

# Supplementary Information

## Interlaboratory evaluation of a user-friendly benchtop mass spectrometer for multiple-attribute monitoring studies of a monoclonal antibody

Claire I. Butré<sup>1</sup>, Valentina d'Atri<sup>2,3</sup>, Hélène Diemer<sup>5,6</sup>, Olivier Colas<sup>4</sup>, Elsa Wagner<sup>4</sup>, Davy Guillaume<sup>2,3</sup>, Alain Beck<sup>4</sup>, Sarah Cianferani<sup>5,6</sup>, Arnaud Delobel<sup>1</sup>

<sup>1</sup> Quality Assistance sa, Techno Parc de Thudinie 2, 6536 Thuin, Belgium

<sup>2</sup> School of Pharmaceutical Sciences, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva 4, Switzerland

<sup>3</sup> Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva 4, Switzerland

<sup>4</sup> Biologics CMC and Developability, IRPF, Centre d'immunologie Pierre Fabre, 5 avenue Napoleon III, 74160 Saint-Julien en Genevois, France

<sup>5</sup> Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbourg, CNRS, IPHC UMR 7178, 67000 F-Strasbourg, France

<sup>6</sup> Infrastructure Nationale de Protéomique ProFI – FR2048, 67087 Strasbourg, France

Correspondence: alain.beck@pierre-fabre.com ; arnaud.delobel@quality-assistance.be

### Table of contents

<b>Table S1</b>	List of the seven peptides that have been monitored during the MAM study.	S2
<b>Figure S1</b>	Amino acid sequence of nivolumab light chain and heavy chain.	S3
<b>Figure S2</b>	Retention times of the seven peptides monitored during the MAM study.	S4
<b>Figure S3</b>	Chromatographic separation between peptide GFYPSDIAVEWESNGQPENNYK and its deamidated variants.	S5
<b>Figure S4</b>	Determination of the deamidation of peptide GFYPSDIAVEWESNGQPENNYK in the different labs in control and high pH stressed samples.	S5

**Table S1.** List of the seven peptides that have been monitored during the MAM study. For each peptide, location, amino acid sequence and occurring modification (oxidation, deamidation, glycosylation, or C-terminal truncation) are reported. HC stands for heavy chain.

PEPTIDE	AMINO ACID SEQUENCE	MODIFICATION TYPE
HC (24-38)	ASGITFSNSGMHWVR	Oxidation
HC (77-87)	NTLFLQMNSLR	Oxidation
HC (242-248)	DTLMISR	Oxidation
HC (282-310)	TKPREEQFNSTYRVVSVLTVLHQDWLNGK	Glycosylation
HC (334-353)	GQPREPQVYTLPPSQEEMTK	Oxidation
HC (364-385)	GFYPSDIAVEWESNGQPENNYK	Deamidation
HC (433-440)	SLSLSLGK	C-term Lys truncation

**Figure S1.** Amino acid sequence of nivolumab A) light chain and B) heavy chain.

A) Light Chain (LC) sequence:

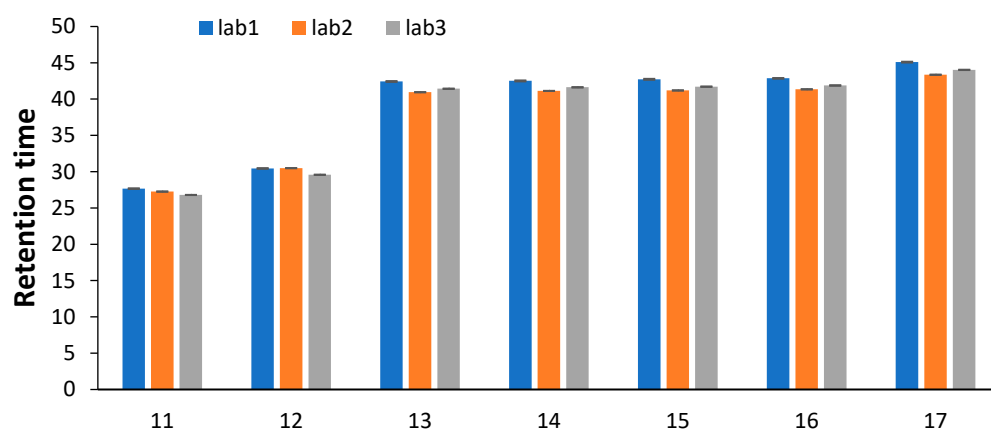
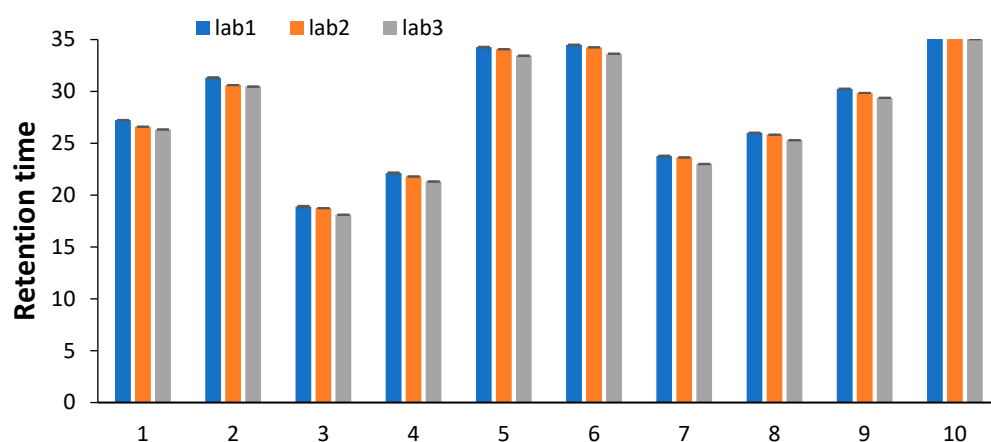
1 EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD  
51 ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SSNWPRTFGQ  
101 GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV  
151 DNALQSGNSQ ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG  
201 LSSPVTKSFN RGEK

B) Heavy Chain (HC) sequence:

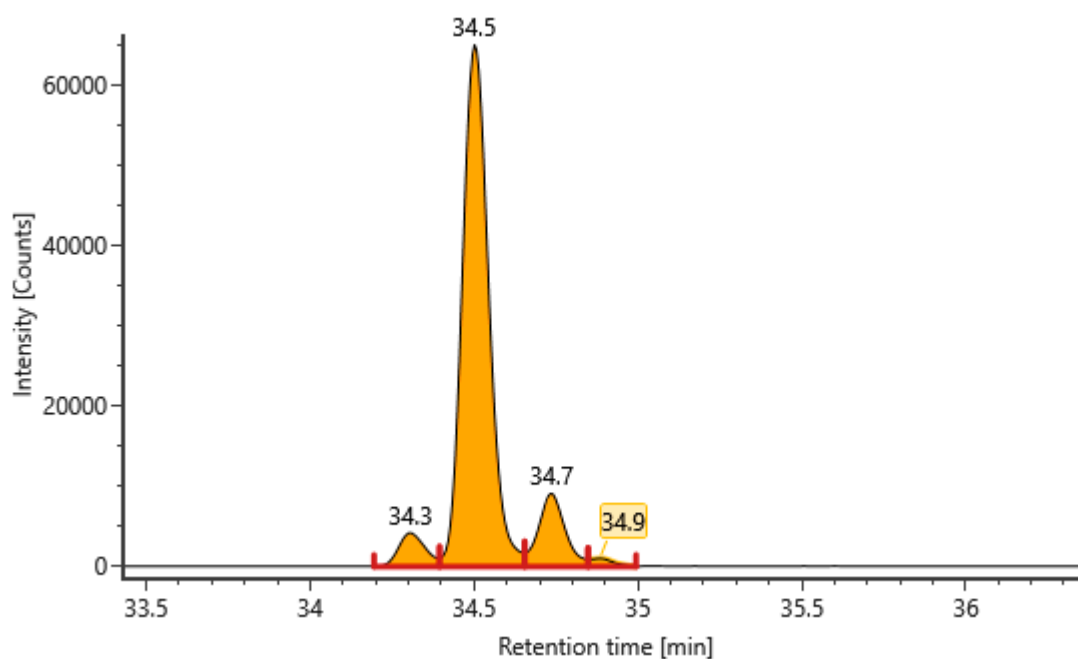
1 QVQLVESGGG VVQPGRLRL DCKASGITFS NSGMHWVRQA PGKGLEWVAV  
51 IWYDGSKRYV ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCATND  
101 DYWGQGTLLV VSSASTKGPS VFPLAPCSRS TSESTAALGC LVKDYFPEPV  
151 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TKTYTCNVDH  
201 KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP KDTLMISRTF  
251 EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN STYRVVSVLT  
301 VLHQDWLNGK EYKCKVSNKG LPSSIIEKTIS KAKGQPREPQ VYTLPPSQEE  
351 MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV LDSDGSFFLY  
401 SRLTVDKSRW QEGNVFSCSV MHEALHNHYT QKSLSLSLGK

**Figure S2.** Retention times of the seven peptides monitored during the MAM study and graphical representation of the reported values. Relative standard deviation (RSD) values calculated on n = 3.

			LAB1		LAB2		LAB3	
			Average	RSD	Average	RSD	Average	RSD
1	HC (24-38)	Oxidation (M)	27.23	0.02	26.60	0.01	26.33	0.02
2		Control	31.32	0.05	30.61	0.01	30.47	0.02
3	HC (242-248)	Oxidation (M)	18.90	0.07	18.74	0.00	18.11	0.01
4		Control	22.13	0.06	21.80	0.01	21.31	0.01
5	HC (364-385)	Deamidation (N)	34.28	0.04	34.07	0.01	33.45	0.02
6		Control	34.48	0.04	34.25	0.01	33.64	0.02
7	HC (334-353)	Oxidation (M)	23.76	0.06	23.64	0.01	23.00	0.02
8		Control	25.99	0.04	25.83	0.01	25.30	0.02
9	HC (77-87)	Oxidation (M)	30.25	0.04	29.85	0.01	29.38	0.02
10		Control	35.94	0.05	35.33	0.01	35.04	0.02
11	HC (433-440)	K truncation	27.67	0.04	27.27	0.01	26.81	0.01
12		No K truncation	30.44	0.05	30.49	0.01	29.58	0.02
13	HC (282-310)	G2F	42.44	0.06	40.96	0.01	41.44	0.02
14		G1F	42.52	0.06	41.14	0.01	41.63	0.03
15		G0F	42.72	0.06	41.21	0.01	41.72	0.03
16		G0	42.87	0.05	41.35	0.01	41.88	0.03
17		No glycan	45.11	0.06	43.36	0.01	44.03	0.03

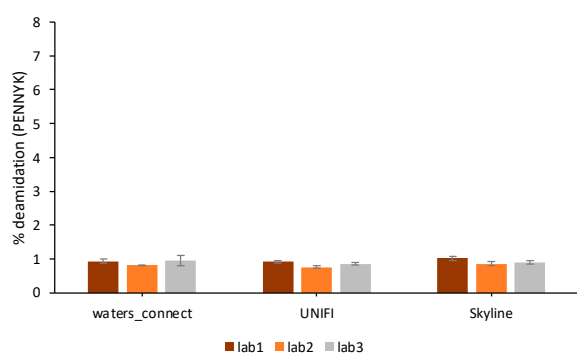


**Figure S3.** Chromatographic separation between peptide GFYPSDIAVEWESNGQPENNYK and its deamidated variants.



**Figure S4.** Determination of the deamidation of peptide GFYPSDIAVEWESNGQPENNYK in the different labs for A) control and B) high pH stressed samples.

A) Control sample



B) High pH stressed sample

