ALMAC

Partnering to Advance Human Health

Case study

Precision in Biologic Development: Leveraging Orthogonal Techniques for Superior Insights

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Introduction

In biologic development, having precise analytics is key to maintaining product quality and driving progress in clinical research. At Almac Sciences, our commitment to innovation is reflected in the rigorous development and validation of orthogonal analytical techniques. This article highlights a recent project focused on quantifying maleimide reactivity as a critical parameter in conjugation chemistry using advanced chromatographic methods, and shares key lessons learned from evaluating commercial solutions.

Evaluating commercial solutions:

The project commenced with the assessment of the Amplite® Colorimetric Maleimide Quantitation Kit, a commercial assay designed for quantifying maleimide groups. Despite its intended purpose, our evaluation revealed significant limitations. The kit produced random absorbance values across dilution series, with no consistent or theoretical correlation to expected molar ratios. After consulting with the Amplite kit technical support team, it was clarified that the kit is designed primarily for protein conjugation reactions and has not been fully evaluated for use with peptides, which have different physicochemical properties. This lack of reproducibility and interpretability led to its discontinuation for our application, underscoring the necessity of validating commercial kits within the context of specific biologic workflows.

Absorbance 1	Absorbance 2	Absorbance 3
2.797	1.148	1.139
2.102	2.168	0.733
0.732	0.129	0.129
1.708	1.750	0.923
0.923	2.948	2.866

Chromatography method development: through orthogonality

Recognising the need for a more robust approach, an orthogonal chromatographic method to quantify maleimide reactivity conjugated to peptide is developed. The method centers on the interaction between maleimide and thiol-containing compounds, such as cysteine and mercaptoethylamine (MEA). The maleimide group is known to selectively react with sulfhydryl-containing species like cysteine and MEA. Upon conjugation, the resulting product is expected to exhibit reduced polarity compared to the unreacted Mal-Furin-CMV peptide. This change in polarity is anticipated to produce distinct chromatographic behaviour, enabling the separation of conjugated and unconjugated species based on retention time shifts in reverse-phase chromatography.

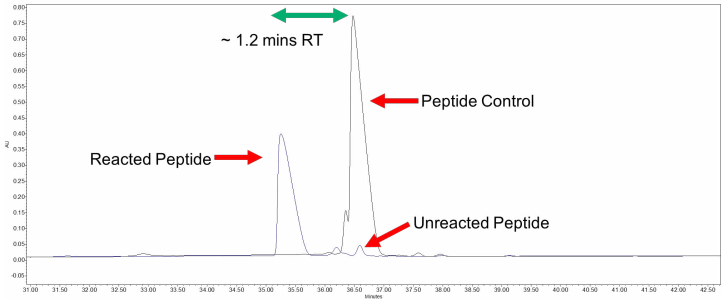
Key findings

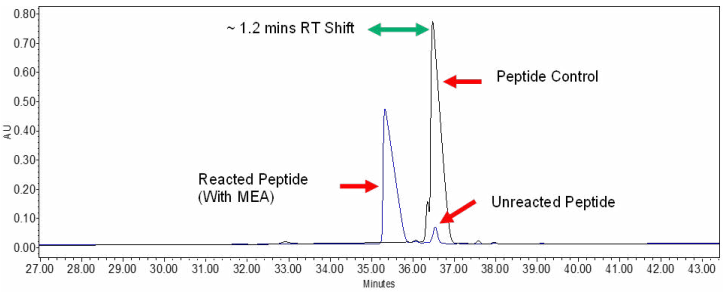
• High reactivity and specificity:

Both cysteine and MEA demonstrated strong reactivity with maleimide, with cysteine consistently achieving conversion rates above 98-99%. The retention time shift confirmed successful conjugation and method specificity.

• Reproducibility and robustness:

The method showed excellent reproducibility, with consistent results across experiments and conditions. Cysteine was ultimately preferred due to superior performance.





Conclusion

This orthogonal approach to maleimide quantification exemplifies Almac's commitment to analytical excellence. The developed chromatography method enables precise monitoring of conjugation efficiency, supporting advanced biologic development and quality assurance.

By critically evaluating commercial solutions and developing robust orthogonal methods, Almac Sciences continues to advance precision in biologic development. This project demonstrates the value of innovation, and continuous optimisation in delivering reliable analytical results for the next generation of biologics.

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