QUALITY OF ISOTOPICALLY LABELLED INTERNAL STANDARDS FOR PEPTIDE QUANTIFICATION

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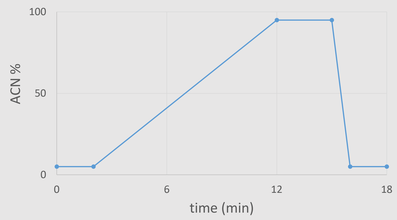
INTRODUCTION OBJECTIVE

Isotopically labelled internal standards (ILIS) allow the quantification of peptides in LC-MS using MRM. However, the quality of the ILIS is critical in quantitative peptidomics and its material- and time-efficient determination of its chemical and isotopic purity is an often neglected challenge. A fast UPLC-UV/single quadrupole MS method and algorithm was developed for the chemical and isotopic characterisation.

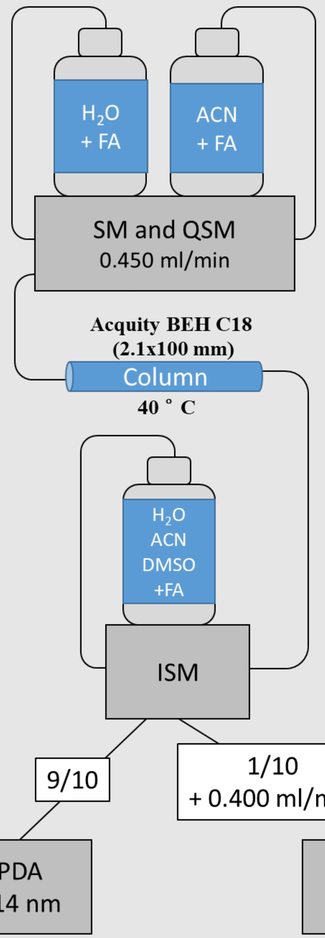
EXPERIMENTAL & RESULTS

Gradient scheme



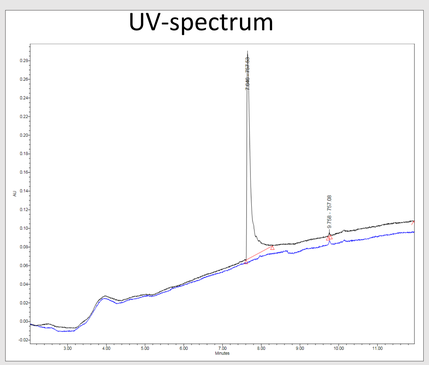
Settings

- **Injection volume** 2 µl
- **Sample temperature** 20 °C
- **Cone voltage** 15 V
- **Capillary voltage** 0.8 kV



CHEMICAL PURITY

UV-spectrum



Sequence	Chemical purity	LoD
SI*FTLVA	100.0 %	16.1 µM (1.36 %)

ISOTOPIC PURITY

ALGORITHM

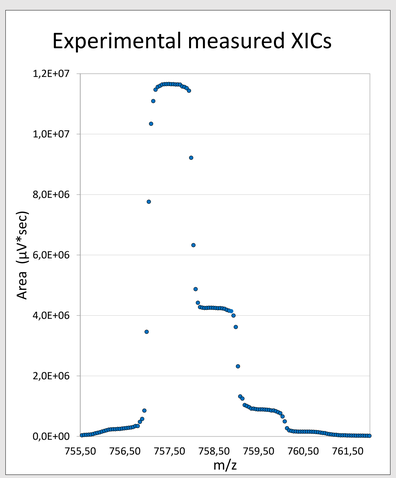
XIC = accumulation of all the observed ions within a specific window (c)

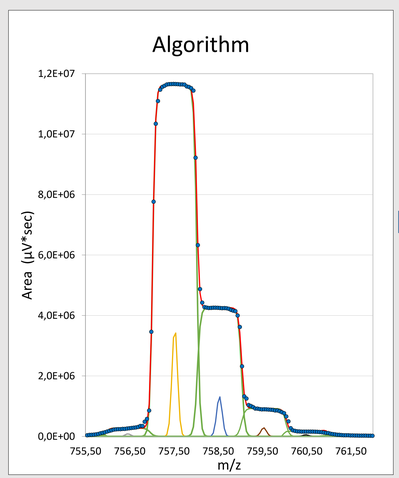
$$Y(x_i) = \sum_{k\in\mathbb{Z}} \int_{-c}^{c} a_k \cdot e^{-\frac{1}{2}\left(\frac{x-m_k}{\alpha}\right)^2} dx = \sum_{k\in\mathbb{Z}} A_k \left[\frac{1}{1+e^{-\left(\frac{(x+c)+m_k}{b_k}\right)}} - \frac{1}{1+e^{-\left(\frac{(x-c)+m_k}{b_k}\right)}} \right]$$

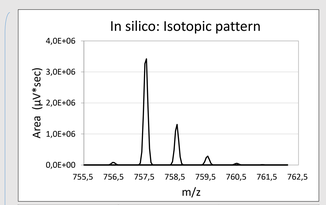
Determine: A_k, m_k, b_k using nonlinear least squares (+physical constraints)

$$y(x) = \sum_{k\in\mathbb{Z}} f'\left(\frac{A_k}{1+e^{-\left(\frac{x+m_k}{b_k}\right)}}\right) = \sum_{k\in\mathbb{Z}} a_k \cdot e^{-\frac{1}{2}\left(\frac{x-m_k}{\alpha}\right)^2}$$

RESULTS

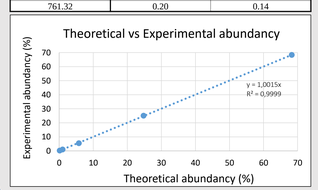






Sequence	Isotopic purity	LoD
SI*FTLVA	98.1 %	197.6 nM (0.15%)

Exact isotopic mass	Relative concentration (%)	Theoretic isotopic abundancy (%)
755.45	0.35	-
756.46	1.60	-
757.52	**67.40**	68.27
758.53	24.69	24.79
759.53	5.35	5.77
760.48	0.99	1.01
761.32	0.20	0.14



DISCUSSION CONCLUSION

XICs were obtained with moving the target values, from a TIC covering the whole range of the possible m/z values of the studied peptides. The increments were much smaller than the equipment filter of range ± 0.5 m/z window. A general algorithm is presented using these moving XICs. The obtained isotope pattern matched the theoretical pattern. These results prove the cost-efficient effectiveness of a simple single-quadrupole MS system during the quality control of isotopically labelled peptides.