Development of a production process for a candidate BSA reference material.

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Objectives

Summary
Here we present the development of the production process for a candidate CRM of BSA using fast protein liquid chromatography (FPLC) and a comparison between our candidate and a BSA CRM of NIM. The candidate produced has high purity (≥ 99.1%). Sufficient purity and quality were achieved to continue scale-up and certification efforts in future works.

Background
BSA is a universally accepted standard for total protein quantification. Its target application as a daily working standard is for “quantification of total serum proteins” and also proteins Biotechnology productions, in colorimetric methods.

INTI, INMETRO and CENAM, work in this project, under the support of Inter-American Metrology System (SIM). This development will provide a CRM useful for regional calibration. This is the starting point for Protein CRM production and certification of Latin American & Caribbean countries according ISO NORMS 17.034.

BSA quantification by amino acid analysis ID-MS
The overlapped chromatograms between amino-acids predicted and amino-acid hydrolyzed (– and –) and a low variation coefficient between replicates ≤ 2% are an additional clue that correlated with the high purity previously found.

Purity & DDA analysis
<table>
<thead>
<tr>
<th>Sample</th>
<th>HPLC-UV - BSA</th>
<th>RSD %</th>
<th>MS - Purity - BSA</th>
<th>RSD %</th>
<th>N° proteins identified by DDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA NIM</td>
<td>99.96%</td>
<td>0.01</td>
<td>99.96%</td>
<td>0.01</td>
<td>11</td>
</tr>
<tr>
<td>21-BSA</td>
<td>99.13%</td>
<td>0.17</td>
<td>99.49%</td>
<td>0.02</td>
<td>28</td>
</tr>
</tbody>
</table>

The purity of the candidate by mass spectrometry or by HPLC-UV was ≥ 99% and the same for CRM from NIM.

More traces of proteins on average appeared in the Candidate, by Bottom up proteomics, blasted against bovine plasma proteins.

Analysis & conclusion
21-BSA candidate was chosen between four candidates. On this fast screening, 3 point were achieved for the selection of the candidate. 1. High Purity (≥ 99.1%). 2. High production yield more than 30 g of candidate per liter of resin used. 3. Low cost and local production of the raw material used to produce the candidate (see production).

In all cases, the candidate showed purity ≥ 99.1% (see analytic and comparison tables and SDS-PAGE figure), regardless of the method used. Also, on top of the amino acid analysis a low standard deviation was found between 5 different amino acids. This Candidate under development has therefore been highly purified.

Other plasma proteins were detected in the candidate (by DDA analysis blasted against bovine plasma proteins). To move forward with this project, less than 1% of impurities was considered acceptable. This development sets the basis for the production and certification efforts in our next step.

To achieve the use of the reference material, a commutability study with colorimetric methods should be performed.

Identification of the candidate
The majority of the sequence was found; this was a key step to characterize the candidate and for the quantification of BSA by amino-acid analysis. Also, BSA was identified.

BSA coverage sequence detected 87,52 %

FPLC production process N° 21

BSA Fraction collected
Conductivity Salt % UV 280nm

Amount of 21-BSA candidate produced: 0.162g, purity ≥ 99%

Inter-comparison
In order to compare amino-acid analysis results, we are interested on performing a ID-MS comparison with other NIMs, during 2019. Contact the author.

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