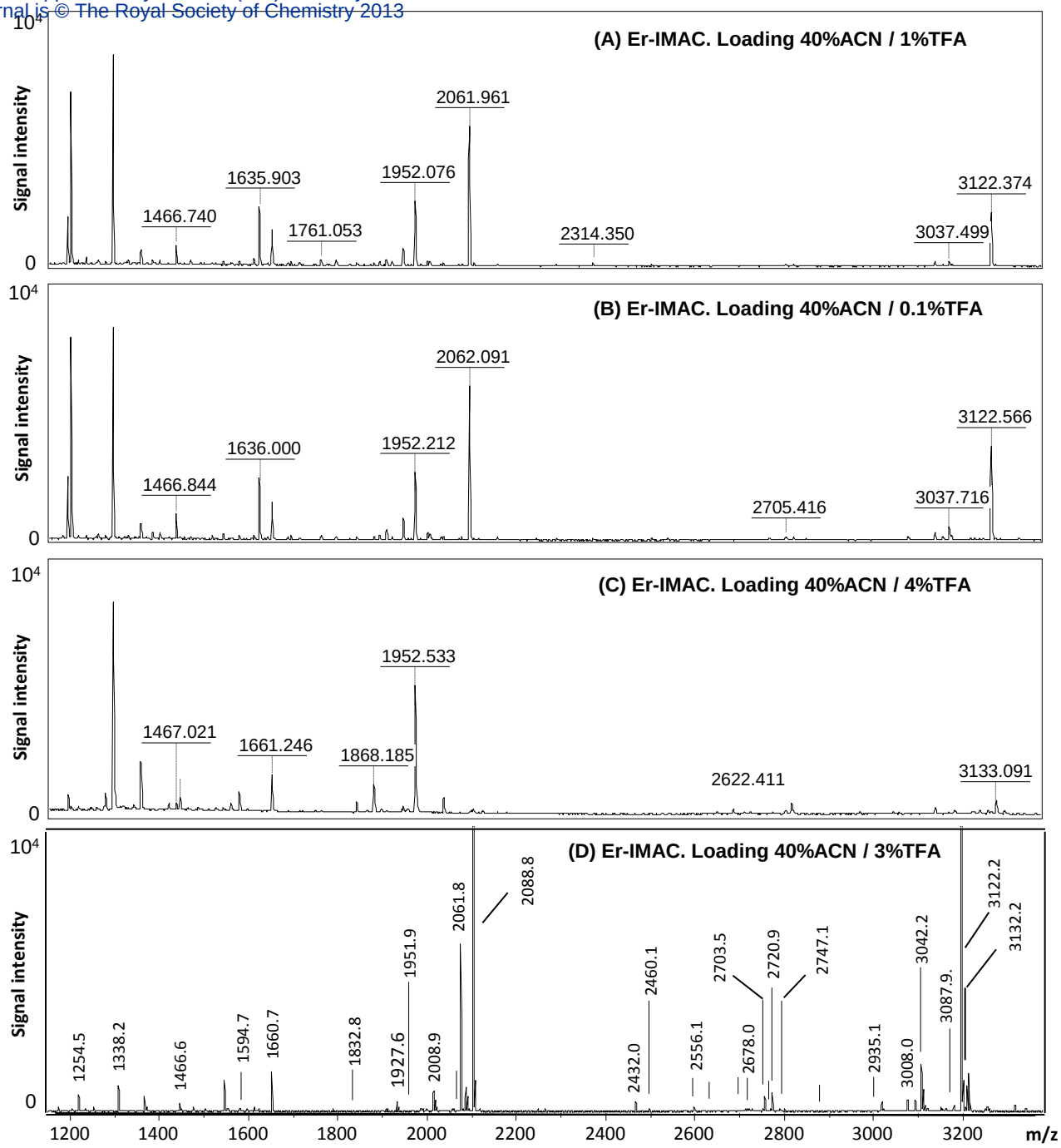
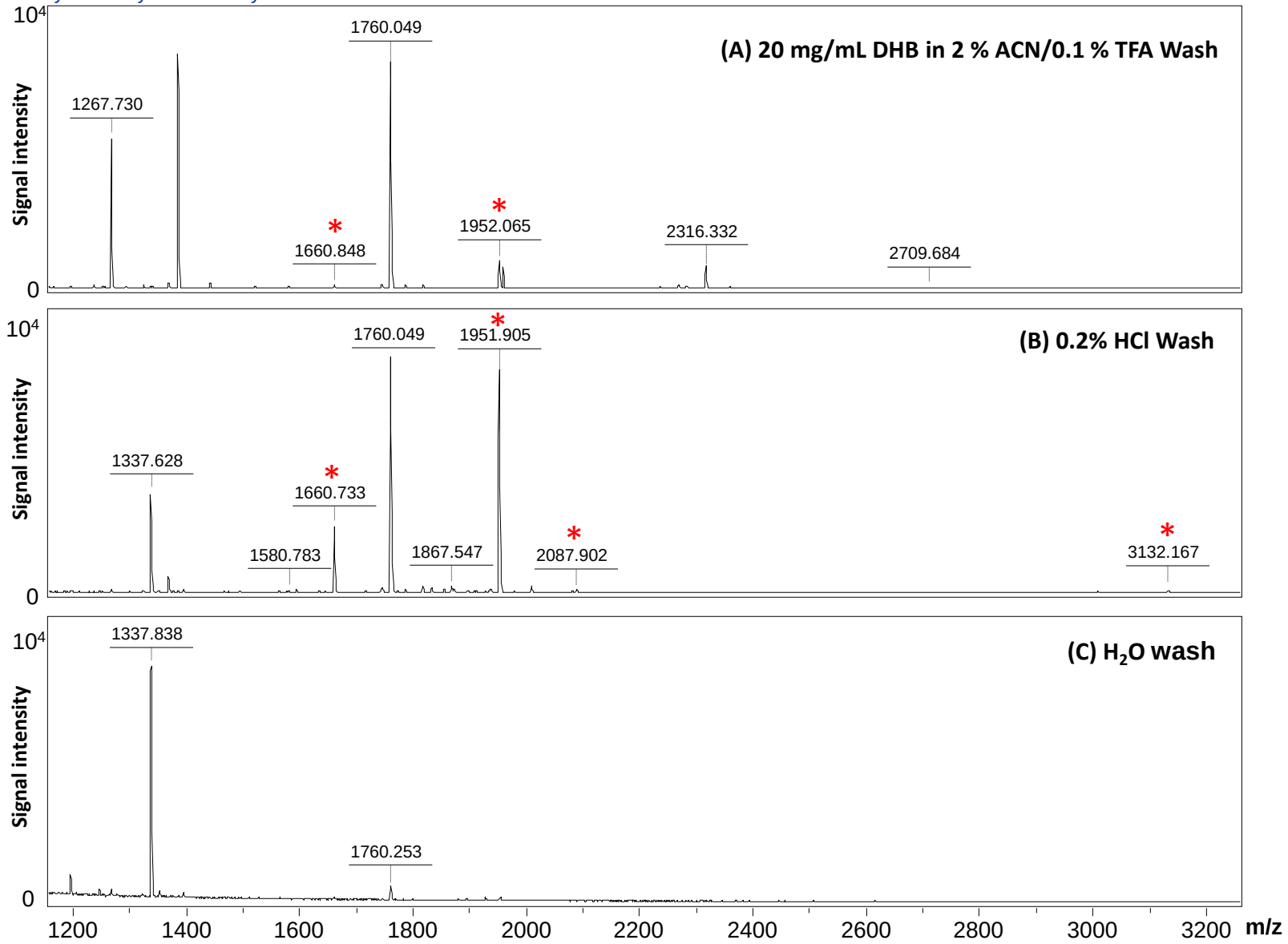
Supplementary Table-1: Different buffers, used during the optimization of phosphopeptides enrichment process. (highlighted in green → delivered best results)

Loading buffers	Washing buffers	Elution buffers
40%ACN/ 0.1%TFA	20 mg DHB/ mL / 2%ACN / 0.5 % TFA	100 mM Imidazole
40%ACN/ 1%TFA	0.2% HCl	0.5 % NH_4OH
40%ACN/ 4%TFA	H_2O	
40%ACN/ 3%TFA		

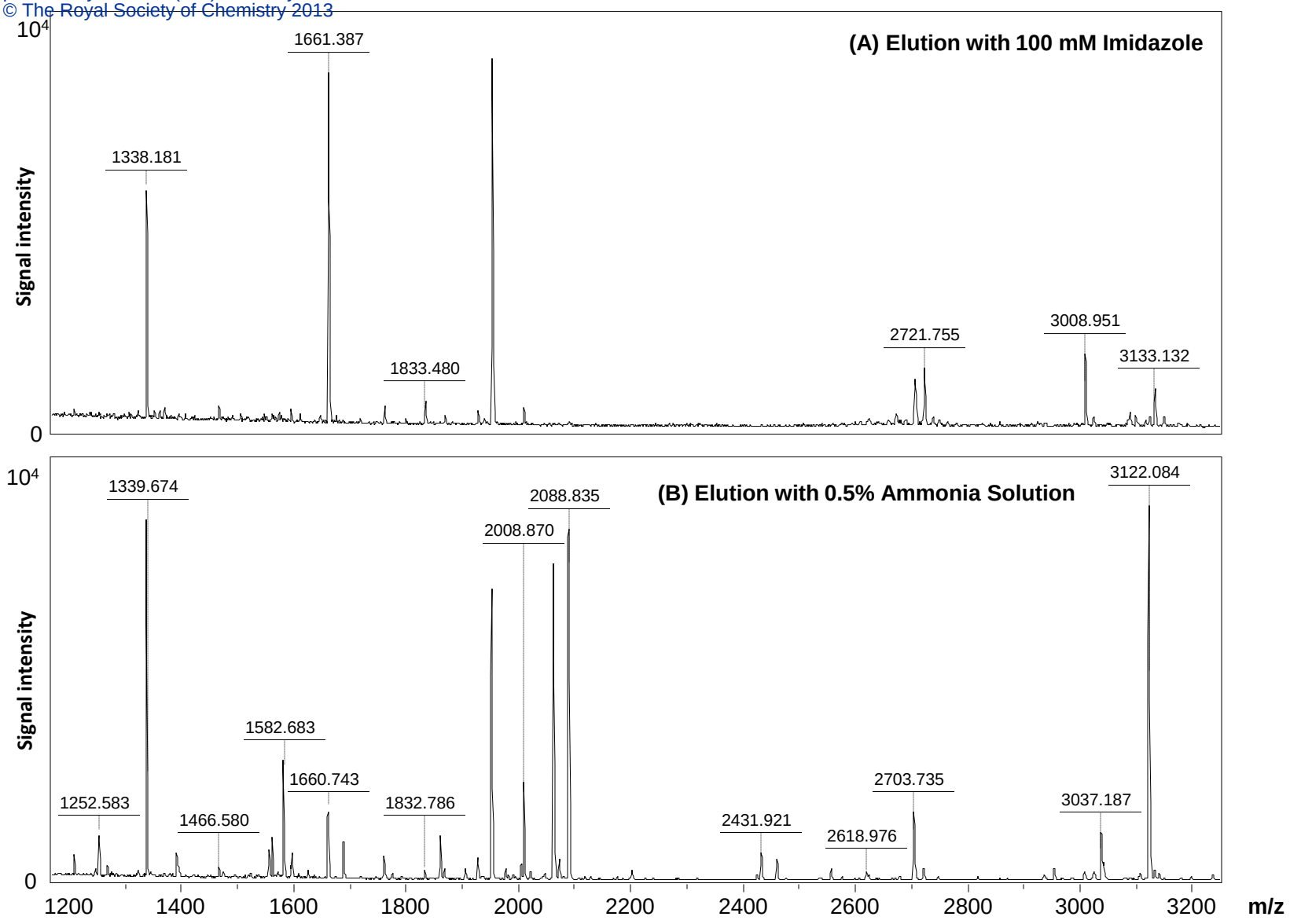


Supplementary figure 3:

MALDI mass spectra of phospho-peptide enrichment by Er-IMAC using different loading buffers **(A-D)**. The maximum number of phosphopeptides could be isolated using 40% ACN/3% TFA as a loading buffer (D). DHB matrix. 400 shots. 337 nm laser, Ultraflex I (Bruker).



Supplementary figure 4: MALDI mass spectra of the different washings buffers. During washing **(A)** and **(B)**, some of the phosphopeptides get lost (marked with red asterisk). A neutral wash with deionized water **(C)** was observed to cause no loss of phosphopeptides. DHB matrix. 400 shots. 337 nm laser, Ultraflex I (Bruker).



Supplementary figure 5: MALDI Mass spectra of two different elution buffers. Only a small number of phosphopeptides can be eluted with 100 mM imidazole **(A)**. Highest number of phosphopeptides could be enriched with 0.5% ammonia solution **(B)**.