3]	-	PCR	85	1.3 – 68.4	14
Fluorophore-modified[4,5]	Fluorophores Alexa 488 & 555	FRET	150	5 – 20	12

1. 5'-agc tcc aga aga taa att aca ggg gcc ggg gtg gct cag gca agg ggt tga cct gtc gta ggg att gtt tta aca act agg ata cta tga ccc c-3'
2. 5' - ggt ggg ggt gg-3'
3. 5'-agc tga cac agc agg ttg gtg ggg gtg gct tcc agt tgg gtt gac aat acg tag gga cac gaa gtc caa cca cga gtc gag caa tct cga aat-3'
4. 5'-ggt ggg ggt gg-3'
5. 5'-ggt ggg ggt gg-3'

‡ Horseradish peroxidase enzyme

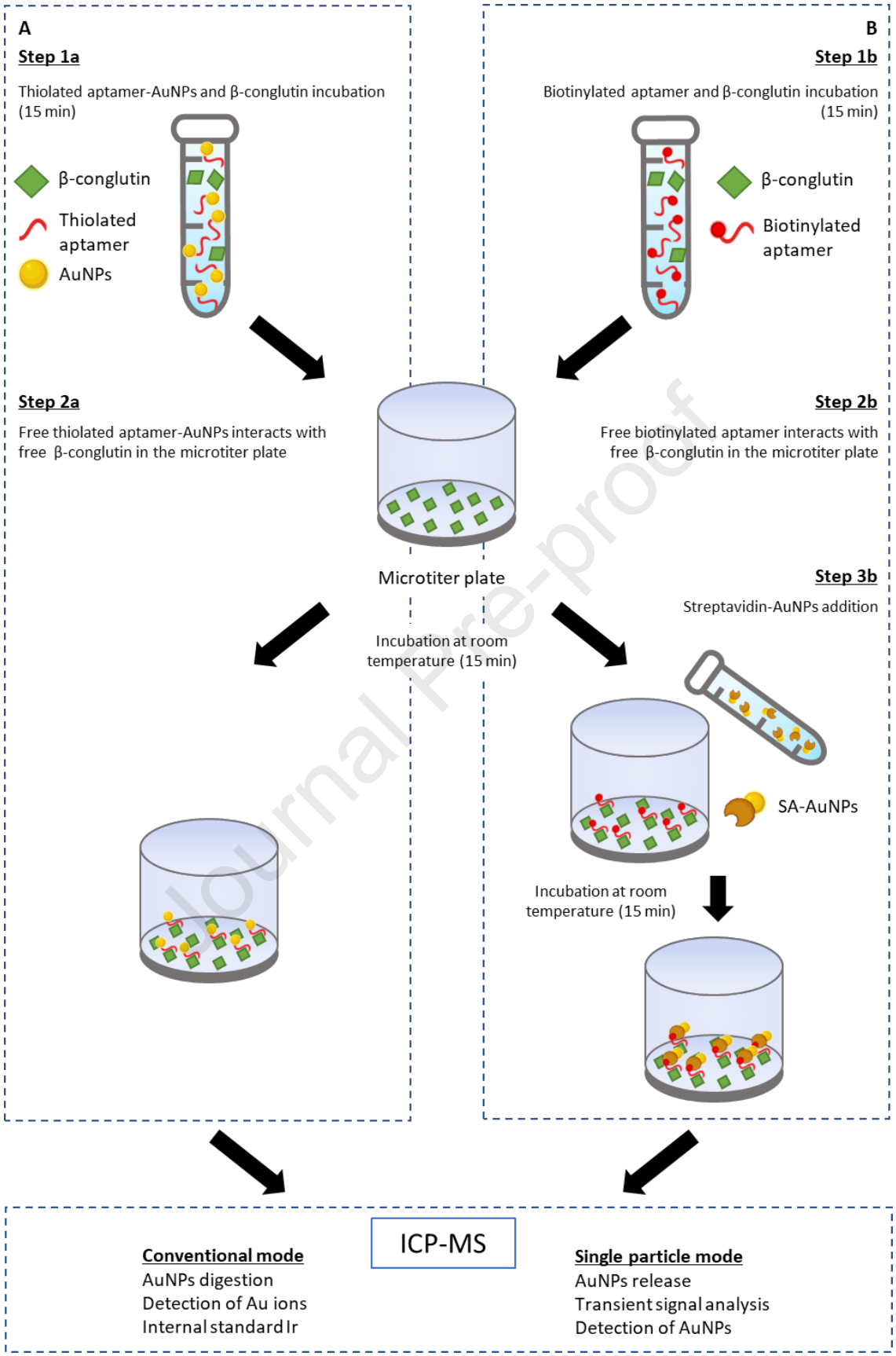

Figure 1. Schematic of the competitive aptamer assays based on ICP-MS detection for β-conglutin determination.

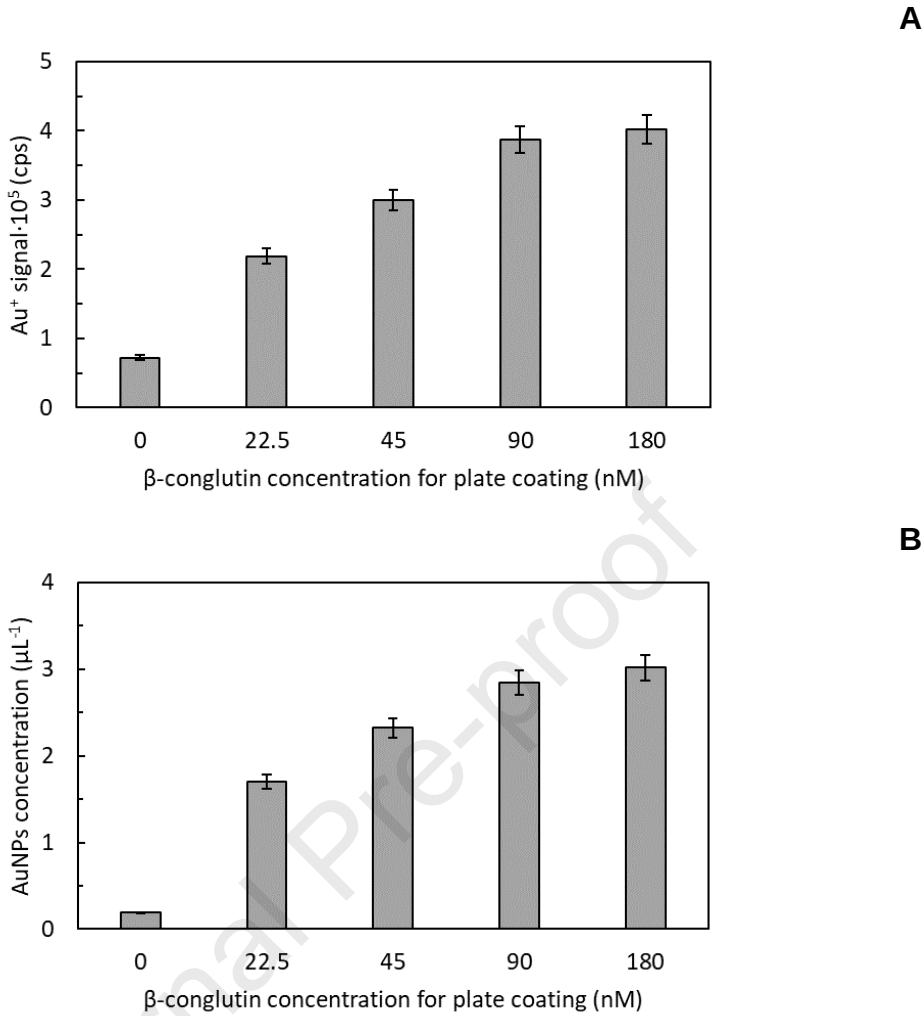

Figure 2. Influence of coating β-conglutin concentration on analyte signal for the thiolated aptamer assay operating (A) conventional ICP-MS detection and (B) spICP-MS detection. Thiolated aptamer concentration 5 nM. Values are expressed as mean ± standard deviation, n = 3.

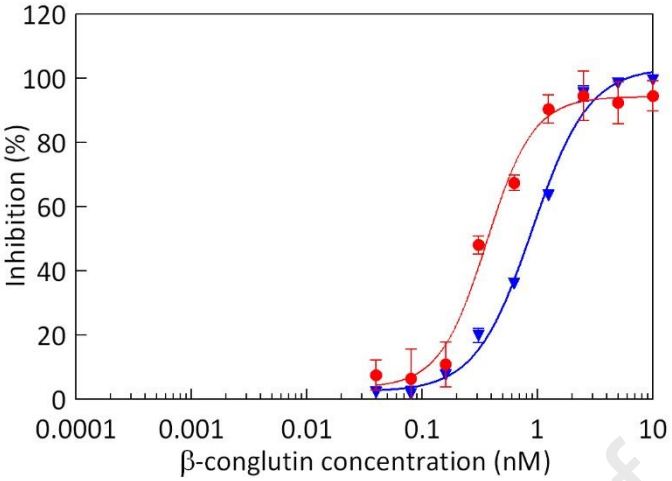

Figure 3. β-conglutin calibration curves for the thiolated aptamer assay using different ICP-MS detection modes: (▼) conventional detection; (●) single particle detection. β-conglutin plate coating concentration 90 nM; thiolated aptamer concentration 0.3 nM. Error bars represent standard deviation for three replicates.

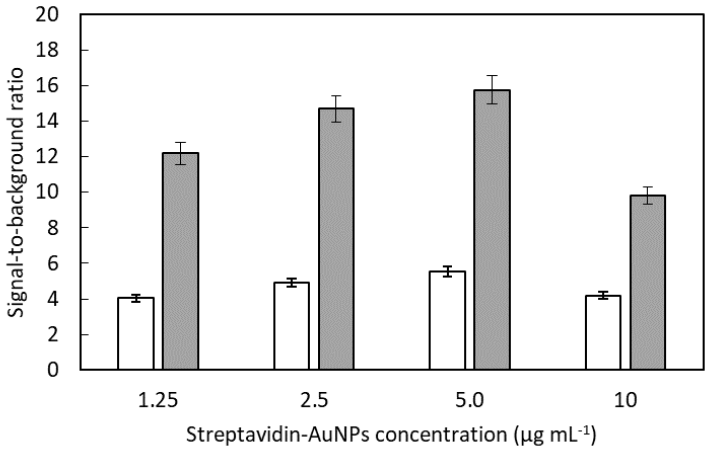

Figure 4. Influence of SA-AuNPs concentration on signal-to-background ratio operating the biotinylated aptamer assay with conventional ICP-MS (white bars) and spICP-MS detection (grey bars). β-conglutin plate coating concentration 90 nM; biotinylated aptamer concentration 0.3 nM. Values are expressed as mean ± standard deviation, n = 3.

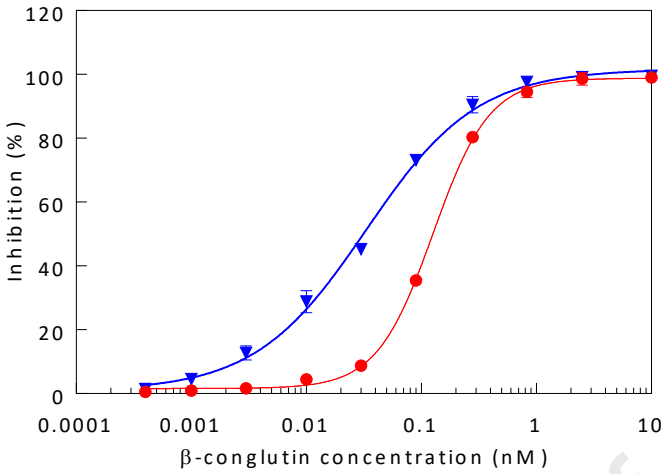

Figure 5. β-conglutin calibration curves for the biotinylated aptamer assay using different ICP-MS detection modes: (▼) conventional detection; (●) single particle detection. β-conglutin plate coating concentration 90 nM; biotinylated aptamer concentration 0.3 nM; streptavidin-AuNPs concentration 5 µg mL^{-1}. Error bars represent standard deviation for three replicates.

Supplementary material

Table S1. Anion-exchange chromatography procedure used for β-conglutin purification. Buffer: 50 mM Tris-HCl (pH 7.5).

Time (min)	% A (Buffer)	% B (1 M NaCl)	Flow rate (mL min^{-1})
0	100	0	0.2
50	100	0	0.2
600	20	80	0.2
610	0	100	0.2
615	100	0	0.2

Table S2. Influence of the biotinylated aptamer concentration on analyte signal for blank and 10 nM β-conglutin standards. Conventional detection signals are expressed as Au^+ signal in cps. Single particle ICP-MS detection signals are expressed as AuNPs number of events. SA-AuNPs concentration: 5 µg mL^{-1}. Values are expressed as mean ± standard deviation, n = 3.

	ICP-MS (Au^+ signal·10^5, cps)			**spICP-MS (number of events)**		
Standard	Blank	10 nM	SBR	Blank	10 nM	SBR
Aptamer concentration (nM)						
0.08	1.60 ± 0.08	0.28 ± 0.10	5.7	65 ± 3	15 ± 3	4.2
0.15	2.94 ± 0.06	0.52 ± 0.06	5.3	148 ± 5	37 ± 10	3.9
0.30	15.22 ± 0.04	0.92 ± 0.08	16.5	490 ± 30	63 ± 12	7.8
0.60	28.46 ± 0.14	2.20 ± 0.16	12.9	650 ± 80	95 ± 14	6.8
1.25	37.2 ± 0.4	4.68 ± 0.06	7.9	880 ± 120	200 ± 50	4.3

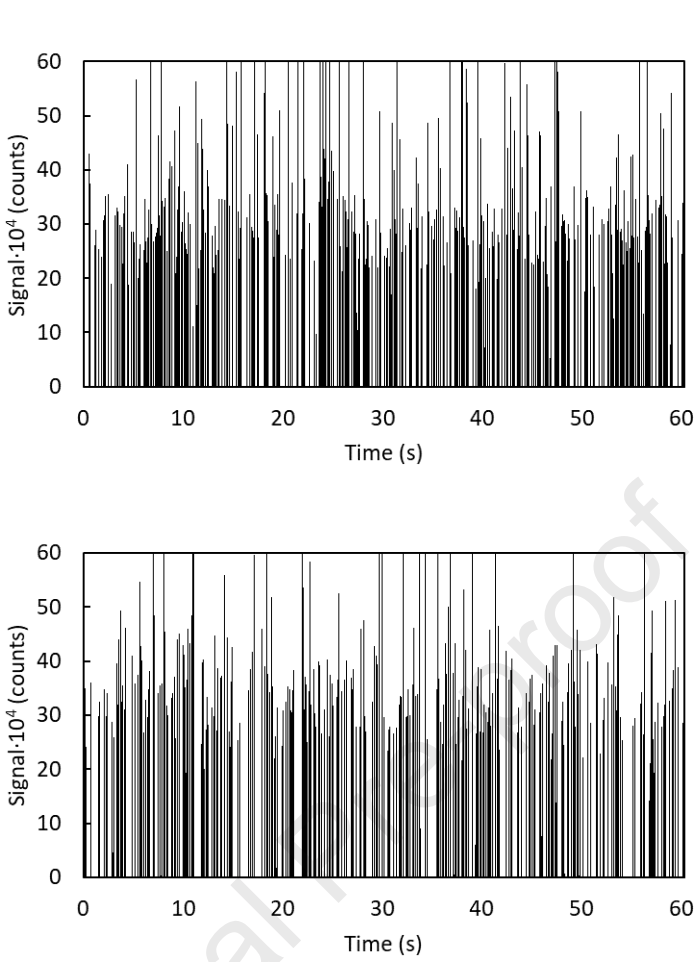

Figure S1. Single particle ICP-MS spectra obtained for (A) a blank standard and (B) a 0.1 nM β-conglutin standard. β-conglutin plate coating concentration 90 nM; thiolated aptamer concentration 0.3 nM.

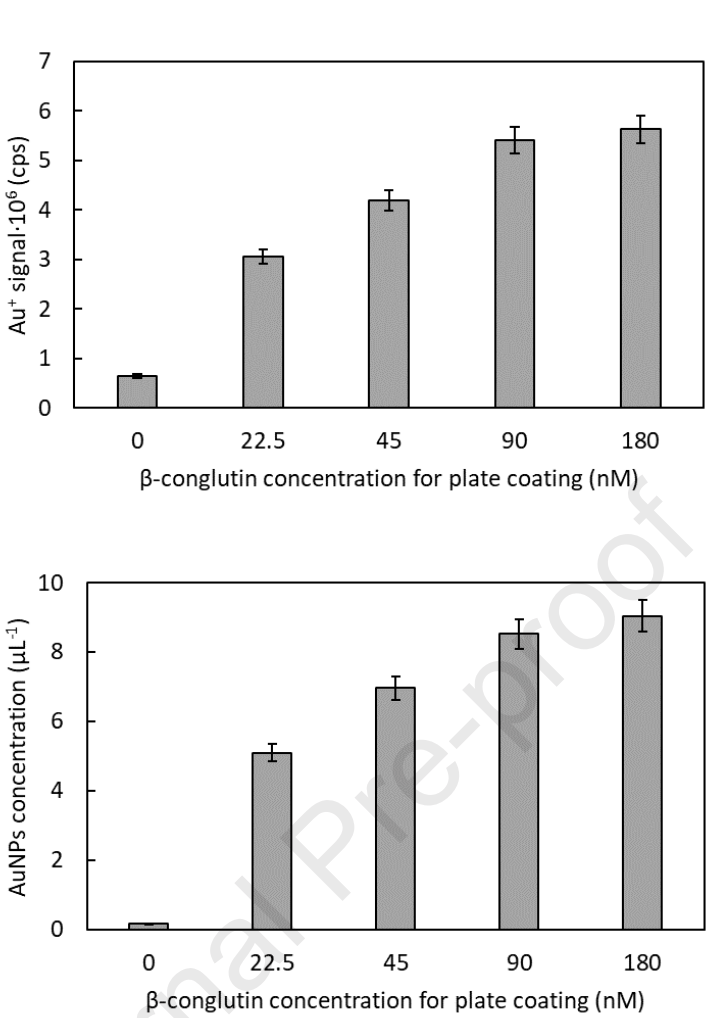

Figure S2. Influence of coating β-conglutin concentration on analyte signal for the biotinylated aptamer assay operating (A) conventional ICP-MS detection and (B) spICP-MS detection. Biotinylated aptamer concentration 5 nM; SA-AuNPs concentration 5 µg mL^{-1}. Values are expressed as mean ± standard deviation, n = 3.

Highlights

- Different aptamer assays based on ICP-MS detection are evaluated for β-conglutin determination.
- Biotinylated aptamers with Streptavidin-AuNPs afford better results than AuNPs-thiolated ones.
- Conventional ICP-MS detection mode provides better LoDs than the single particle one.
- The use of ICP-MS results in a 10 to 100-fold LoD improvement with regard other detection approaches.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: