

QUALITY CONTROL ANALYSIS OF GLYCOPROTEINS



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1. Introduction

Biopharmaceuticals are of ever increasing importance in the pharma industry, with the current market share of over 20% expected to rise steadily year after year¹.

The efficacy and safety of protein based pharmaceuticals are influenced not only by primary, secondary and tertiary structure, but also by various post translational modifications (PTM), with glycosylation being the most common. Various techniques exist to characterise glycosylation in terms of occupancy of glycosylation sites, and identification of glycoforms, however full characterisation is time consuming and requires a range of techniques and instrumentation (Fig 1).

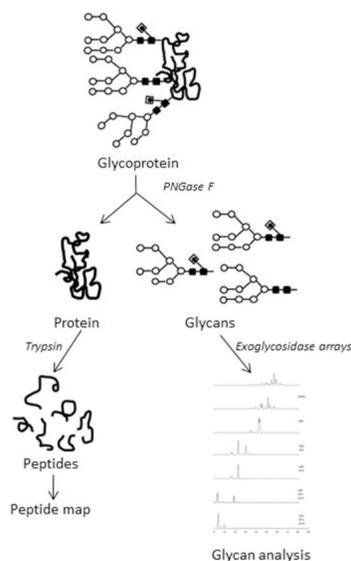


Figure 1: Characterising protein glycosylation

Once a glycoprotein is fully characterised, ongoing quality control analysis would preferably utilise simpler and faster modes of analysis, which are nevertheless able to detect any changes in the degree and pattern of glycosylation.

Here we compare two techniques for the analysis of intact glycoprotein Ribonuclease (RNase) B.

2. Methods

- **nanoLC/QTOF**
Agilent 6530 QTOF with chip cube technology
Agilent Zorbax 300SB-C18 chip (5µm 30Å 75µm x 150mm with 40nL enrichment column)
- **HPCE/UV**
deltaDOT High Performance Capillary Electrophoresis system with 512 diode array detector
50cm *50µm bare fused silica capillary
SDS run buffer

The methods were applied to the analysis of mixtures of RNase B and its nonglycosylated form, RNase A.

3. Results

Extracted ion chromatograms of the separation of RNase A and RNase B by nanoLC/QTOF are shown in Fig 2.

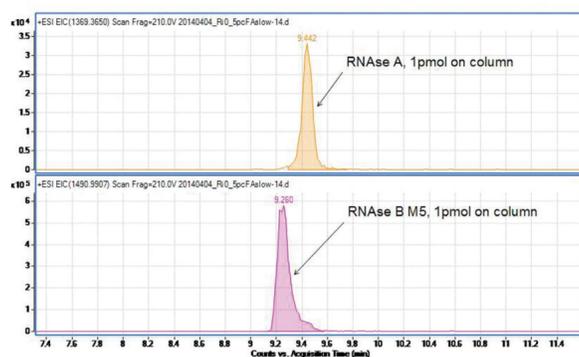


Figure 2: nanoLC/QTOF of a mixture of RNase A and RNase B

The mass spectra of RNase A and the glycoforms of RNase B in Fig 3 demonstrate how the glycoforms are clearly distinguishable by their mass/charge ratio and their relative abundance.

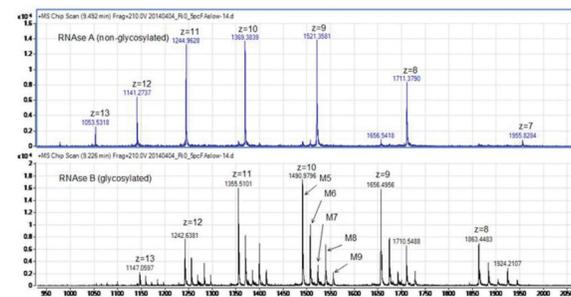


Figure 3: Mass spectra of RNase A (top trace) and 5 glycoforms of RNase B (bottom trace)

The HPCE/UV chromatograms of mixtures of RNase A and RNase B in Fig 4 exhibit clear separation of RNase A and near baseline separation of the five glycoforms of RNase B.

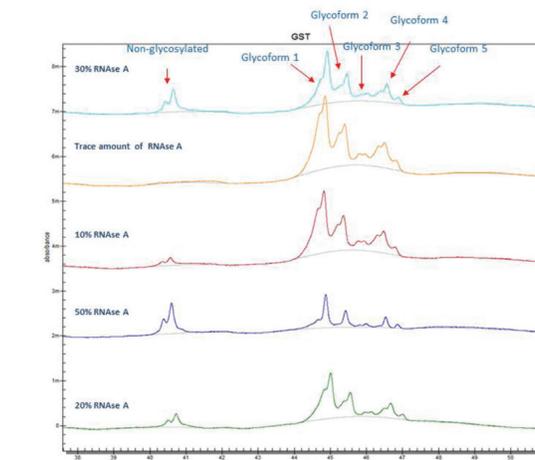


Figure 4: HPCE/UV of mixtures of RNase A and RNase B containing trace amounts, 10%, 20% 30% and 50% RNase A

Figures 5 and 6 depict the results of repeat analysis (n=5) by nanoLC/QTOF and HPCE/UV of samples containing varying amounts of RNase A and glycoforms of RNase B.

The techniques gave comparable results in terms of standard deviation and relative abundance of the analytes. Any small differences between the percent of total peak area observed by MS and UV are due to different response factors between the two detectors.

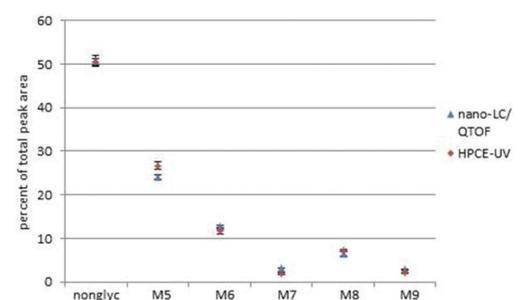


Figure 5: Percent of total peak area in a sample containing a mixture of nonglycosylated RNase A and 5 glycoforms of RNase B by nanoLC/QTOF (blue) and HPCE/UV (red). Error bars signify the standard deviation of 5 repeat injections

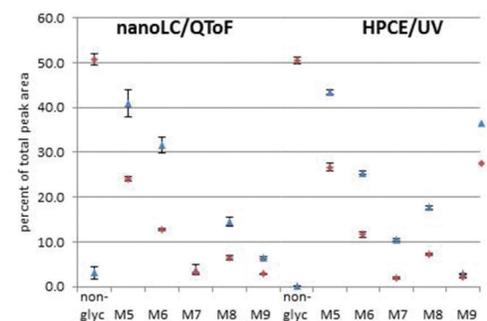


Figure 6: Percent of total peak area in two samples of RNase A/B. Sample A contains predominantly glycosylated RNase, whereas sample B contains a mixture of RNase A and 5 glycoforms of RNase B

4. Conclusion

The comparison of nanoLC/QTOF and HPCE/UV for the analysis of glycoprotein RNase B showed that both techniques are amenable for monitoring the glycosylation pattern for changes such as loss of glycosylation or changes in the relative abundance of the various glycoforms.

5. References

- ¹ EvaluatePharma World Preview 2015, Outlook to 2020. 8th edition, June 2015.

6. Acknowledgements

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HPCE/UV analysis was carried out by deltaDOT Ltd, London.