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IMPACT OF HIGH TEMPERATURE AND SHAKING ON CHARGE VARIANTS OF ADALIMUMAB (HUMIRA ®) ASSESSED BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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INTRODUCTION

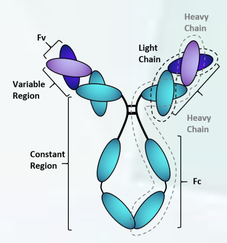
Monoclonal antibodies (mAbs) are biopharmaceuticals widely used for the treatment of highly prevalent diseases such as cancer and autoimmune diseases.

Adalimumab (Humira® 100 mg/mL) is a recombinant human IgG1 mAb glycoprotein consisting of 1330 amino acids that is specific for human tumor necrosis factor (TNF). **Humira® is used for the treatment of psoriasis, rheumatoid arthritis and Crohn's disease in adults and children.**

Humira® is injected under the skin by a prefilled syringe. The dose for a child is calculated according to the child's weight and the medicine should be redosificated in other different prefilled syringes to be selfadministered at home in different days.

Adalimumab has low stability after the vial is open and therefore it is necessary to study its stability under the usual conditions which the prefilled syringes will be exposed. In this context, **it is important to provide rigorous and reliable analytical methods which allows the rigorous mAbs characterization.**

The structural changes in the mAbs appeared during its manipulation could be detected by the charge variant characterisation, for this purpose a **(SCX)UHPLC- UV-(NATIVE)HRMS** has been applied.



AIM AND OBJETIVES

The objective of this work is to characterize the charge variants of adalimumab under stress conditions, i.e. high temperature (60º) and smooth shaking, by liquid chromatography coupled to UV and mass spectrometry detection in order to assay impact when mishandling adalimumab in pre-filled syringes.

METHODOLOGY

adalimumab 100mg/mL

Aliquots of 0,1 ml of adalimumab

Temperature stress (60°C, 3 h)

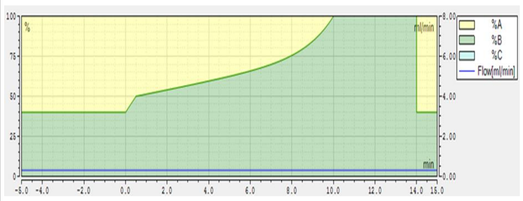
Smooth shaking stress (room temp. 1 h)

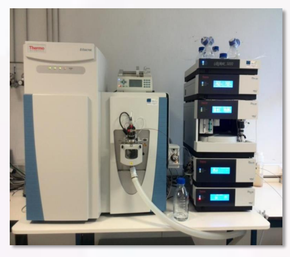
(SCX)UHPLC-UV-(NATIVE)HRMS

Analysis

Chromatographic separation

MAbPac SCX-10 RS 2.1mm X 50mm

Separation conditions	
Colunm temp.	30 °C
Flow	0.3 ml/min
Mobile phase A	25 mM NH4HCO3 in 30 mM de AcOH (pH 5.3)
Mobile phase B	10 Mm NH3 in 20mM AcOH (pH 10.8)
Gradient	

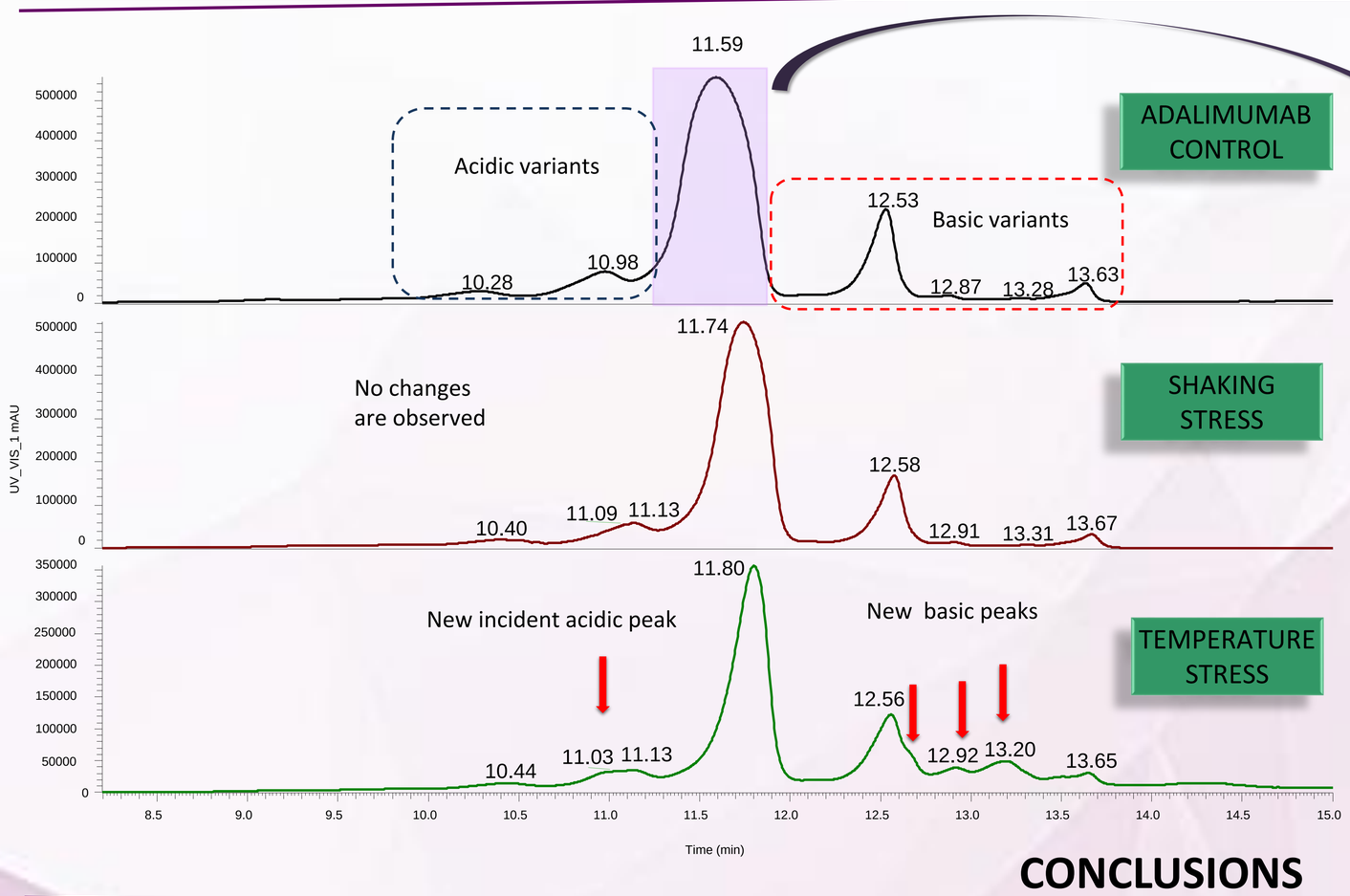


A Thermo Scientific™ Q-ExactivePlus Hibrid Quadrupole-Orbitrap™ mass spectrometer, coupled to a Thermo Scientific ™ 3000 ultimate was used for the analysis.

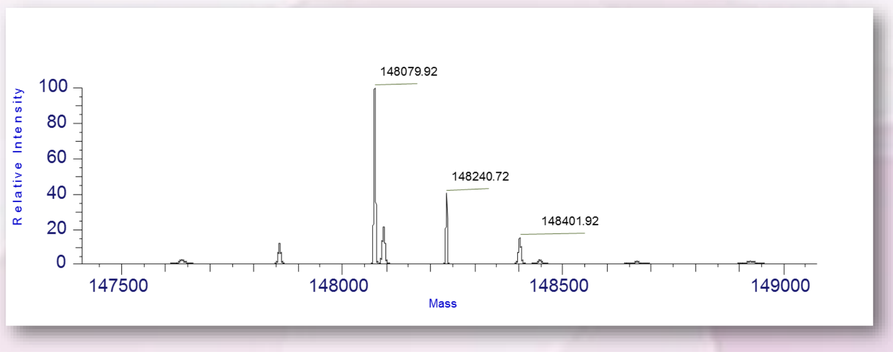
The data treatment was performed by BioPharma Finder 3.1 software.

Detection conditions	
Mass spectrometer conditions	
Resolution	17500
Scan range	2500-6000 m/z
Capilary temperature	275 °C
Ultraviolet conditions	
λ	214 nm

RESULTS



Deconvoluted mass spectra



mAb	Linked glycan	Theoretical average mass (Da)	Experimental mass (DA)	Mass difference (ppm)
ADA	0K-G0F/G0F	148080,55	148079,92	4,3
	0K-G1F/G0F	148242,69	148240,71	13,4
	0K-$(G1F)_2$ o G0F/G2F	148404,94	148401,92	19,6

CONCLUSIONS

✓ **The exposition to 60 °C modified the chemical structure of adalimumab. The increase of positive charges in the primary structure indicated the increase of basic variants. On the other hand, the agitation of adalimumab solution did not affect the charge variant profiles.**

✓ **It is highly recommended to keep refrigerated the pre-filled syringes during their transport and storage. No particular recommendation should be taken respecting the agitation control.**

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