Section 1

The Coulomb potential of two elementary charges at large separation, i.e., before the electron densities overlap is given by:

\[ E_{pot} = \frac{1}{4\pi\varepsilon_0} \cdot \frac{e^2}{r} \]

where \( \varepsilon_0 \) is the vacuum permittivity, \( e \) is the elementary charge and \( r \) is the distance between the charges. For this example a distance of 4 Å should be achieved so that a protonation reaction could actually occur. The resulting potential energy is 3.6 eV. For two ions to approach each other at that distance the center-of-mass collision energy \( E_{kin,com} \) needs to be equal or greater than that value.

\[ E_{kin,com} = \frac{1}{2} \mu v_r^2 \]

where \( v_r \) is the relative velocity and \( \mu \) is the reduced mass. The reduced mass is calculated as follows

\[ \mu = \frac{m_1 \cdot m_2}{m_1 + m_2} \]

where \( m_1 \) and \( m_2 \) are the masses of the particles. For a heavy analyte ion (e.g. Substance P) colliding with a light acid, such as \( \text{H}_3\text{O}^+ \), \( \mu \) is approximately equal to the mass of the lighter collision partner, thus, 19 Da in this example. Given the potential energy of 3.6 eV and the reduced mass of 19 Da, the relative velocity needs to be equal or larger than

\[ v_r = \sqrt{\frac{2 \cdot 3.6 \text{ eV}}{19 \text{ Da}}} = 6050 \text{ m s}^{-1}. \]
This is very unlikely - or better impossible - to occur in an API source. For comparison, the average relative velocity for this example, given that both collision partners follow a Maxwell-Boltzmann velocity distribution according to a temperature $T$ of 300 K, is

$$v_r = \sqrt{\frac{8k_BT}{\pi\mu}} = 578 \text{ m/s}$$

where $k_B$ is the Boltzmann constant. It is pointed out that even in high absolute electrical field gradients, e.g., caused by a corona needle electrode or an ESI emitter, the reduced electrical field strength remain way too small to get anywhere near the velocity required for an additional charge transfer step. The proportion of particles with a velocity $\geq 6050 \text{ m/s}$ can be calculated as

$$P(v_r \geq 6050 \text{ m/s}) = \int_{6050 \text{ m/s}}^{\infty} 4\pi \left(\frac{\mu}{2\pi k_BT}\right)^3 v_r^2 \exp\left(-\frac{\mu v_r^2}{2k_BT}\right) dv_r = 3.8 \times 10^{-60}.$$ 

Thus, formation of a doubly protonated ion via gas-phase protonation of a singly protonated analyte ion is negligible.
Section 2

Experiments were conducted with a custom built nano electrospray ionization (nESI) source, depicted in Figure S1. The diagram in Figure S2 displays the gas flows in the experimental setup.

Figure S1: Half section of the custom nano electrospray source

Figure S2: Sketch of the ion source and gas supply
Section 3

This section contains control spectra without any gas phase modifier present and the mixing ratio dependent relative intensities of protonated Substance P (SP\(^{2+}\), SP\(^{3+}\)) and Substance P-modifier clusters (if existing) and representative mass spectra of the corresponding experiments.

*Figure S3: Mass spectra of SP without gas phase modifiers; SP only (top), SP + DMSO as SCA (center) and SP + sulfolane as SCA (bottom)*
Figure S4: Acetonitrile mixing ratio dependent intensities of SP species (top) and corresponding mass spectrum with 9.5 % acetonitrile (bottom)

Figure S5: Acetone mixing ratio dependent intensities of SP species (top) and corresponding mass spectrum with 11.0 % acetone (bottom)
Figure S6: Diethyl ether mixing ratio dependent intensities of SP species (top) and corresponding mass spectrum with 15.5 % diethyl ether (bottom)

Figure S7: Methanol mixing ratio dependent intensities of SP species (top) and corresponding mass spectrum with 1.9 % methanol (bottom)
Figure S8: Ethanol mixing ratio dependent intensities of SP species (top) and corresponding mass spectrum with 7 % ethanol (bottom)

Figure S9: Water mixing ratio dependent intensities of SP species (top) and corresponding mass spectrum with 2.8 % water, the peak at m/z 462.1 is a contamination (bottom)
Figure S10: Ammonia mixing ratio dependent intensities of SP species (top) and corresponding mass spectrum with 0.01 % ammonia (bottom)

Figure S11: Methanol mixing ratio dependent intensities of SP species with 1 % DMSO added as SCA (top) and corresponding mass spectrum with 3.8 % methanol (bottom)
Figure S12: Acetonitrile mixing ratio dependent intensities of SP species with 1 % DMSO added as SCA (top) and corresponding mass spectrum with 1.35 % acetonitrile (bottom)

Figure S13: Methanol mixing ratio dependent intensities of SP species with 1 % sulfolane added as SCA (top) and corresponding mass spectrum with 0.76 % methanol (bottom)
Figure S14: Acetonitrile mixing ratio dependent intensities of SP species with 1 % sulfolane added as SCA (top) and corresponding mass spectrum with 4.05 % acetonitrile (bottom)
Section 4

Figure S15: Figure 2 with error bars
Figure S16: Figure 3 with error bars
Figure S17: Figure 4 with error bars
Figure S18: Figure 5 with error bars
Figure S19: Figure 6 with error bars