Informed manufacturing process development of a recombinant botulinum neurotoxin through the use of a sensitive and reproducible sandwich ELISA

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Introduction

Development of a downstream process to purify therapeutic proteins requires quantitation of the target protein in order to calculate column capacities and evaluate yields and losses. During the initial stages of purification, samples comprise a complex mixture of cellular proteins, DNA and RNA along with the target. Quantitation of the target protein within this mixture requires a method specific to the target protein.

Immunoassay techniques provide a quantitative method specifically detecting target analytes within complex mixtures. Ipsen Bioinnovation Ltd. has developed a sandwich ELISA with ng/mL levels of sensitivity for a recombinant Botulinum neurotoxin of the E serotype (BoNT/E). The development of a capture column for this recombinant BoNT has been successfully achieved, with appropriate sizing and step gradients, using quantitation from this sandwich ELISA.

Methods

ELISA

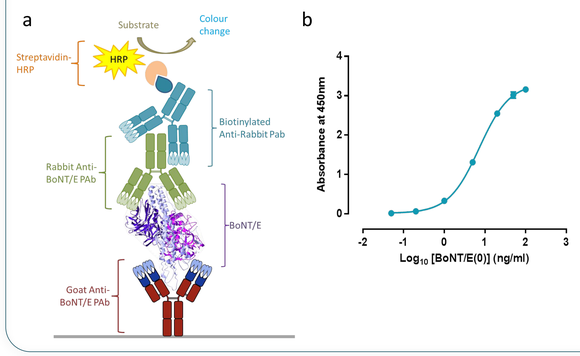
- Detection of the target BoNT was achieved using the sandwich ELISA outlined in Figure 1.
- Matrix effects (interference from other molecules present in the samples) were assessed by addition of purified target BoNT of known concentration (spikes) to cell lysates from host cells containing plasmid without the BoNT gene insertion.
- Assay accuracy was determined from the deviation of the measured concentration from the expected concentration (spike recovery), across the assay range.

Experiments to assess column capacity

- A capture column was loaded with an excess of lysate feedstock and the flow-through fractionated , as shown in the schematic in Figure 2a.
- The concentration of the target BoNT in the lysate loaded onto the column was determined via the sandwich ELISA.
- For efficiency, a constant dilution factor was used for all fractions, such that 10% of the loaded BoNT concentration would be within the linear region of the curve and could be quantitated.
- Column capacity was determined as the total mass of BoNT loaded onto the column when 10% of the target was detected in the flowthrough.

$$\begin{aligned} &Column\ capacity\ (mg\ of\ BoNT) \\ &= Concentration\ of\ BoNT\ in\ Load\ (mg/mL) \\ &\times Volume\ at\ which\ 10\%\ of\ BoNT\ in\ Load\ detected\ in\ Flowthrough \end{aligned}$$

Figure 1. Sandwich ELISA for the detection and quantitation of recombinant BoNT, serotype E, with a schematic representation of the assay format (a) and example dose-response curve obtained (b).



Results

ELISA

- Measured spike concentrations were found to be within 20% of the expected concentration. This level of accuracy was confirmed within each run through incorporation of spiked-samples as intra-assay controls.
- The lower limit of quantitation was empirically determined as 0.5 ng/mL.
- Sample quantitation was targeted between 1 and 10 ng/mL, to fall in the linear region of the curve.

Table 1. Accuracy data for recovery of BoNT from blank lysates

Concentration in Lysate (µg/mL)	Dilution Factor	Final Concentration (ng/mL)	Accuracy of recovery (%)
300	100,000	3	101
200	20,000	10	94
100	10,000	10	94
10	1,000	10	101
5	500	10	100
5	1,000	5	98

Column capacity

- Detection of BoNT at 10% of the loaded concentration (breakthrough) was detected in the flowthrough following loading of 89 mL of clarified lysate.
- Binding capacity was set at 80% of the measured breakthrough volume to ensure complete capture of the target, allowing for potential variation in the load.

Step gradient development

- Target elution profile determined using the sandwich ELISA was calculated and compared to elution profile at 280 nm (Figure 3).
- Salt concentration range over which the target elutes calculated
- Comparison with purity measured by SDS-PAGE allowed maximal clearance of host cell protein impurities, whilst minimising losses of the target BoNT.
- Salt concentrations for stepped elutions were calculated and empirically refined using ELISA data to map the product capture and losses.
- A highly efficient capture process was achieved, capturing 80% of the target BoNT (Figure 3).

Figure 2. Capture column capacity was determined using an excess of lysate and monitoring the flowthrough, as represented in the schematic in (a). ELISA analysis of BoNT in the flowthrough determined the volume at which 10% of BoNT in the load eluted in the flowthrough without binding to the column (b).

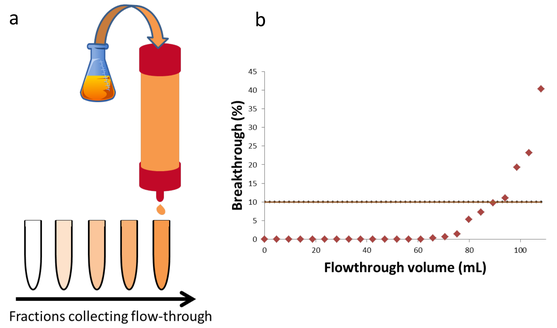
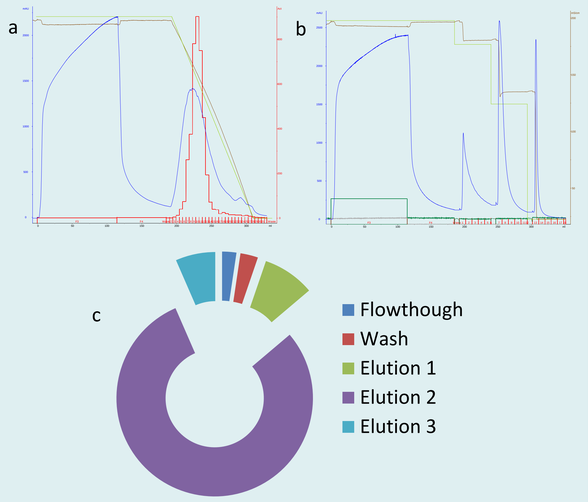


Figure 3. Chromatograms showing elution profiles obtained with (a) a linear NaCl concentration gradient and (b) the step gradient developed using ELISA data.



Traces in blue show the absorbance measured at 280nm (total protein). Traces in green and brown are the programmed gradient and measured conductivity, respectively. The red profile in figure 3a shows the concentration of target BoNT in each fraction, measure by ELISA. The chart in part c shows the proportion of the target BoNT measured in the flowthrough, wash and eluted with each step of the gradient. Eighty percent of the target was recovered in step 2.

Conclusions

- A sensitive sandwich ELISA was developed to quantitate BoNT/E, and shown to be able to accurately quantitate our target BoNT in complex matrices.
- The column capacity for the selected capture column was determined through detection of the target BoNT in the flowthrough, allowing appropriate scaling.
- The elution profile of our target BoNT could be dissociated from the total protein elution profile due to the selectivity of the ELISA, thereby permitting an informed development of the step gradient elution for improved manufacture.

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