# NEW APPROACH USING ON-LINE PURIFICATION AND PROCAINAMIDE FOR HILIC-FLR-MS N-GLYCAN ANALYSIS

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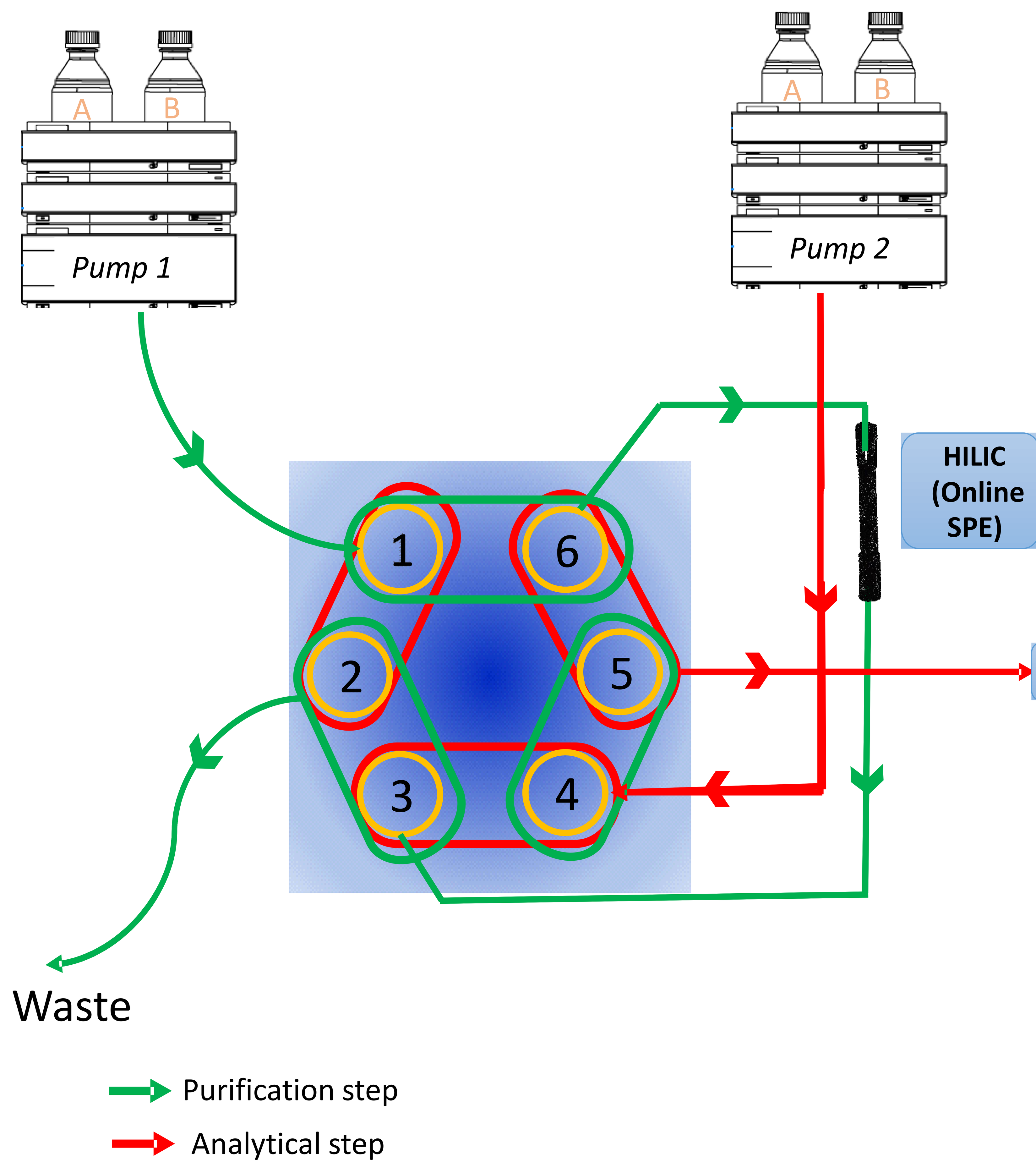
## Introduction:

N-glycosylation is one of the most prominent and ubiquitous post translational modification of protein. It may affect several characteristics of the protein: half-life, immunogenicity, activity . mAbs become the dominant class of approved biotherapeutics du to their successful treatment of a vast array of serious diseases. They are glycoproteins and glycosylation is a critical quality attribute (CQA). Unfortunately, N-glycans monitoring remains a daunting analytical challenge requiring a long preparation protocol except for few recent kits. The aim of our work was to develop and validate a sensitive and robust analytical method with a rapid sample preparation to characterize N-glycans. To this purpose, we use procainamide labelling and on-line SPE purification that were compared to commercial kits RapiFluor MS (RFMS) from Waters and Instant PC from Agilent and applied to NIST mAb standard, a biosimilar comparability study and batch to batch controls.

## Methods :



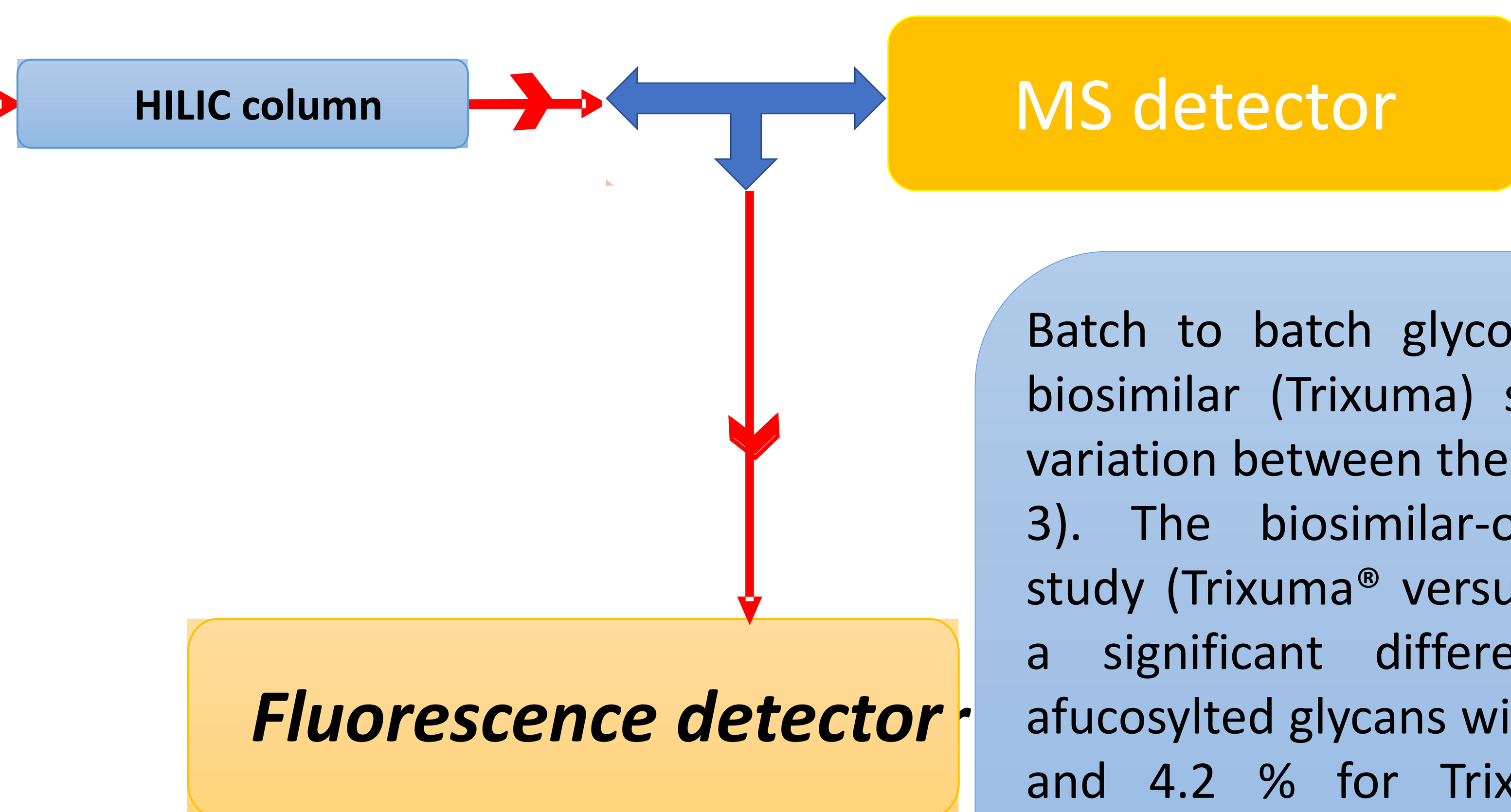




Figure 1: On-line HILIC SPE system coupled ton mass spectrometer (MS and fluorescence (FLD) detector, Green line defined the loading time (purification step), Red line represent the analytical step.

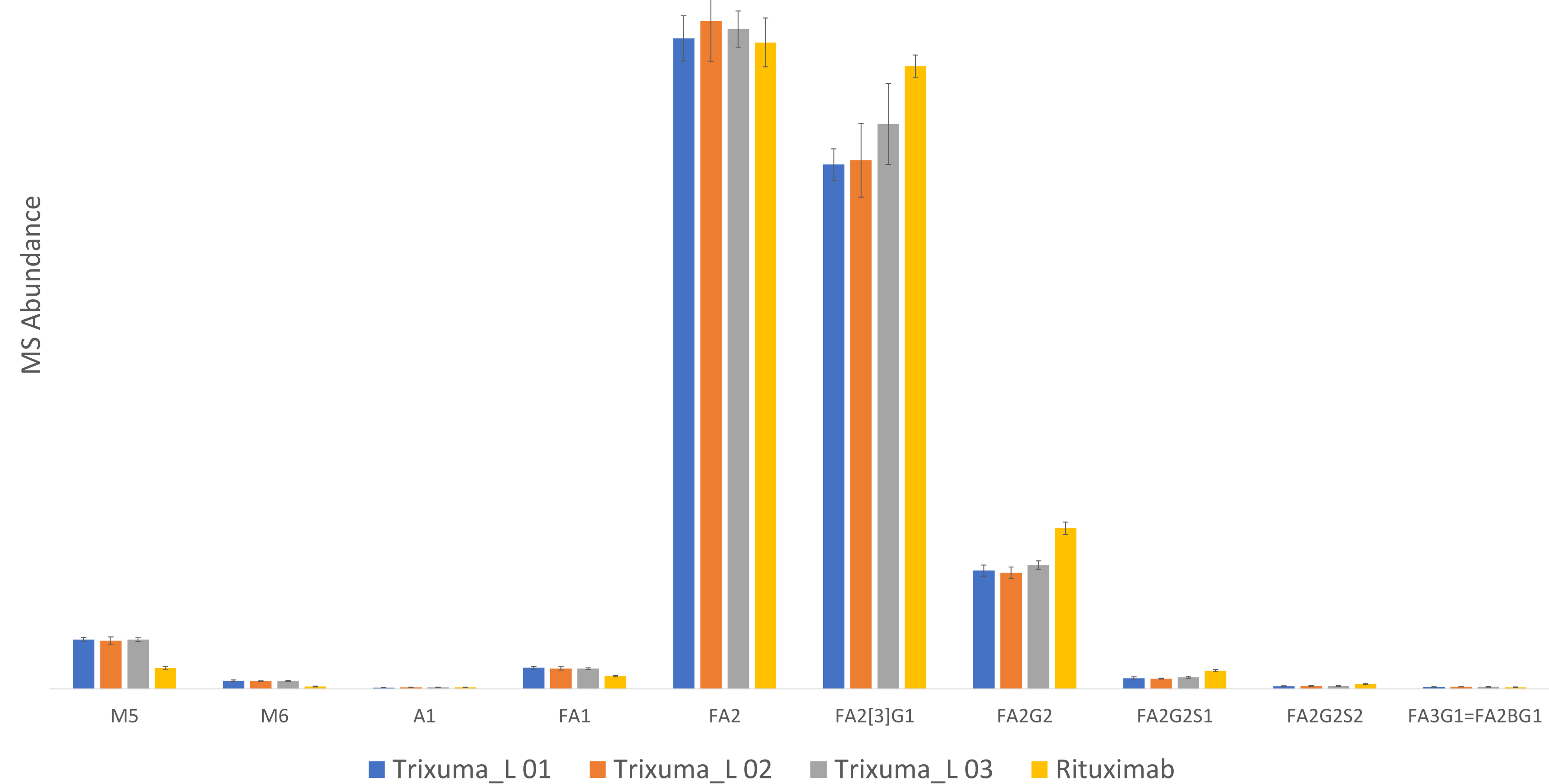



Figure 3: Comparison of batch-to-batch glycosylation of a biosimilar (Trixuma) and his bio-original (Mabthera)

Batch to batch glycosylation analysis of a biosimilar (Trixuma) showed no significant variation between the different batch (Figure 3). The biosimilar-originator comparison study (Trixuma® versus Mabthera®) showed a significant difference concerning the afucosylted glycans with 1.7% for Mabthera® and 4.2 % for Trixuma®. These results correlate with a recent published study on the impact of the glycan microheterogeneity on Fc function [3]

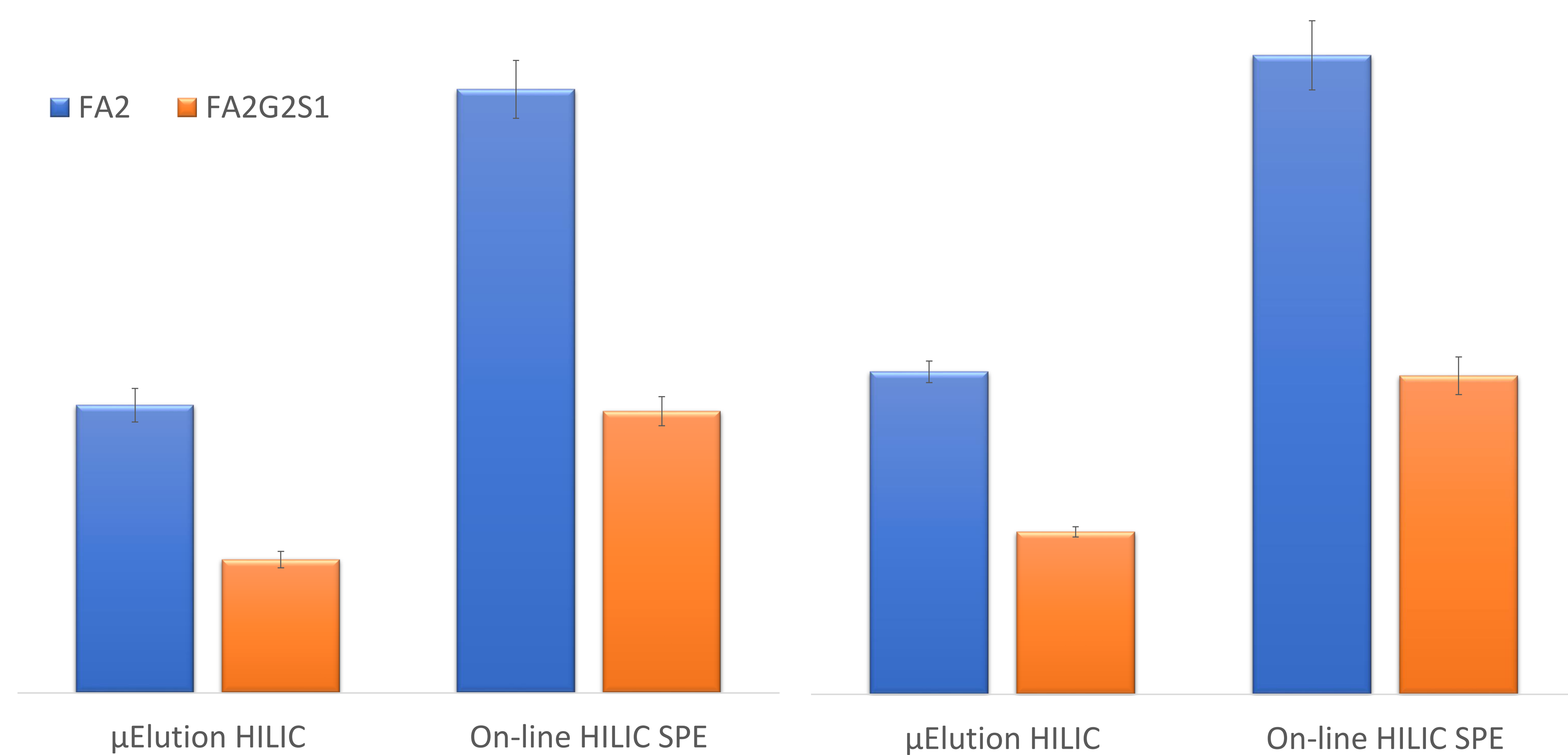



Figure 2 Comparison between µElution HILIC SPE (On the bench) and Online-HILIC-SPE. The error bars represent the standard deviation of the triplicates. FA2 and FA2G2S1 were chosen as readout glycans. A) MS result B) FLD result.

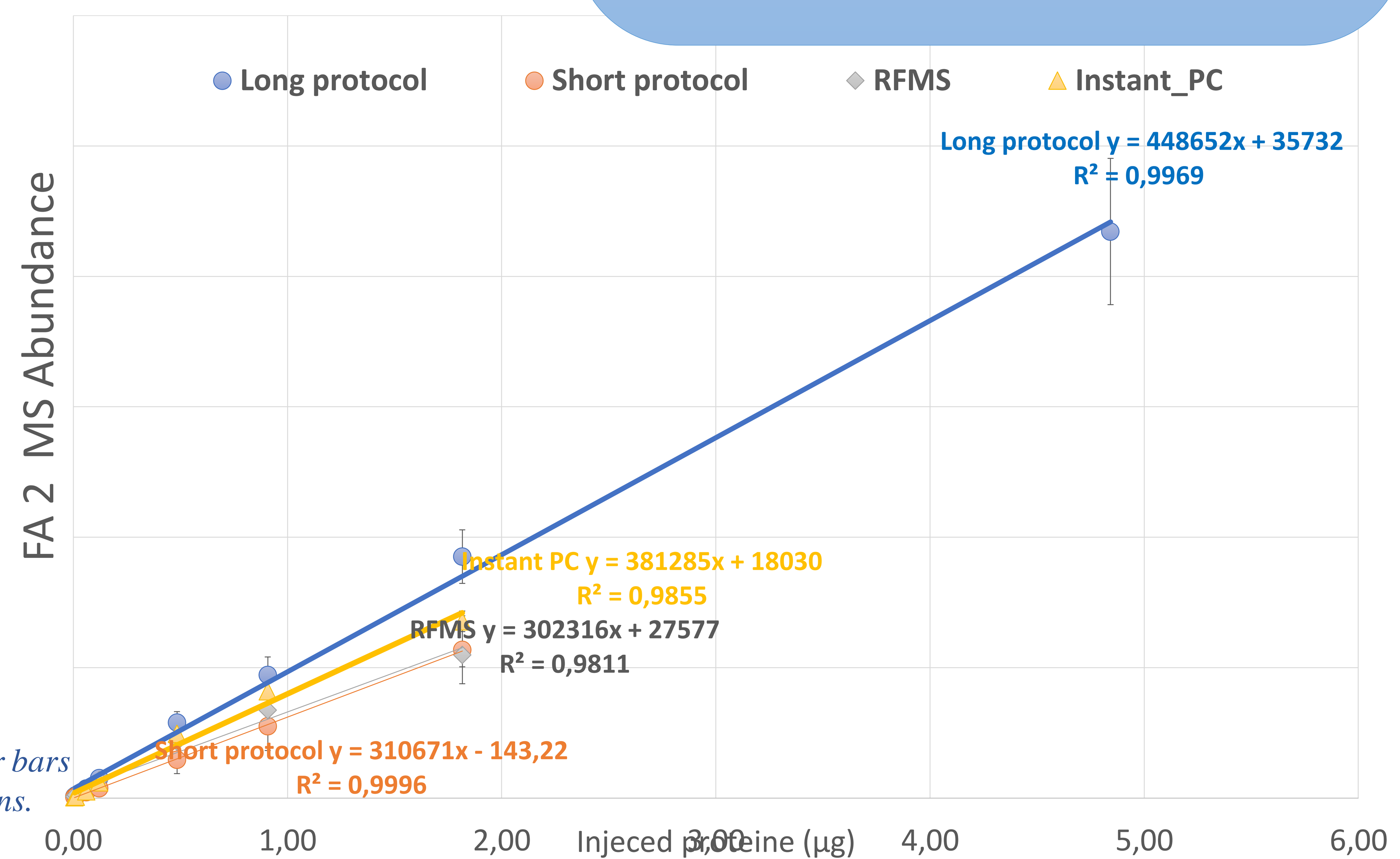



Figure 4: Comparison between the developed approach and commercial kits( Rapifluor MS (RFMS) from Waters and the Instant PC from Agilent) using MS detector

→The developed approach shows interesting result with an equivalent (or better) MS sensitivity comparing two commercial kits (RFMS from Waters and Instant PC from Agilent) (Figure 4).

## Conclusion:

The method has been validated and might be used for N-glycosylation analysis in QC environment. The on-line HILIC SPE purification is a good alternative and is less time consuming than the classical µElution HILIC SPE plate purification. The next step of our work, will consist on the use of this reliable approach for the characterization of the N-glycosylation in several diseases where glycoprotein quantity is lower, and sensitivity is required.

## REFERENCE

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