Electronic Supplementary Material (ESI) for Analytical Methods.


# Development of an Easily Adaptable, High Sensitivity Source for *Inlet* Ionization

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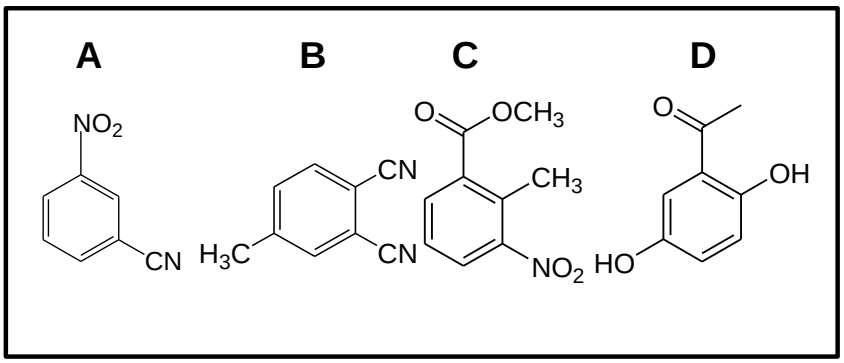
**Scheme S1**. Structure of matrix compounds used for MAI-MS: (**A**) 3-nitrobenzonitrile (3-NBN); (**B**) 4-methyl-phthalonitrile; (**C**) methyl 2-methyl-3-nitrobenzoate, and (**D**) 2,5-dihydroxyacetophenone (2,5-DHAP).



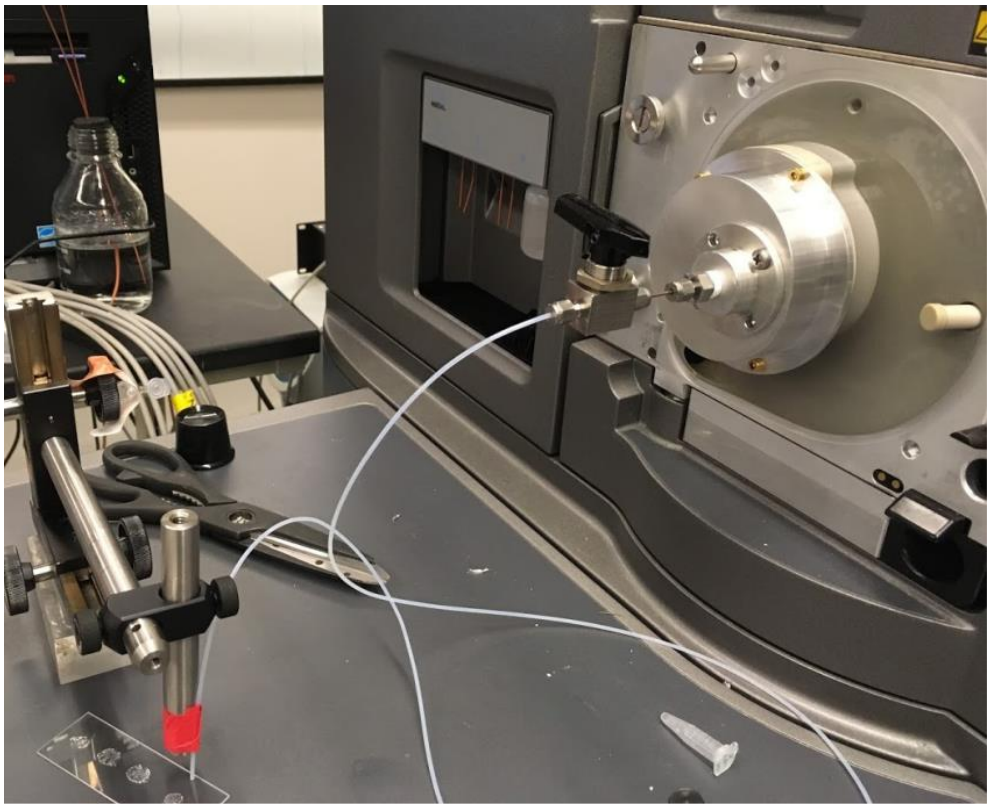


**Figure S1**. Photograph of source 2 on a SYNAPT G2S mass spectrometer. The on/off isolation valve has been replaced by a ball valve permitting to exchange parts of the inlet without having to break the vacuum of the instrument.

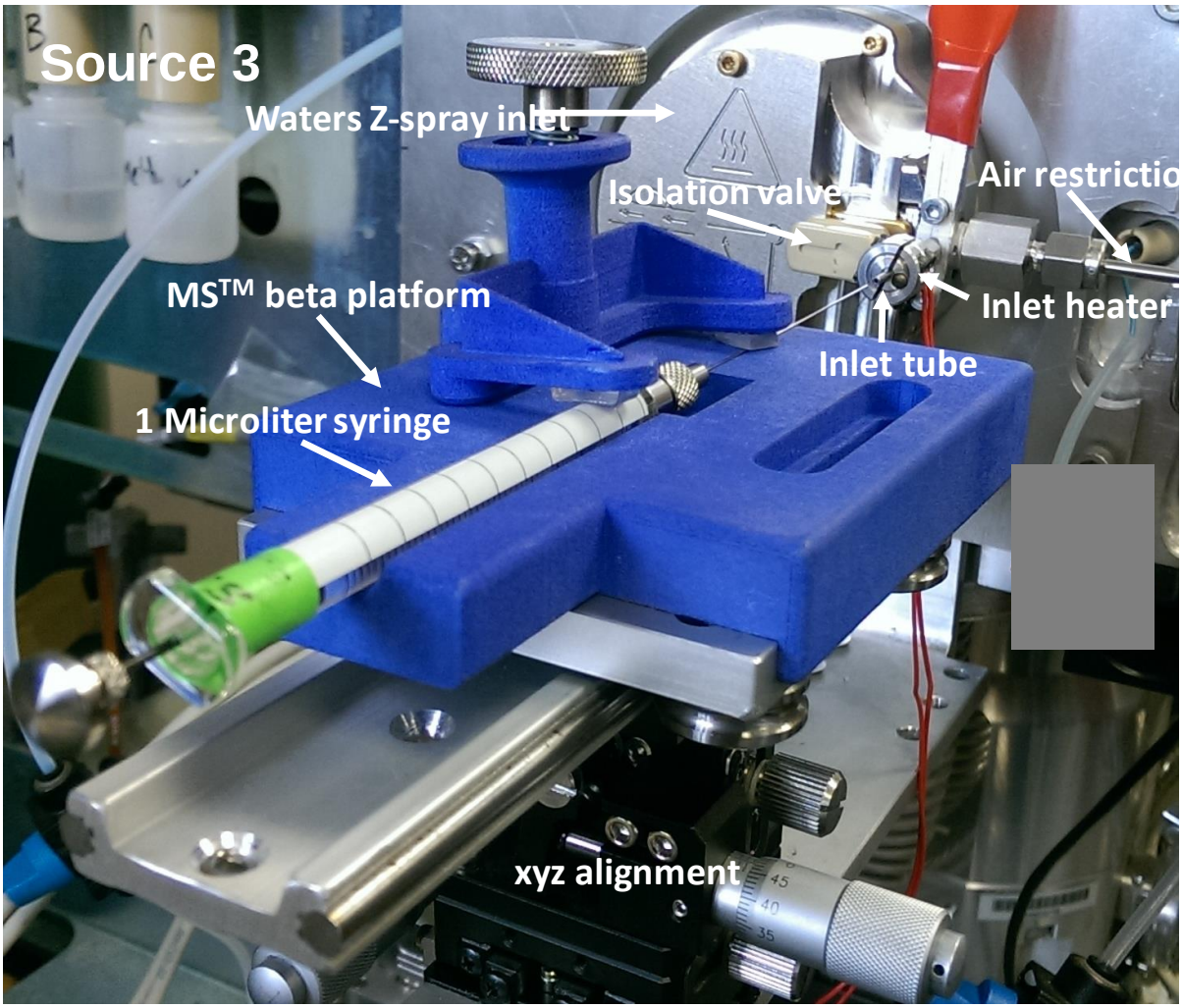



**Figure S2**. Photograph of source 3 of a modified inlet skimmer cone with a perpendicular inlet tube using a setup for 1 µL-dead volume syringe sample introduction method on a Xevo TQ-S mass spectrometer.

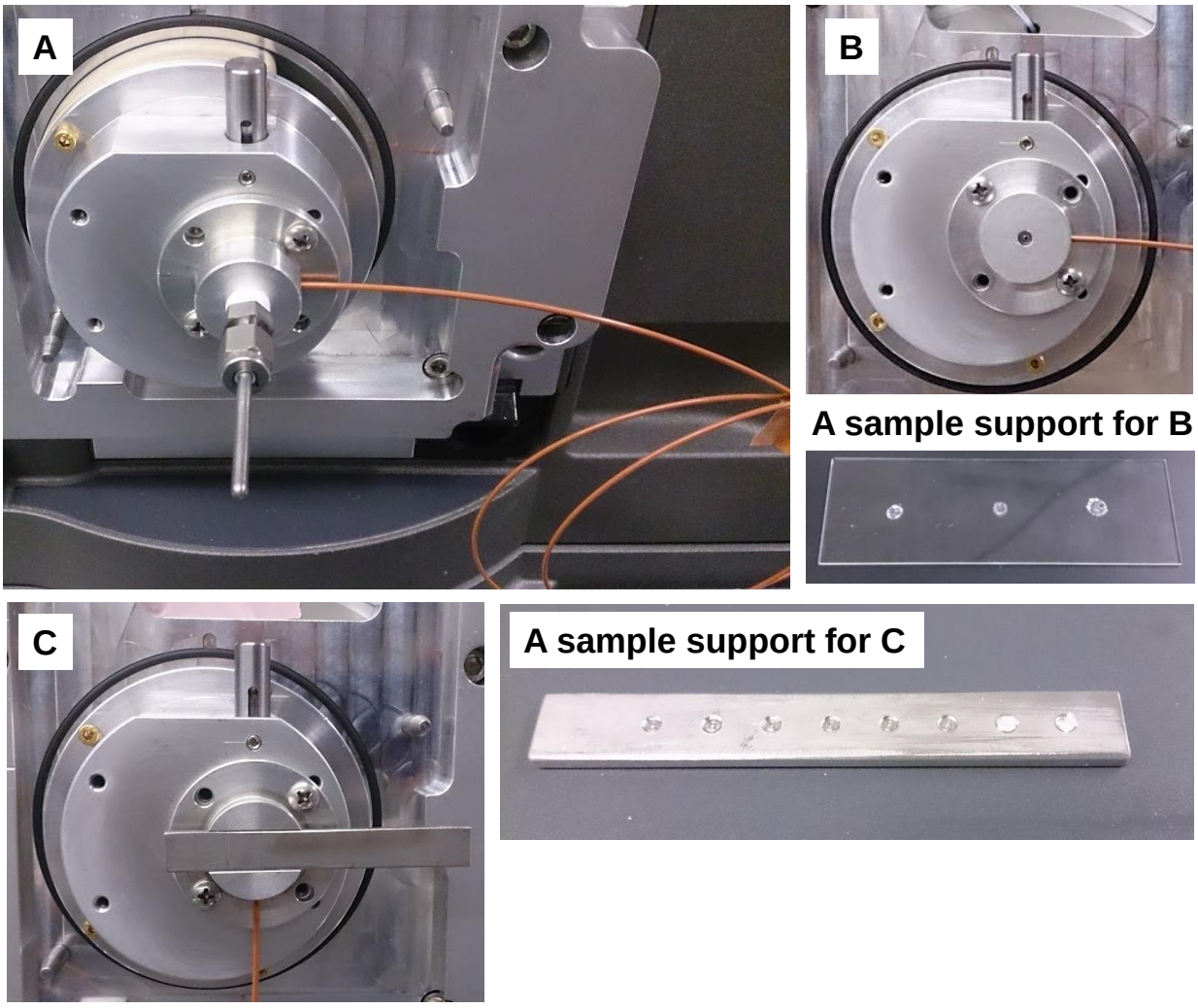



**Figure S3**. Photographs of add-on modifications that can be used with source 1: (**A**) probe introduction; (**B**) surface introduction such as a flat glass plate in which the mini inlet tube is ~2 mm away from the matrix:analyte surface deposited on a surface; (**C**) surfaces with indentation. The inclusion of several features such as (**i**) an on/off isolation valve; (**ii**) an easily changeable flange; (**iii**) a variable diameter entrance aperture; and (**iv**) an air inlet restriction allow for all add-ons to be interchangeable without breaking the vacuum of the mass spectrometer.

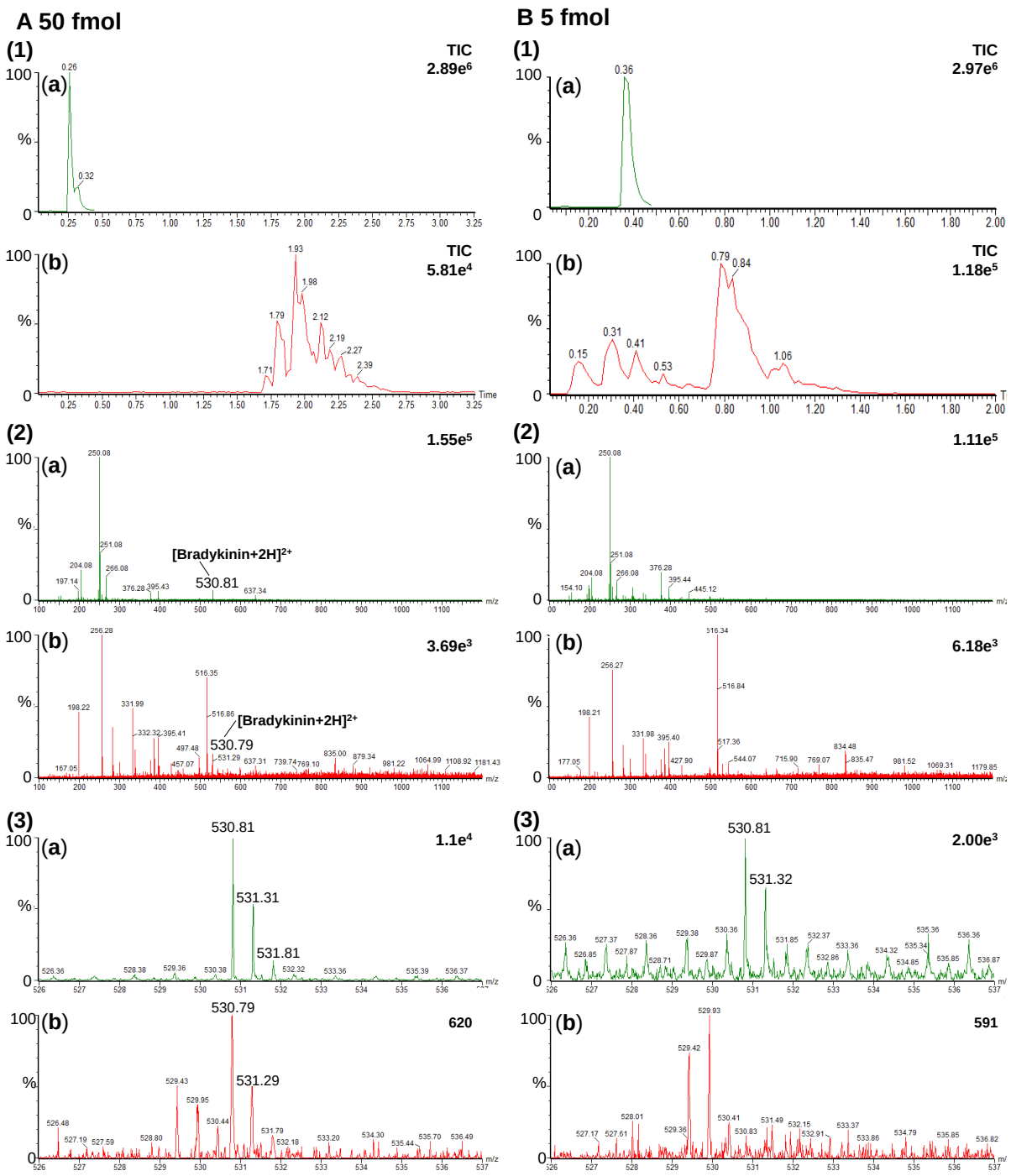



**Figure S4**: MAI mass spectra of bradykinin (monoisotopic MW 1059.56 Da) using (**A**) 50 fmol and (**B**) 5 fmol; (**1**) total ion chronogram (TIC), (**2**) mass spectra, and (**3**) inset mass spectra: (**a**) source 1 with the glass plate non-touch adapter and (**b**) commercial ESI Z-Spray source. Matrix 3-NBN and a SYNAPT G2 mass spectrometer were used. Movie S2 exemplifies the operation.

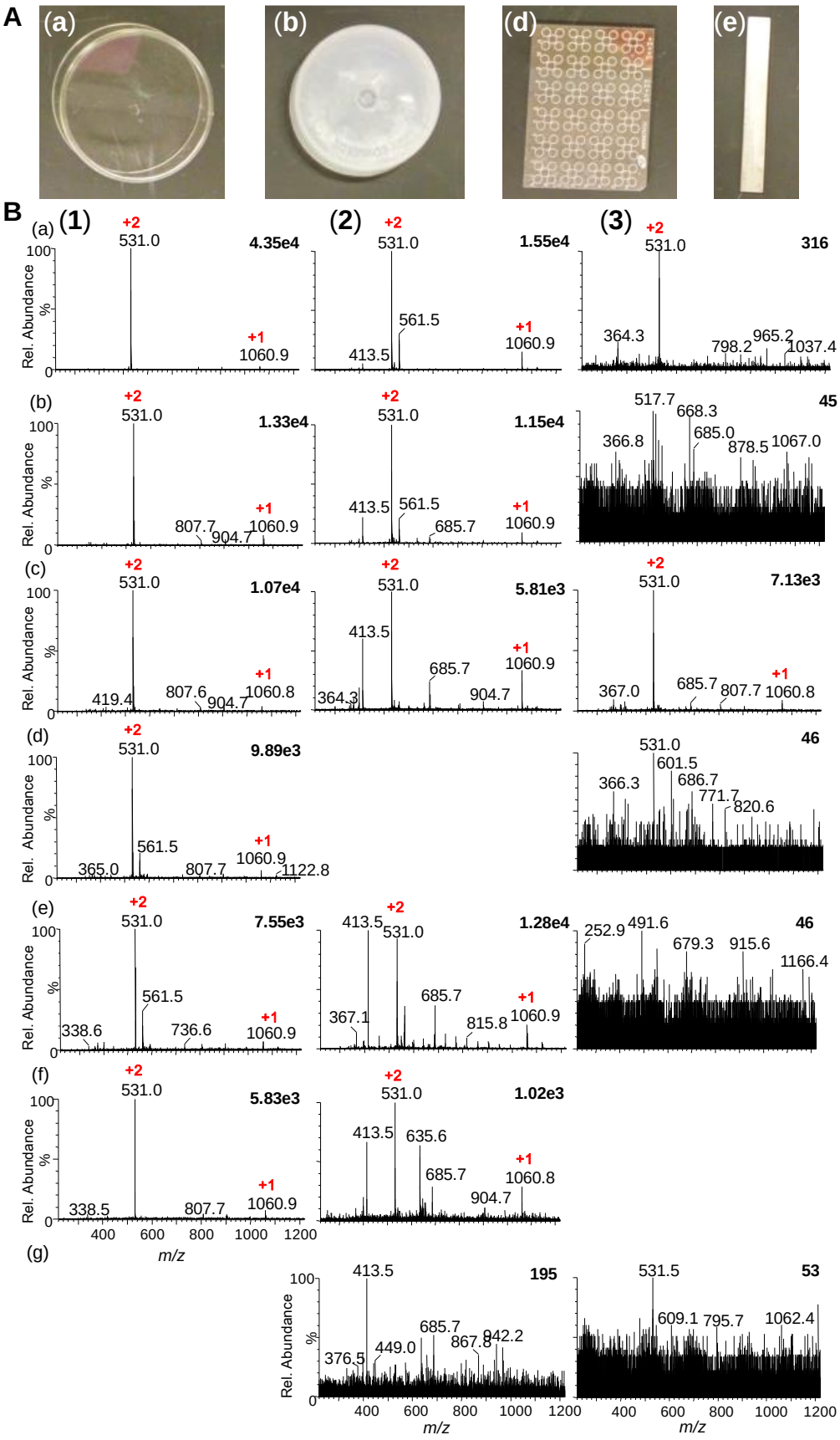



**Figure S5**. Study of the effect of substrate surface and shape of sample holder. (**A**) Photographs. (**B**) Mass spectra of bradykinin (monoisotopic MW 1059.56 Da) using matrix (**1**) 3-NBN, (**2**) 4-methyl-phthalonitrile, (**3**) methyl 2-methyl-3-nitrobenzoate and different sample holders: (**a**) polystyrene, (**b**) polyethylene, (**c**) syringe needle, (**d**) stainless steel MALDI sample plate with indentations, and (**e**) flat stainless steel plate, (**f**) glass plate and (**g**) pipette tip.

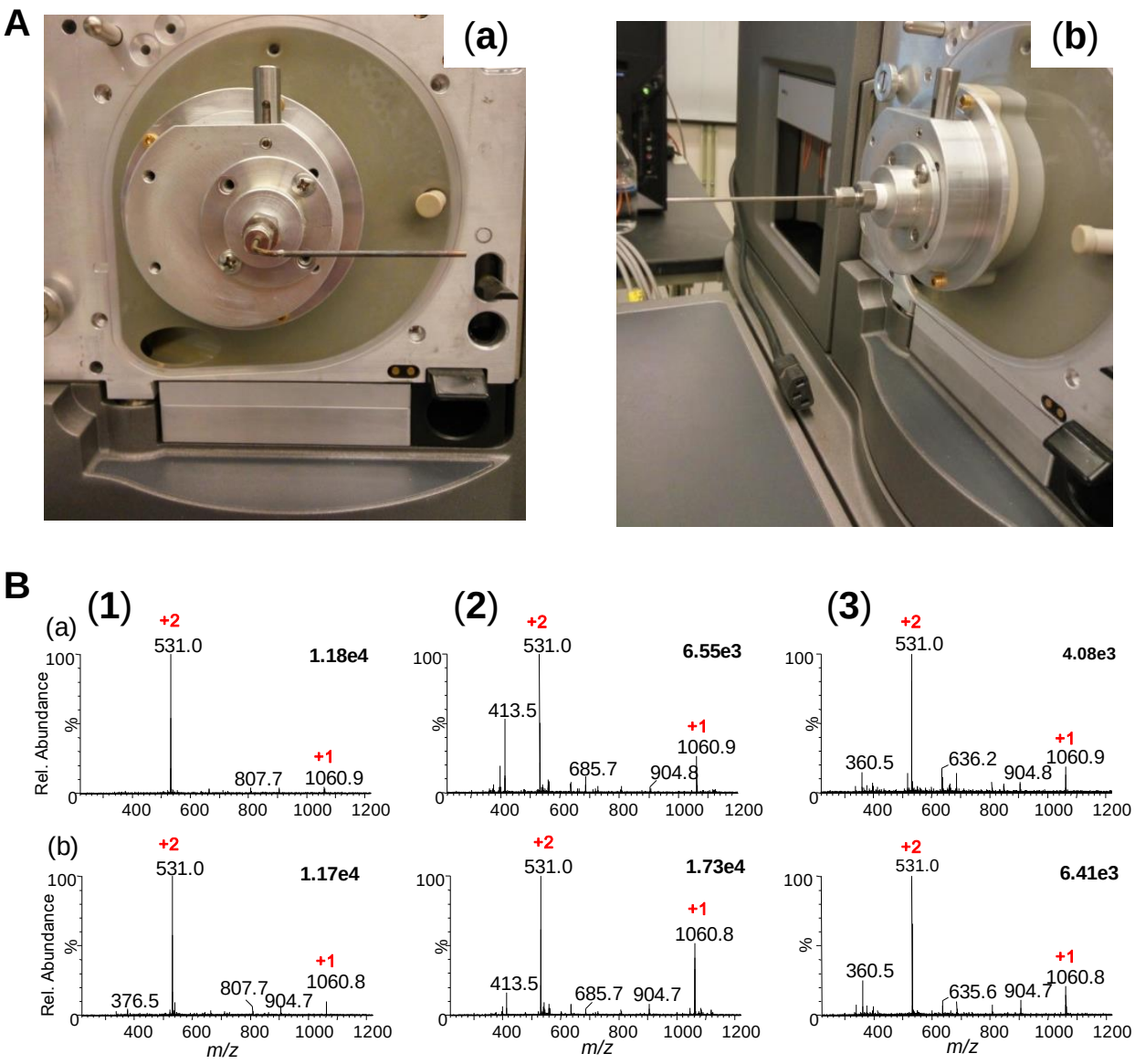



**Figure S6**. Straight and bent tube study. (**A**) Photographs and (**B**) mass spectra of bradykinin (monoisotopic MW 1059.56 Da) using matrix (**1**) 3-NBN, (**2**) 4-methyl-phthalonitrile, and (**3**) methyl 2-methyl-3-nitrobenzoate, with (**a**) bent tube and (**b**) straight tube. Sample introduction used was the syringe method; all data were acquired using a SYNAPT G2S mass spectrometer fitted with source 1.

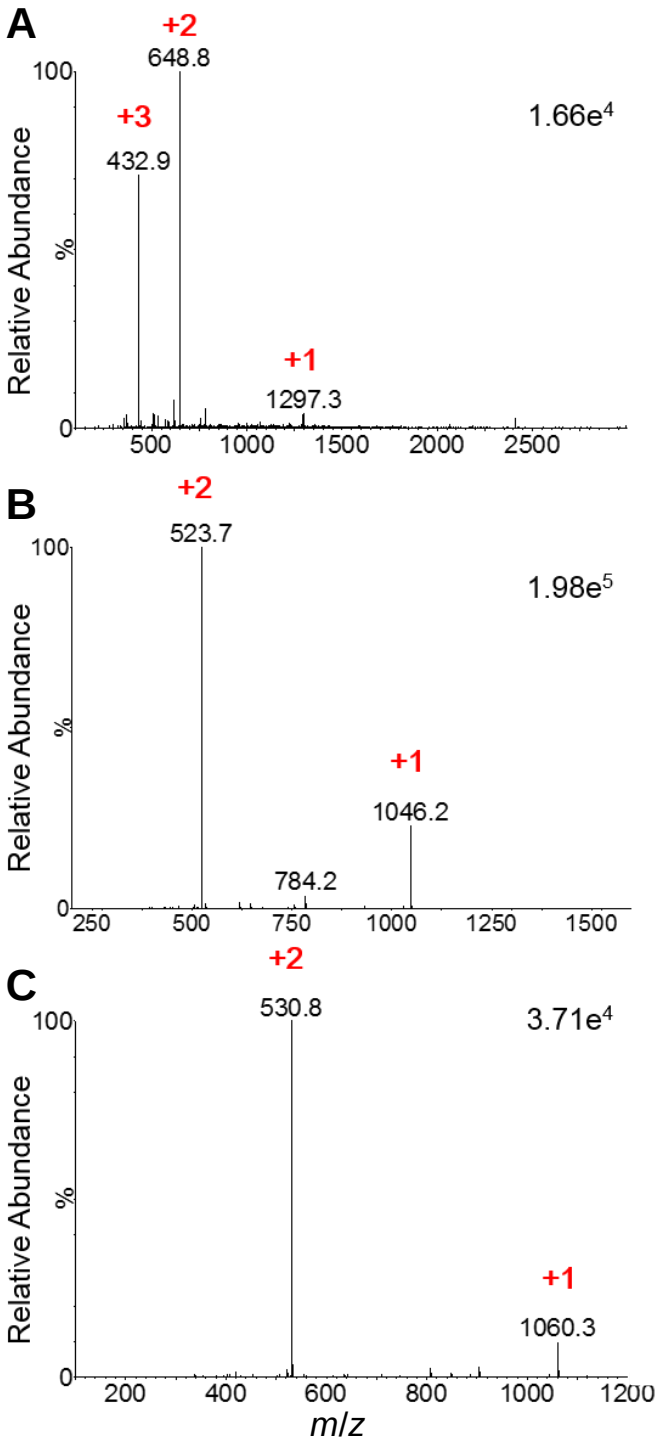



**Figure S7**. MAI mass spectra of (**A**) angiotensin I (monoisotopic MW 1295.68 Da), (**B**) angiotensin II (monoisotopic MW 1045.53 Da), and (**C**) bradykinin (monoisotopic MW 1059.56 Da). The syringe sample introduction method was used to deliver 2.5 pmol of each analyte with 3-NBN as the matrix to the inlet tube of source 1 fitted on a SYNAPT G2S mass spectrometer.

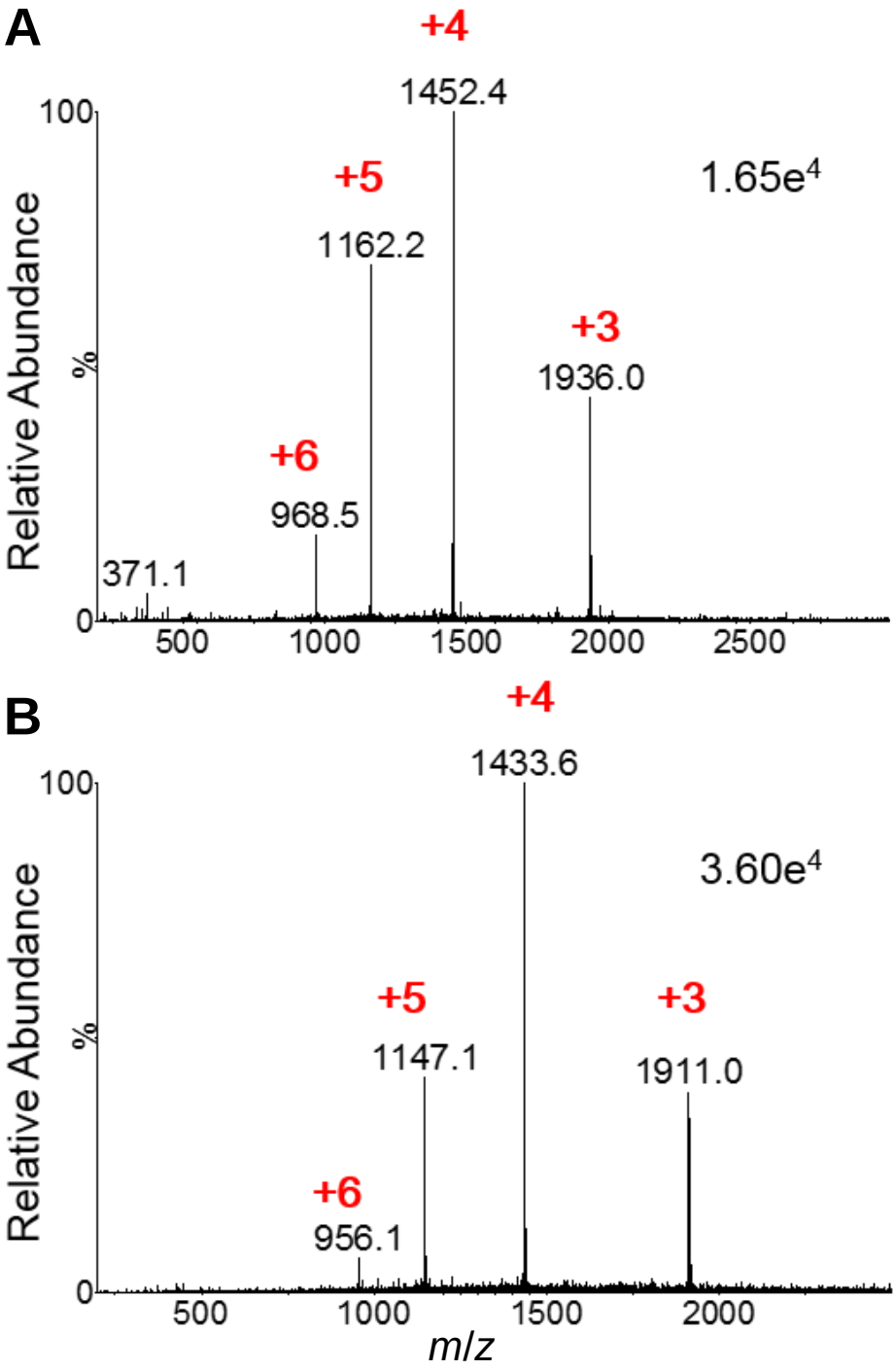



**Figure S8**. MAI mass spectra of (**A**) human insulin (monoisotopic MW 5803.67 Da) and (**B**) bovine insulin (monoisotopic MW 5729.61 Da). The syringe sample introduction method was used to deliver 2.5 pmol of each analyte with 3-NBN as the matrix to the inlet tube of source 1 fitted on a SYNAPT G2S mass spectrometer.

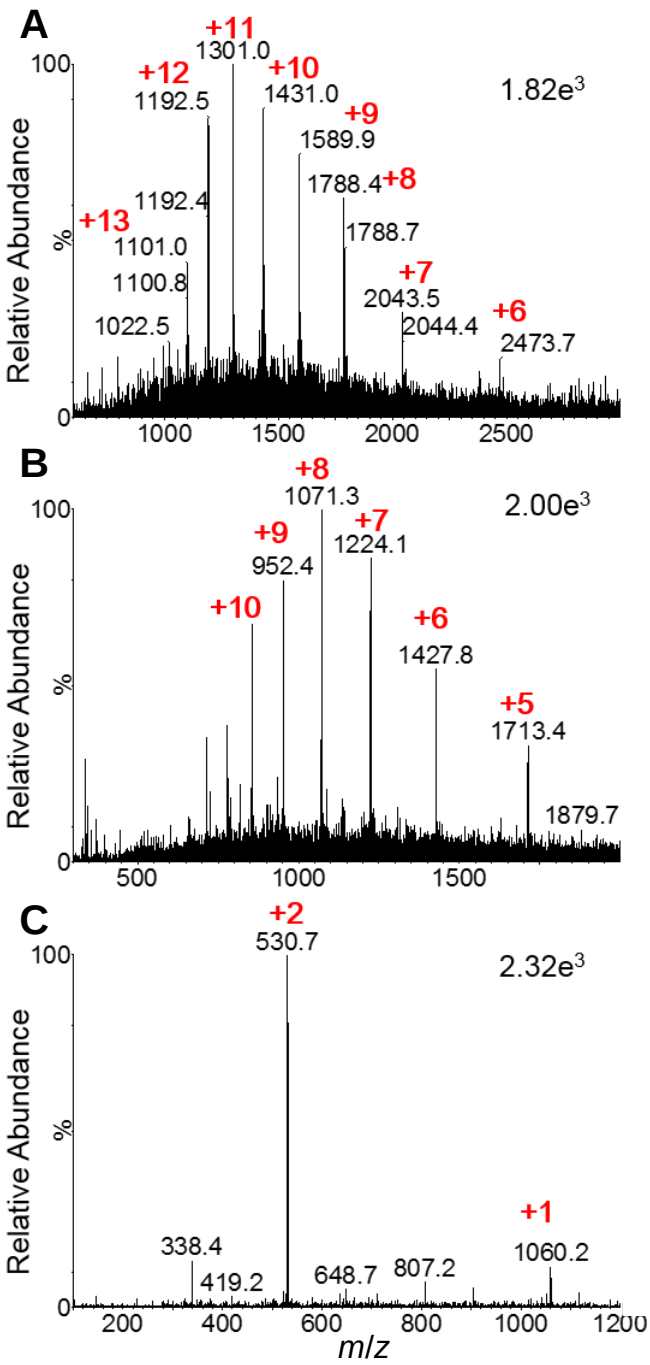



**Figure S9**. LSI mass spectra of 2.5 pmol of (**A**) egg white lysozyme (monoisotopic MW 14305 Da), (**B**) ubiquitin (monoisotopic MW 8559.62 Da), and (**C**) bradykinin (monoisotopic MW 1059.56 Da). Analyte solution (0.5 µL) was spotted on a glass slide followed by 0.5 µL of the matrix mixture and allowed to dry. Matrix:analyte was ablated in transmission geometry.[1] Source 1 fitted with a stainless steel (SS) inlet tube bent to 90$^{o}$ and a binary mixture of 3-NBN:2,5-DHAP (9:1 molar mixture) were used with a SYNAPT G2S mass spectrometer.

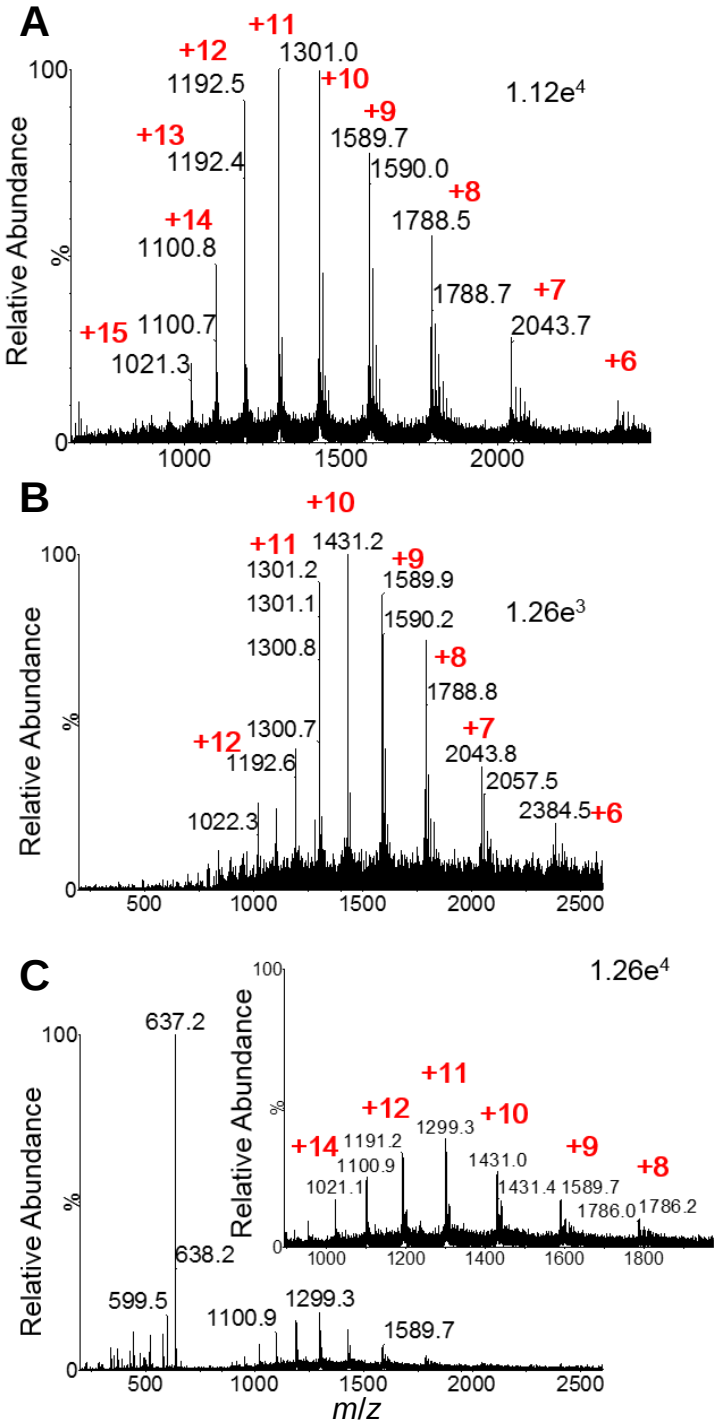



**Figure S10**. MAI mass spectra of egg white lysozyme (monoisotopic MW 14305 Da) using (**A**) source 2, (**B**) source, and (**C**) minimalistic vacuum source with a probe introduction.[2] The syringe sample introduction method was used for (**A**) and (**B**) to deliver 2.5 pmol of each analyte with 3-NBN as the matrix to the inlet tube of source 1

fitted on a SYNAPT G2S mass spectrometer. The minimalistic vacuum source with a probe introduction was also fitted on a SYNAPT G2S mass spectrometer. A 1 µL droplet containing equal volumes of matrix (3-NBN) and analyte (5 pmol $\mu L^{-1}$) was spotted on the stainless steel (SS) probe and allowed to dry prior to insertion of probe to the vacuum source.

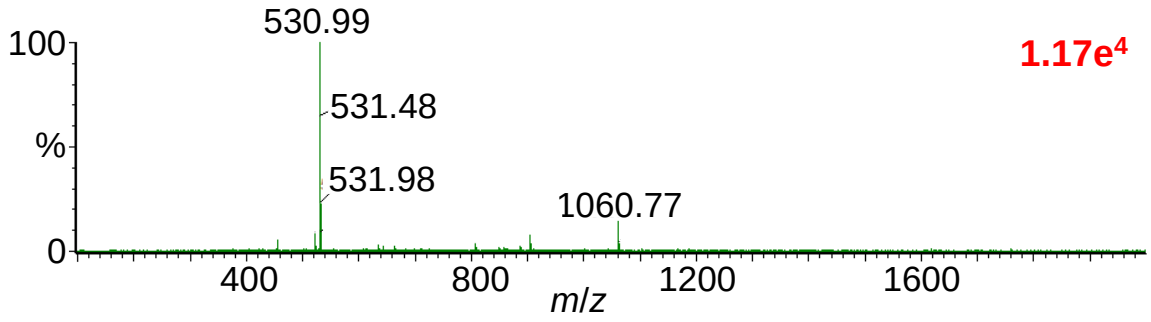



**Figure S11**. Mass spectrum of bradykinin (monoisotopic MW 1059.56 Da) obtained using source 2 with a ball on/off valve add-on modification. The 3-NBN matrix was mixed with the analyte (2.5 pmol) at a 3:1 ratio and 1 µL spotted on a glass slide. Data were acquired using a SYNAPT G2S mass spectrometer.

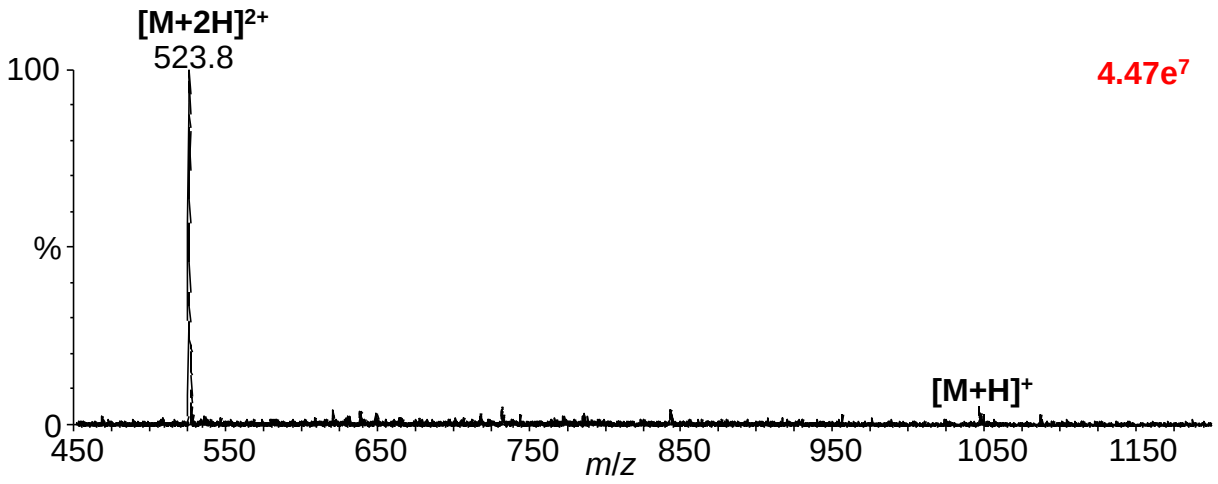



**Figure S12:** MAI mass spectrum of angiotensin II (monoisotopic MW 1045.53 Da) with the matrix:analyte sample applied to a glass coverslip, dried and tapped against the inlet. Source 3, 0.8 pmol analyte, and matrix 3-NBN were used on a Xevo TQ-S mass spectrometer.

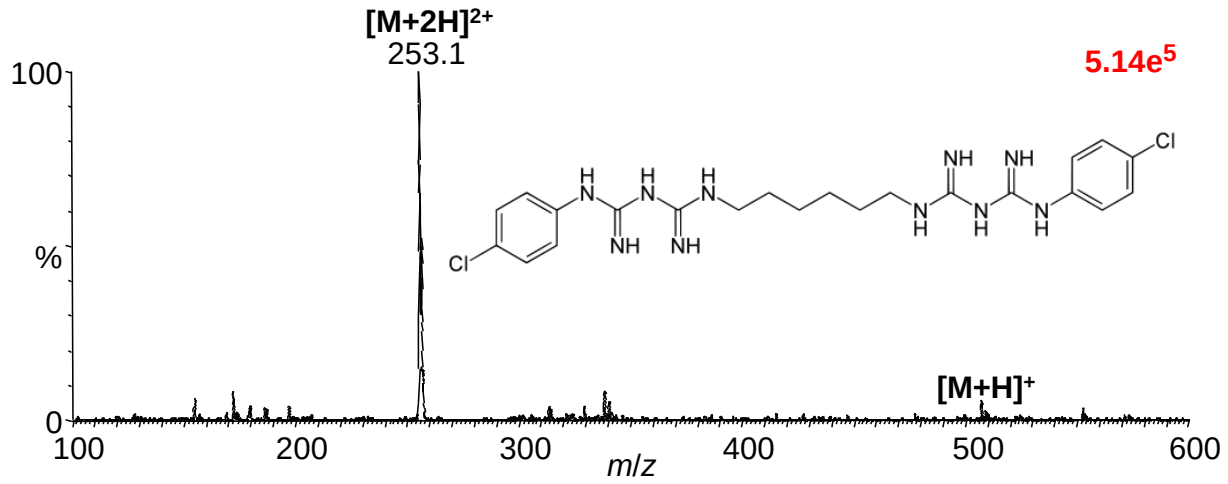



**Figure S13:** MAI mass spectrum of a prescription mouthwash containing chlorhexidine (monoisotopic MW 504.20 Da). Source 3, a solution diluted mouthwash (1:10 in water), and matrix 3-NBN were used on a Xevo TQ-S mass spectrometer.

## References

[1] E. D. Inutan and S. Trimpin, *J. Am. Soc. Mass Spectrom.*, 2010, **21**, 1260–1264.
[2] I. C. Lu, M. Pophristic, E. D. Inutan, R. G. McKay, C. N. McEwen and S. Trimpin, *Rapid Commun. Mass Spectrom.*, 2016, **30**, 2568–2572.