When Mass Isn't Enough:

MS/MS FOR CONFIDENT SIALIC ACID ID

Eliminating Immunogenic Risk from Glycan Profiles with Marker Ion Detection

A late-stage developer preparing for regulatory submission needed to confirm the identity of sialic acid species in their glycan profile. While standard mass spectrometry detected what appeared to be NANA — a sialic acid commonly found in humans — there was no way to rule out isobaric species that could interfere with the result. Specifically, structures like NGNA+Fuc or NANA+Gal have the same molecular mass as NGNA and can appear identical in traditional MS, potentially masking the presence of a non-human, immunogenic form of sialic acid. To eliminate this uncertainty and protect both patient safety and submission timelines, the team turned to Solvias for definitive MS/MS-based confirmation.

THE CHALLENGES

- **Isobaric Interference:** In mass spectrometry, "isobaric" refers to molecules that have the same nominal mass but different structures. NGNA, NANA+Gal, and NGNA+Fuc all share identical molecular weights and can produce overlapping signals in MS. Without further resolution, this makes it impossible to distinguish them based on mass alone.
- **Immunogenicity Risk:** Unlike NANA (N-acetylneuraminic acid), which is the primary sialic acid in humans, NGNA (N-glycolylneuraminic acid) is not naturally expressed in humans and has been linked to unwanted immune responses. If present in a therapeutic glycan profile, NGNA must be excluded from the final product to meet safety expectations.
- **Regulatory Scrutiny:** Global regulators increasingly expect manufacturers to demonstrate that all glycan structures, especially those with known immunogenic potential, are correctly identified. Inaccurate or incomplete sialic acid characterization can lead to regulatory questions or delays during filing, particularly in late-stage development.

OUR COLLABORATIVE APPROACH

- **MS/MS-Based Structural Resolution:** Solvias applied tandem mass spectrometry (MS/MS) to fragment the glycan ions detected in the initial MS analysis. This enabled the team to generate distinct marker ions that are unique to NGNA, allowing it to be confidently identified — or ruled out — based on structure rather than just mass.

- **Differentiating Lookalike Species:** The isobaric combinations NANA+Gal and NGNA+Fuc can mimic NGNA in MS, but not in MS/MS. Solvias' approach enabled a side-by-side comparison of fragmentation patterns, making it possible to discriminate between safe and immunogenic forms even when the precursor ions were indistinguishable.

- **Orthogonal Confirmation of NANA:** MS/MS confirmed that the sialic acid present in the product was NANA — the biologically appropriate form found in human glycoproteins. This confirmation ruled out NGNA and its lookalikes with structural certainty, reinforcing the product's safety profile.

- **Clear Reporting for Regulatory Confidence:** Solvias provided a fully annotated dataset showing marker ion detection and fragmentation profiles, supported by expert interpretation. This ensured the customer could present a clear, defensible case to regulators regarding the absence of immunogenic glycan species.

THE RESULTS

- NGNA definitively ruled out through structural confirmation, not inference.
- Presence of human-compatible NANA confirmed with MS/MS fragmentation.
- Regulatory package strengthened with direct evidence of glycan safety.

CONCLUSION

While standard MS can suggest the presence of safe glycan structures, it cannot always distinguish them from lookalike molecules that pose serious clinical or regulatory risk. This case demonstrated how MS/MS-based sialic acid confirmation is essential when isobaric interference is possible — especially in late-stage products where any ambiguity may delay approval or compromise patient safety. Solvias helped the customer not only protect their submission, but also reinforce product quality and confidence across internal teams, quality reviewers, and global regulatory bodies. As glycan analysis becomes increasingly nuanced, Solvias' MS/MS expertise ensures that what looks safe truly is — on both paper and in practice.

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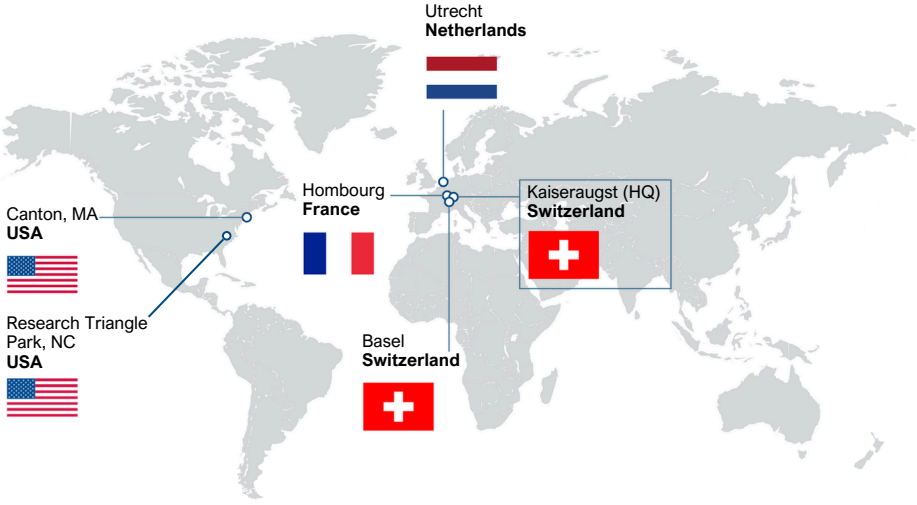

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