Investigations of Analyte-Specific Linear Dynamic Range Limitations in Atmospheric Pressure Ionization Mass Spectrometry

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Abstract. This supplementary information includes the structures of all compounds investigated herein (Chart S-1), a proposed reaction by which quercetin becomes protonated (Scheme S-1), a comparison of the calibration curves obtained with two types of mass analyzers (Figure S-1), a comparison of calibration curves obtained with electrospray ionization mass spectrometry (ESI-MS) in the positive ion mode for two different analytes (Figure S-2), a schematic comparing the ionization processes for HESI and simultaneous HESI/ESI with the dual source (Figure S-3), and details of how equation 1 in the main text was derived and applied to experimental data.

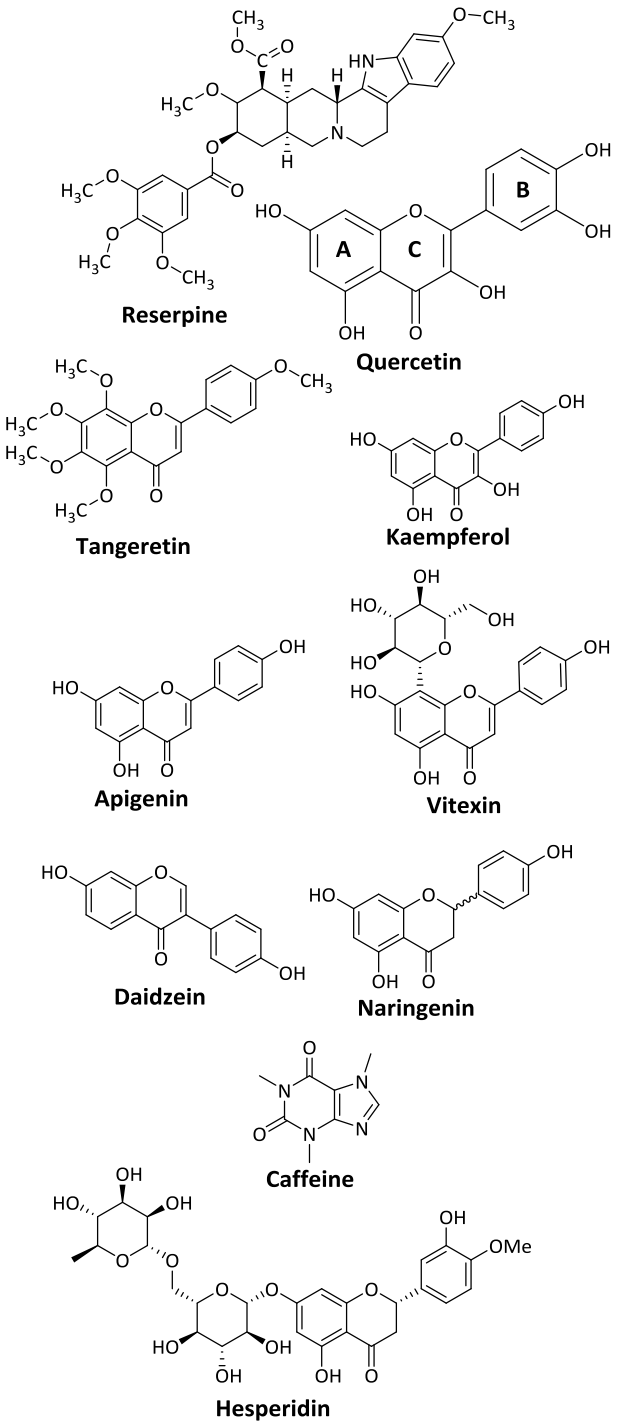
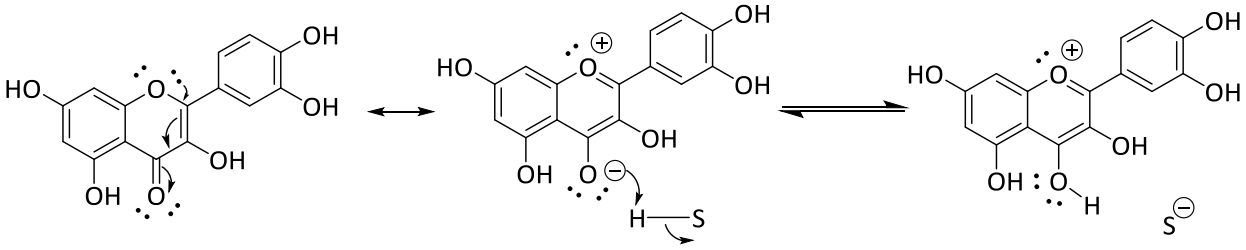


Chart S-1. Structures of analytes studied. Reserpine and caffeine are alkaloids, the rest of the test analyte are flavonoids. Common flavonoid ring (A,B,C) nomenclature is shown for quercetin.



Scheme S-1. Proposed mechanism of quercetin protonation, where SH is a molecule of protic solvent (i.e. H_2O). This reaction is based on quantum chemical predictions that the carbonyl oxygen on the flavonoid C ring is the most basic part of the molecule.[1]

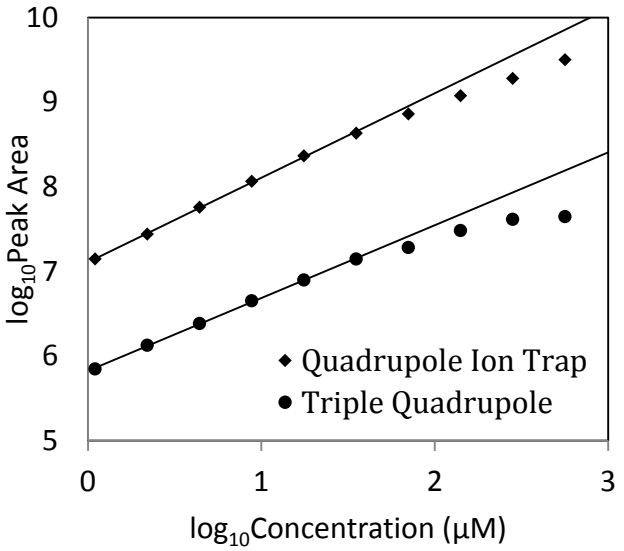


Figure S-1. ESI-MS linear dynamic range for quercetin is limited at high concentration with both quadrupole ion trap (LCQ Deca) and triple quadrupole (TSQ Quantum Access) mass spectrometers. The conditions for the triple quadrupole MS analyses are described under methods in the main text. For the analyses with the ion trap mass spectrometer, the conditions were as follows: 4 kV spray voltage, sheath gas and auxiliary gas at 30 and 10 arbitrary units, capillary temperature of 325 °C, capillary voltage of 13 V, and tube lens offset of -25 V. The ion trap mass spectrometer was interfaced to an Agilent HP1100 HPLC (Palo Alto, CA) with an Alltech Prevail C18 column (3µm particle size 2.1 x 50mm) and a 9 min analysis time. Mobile phase conditions were as follows: 60% A to 40% B from t = 0 to t = 5 min., 100% B from t = 5 min. to t = 5.5 min., maintained until t = 7 min., then initial conditions from t = 7 min. to t = 7.5 min., and maintained until t = 9 min.

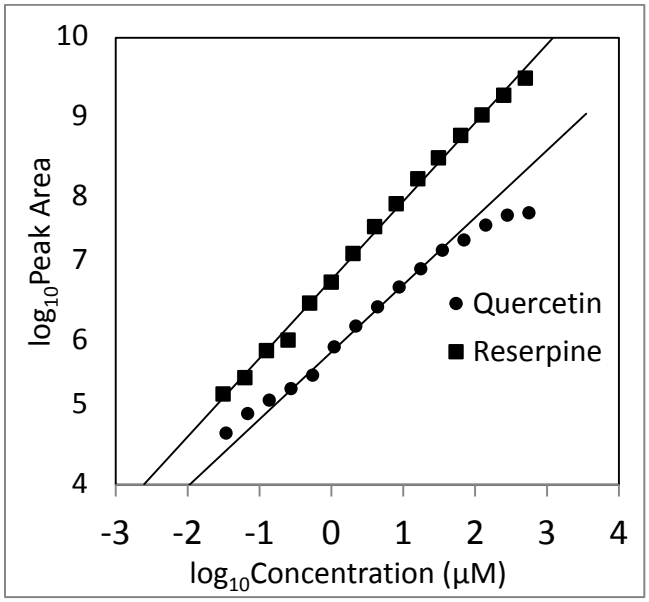


Figure S-2. Comparison of working curves for two analytes, quercetin and reserpine, (see structures in Chart S-1) analyzed by electrospray ionization mass spectrometry (ESI-MS) in the positive ion mode. Reserpine demonstrates a linear relationship between concentration and response over the concentration range investigated, while saturation in response is observed for quercetin. Note that the reserpine response is greater than quercetin response for every concentration.

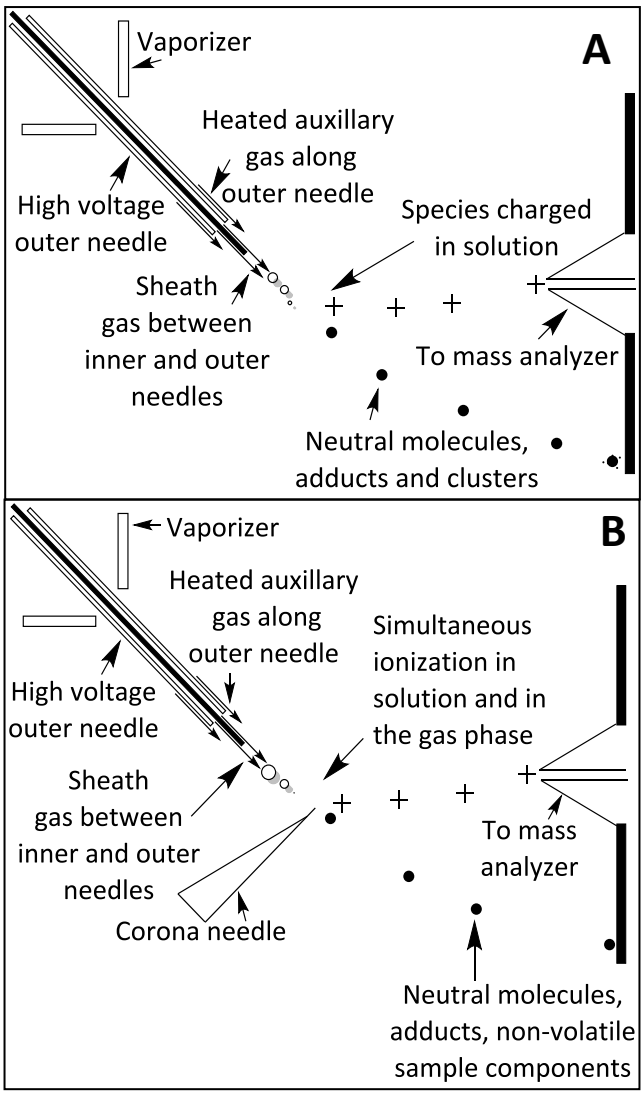


Figure S-3. Comparison of ionization process for a heated electrospray ionization (HESI) source and in the dual source mode (simultaneous HESI/APCI). For the HESI source, voltage is applied at the spray capillary, creating charged droplets. For the dual mode, voltage is applied to both the spray capillary and the corona discharge needle.

Methods for Fit of Experimental Data

The experimental $[MH^+]$ value for quercetin at each standard concentration (C_i) was obtained by substituting the relevant peak area into the equation for the best fit line of the quercetin calibration curve. To obtain predicted values for $[MH^+]$ as a function of C_i, we relied on an equation derived in a previous study (eq 1 main text, shown again here for ease of reference). [2]

$$\left[MH^+\right] = \frac{K_b C_i [H_3O^+]}{K_b [H_3O^+] + K_w} \quad (1)$$

Equation S-1 depends a calculated value of $[H_3O^+]$,[2] which considers the contribution of added acid (in this case 0.1% formic acid). In eq 1, K_b is the thermodynamic equilibrium constant (eq 3) for the protonation of the analyte by the solvent (eq 2), where M is the analyte in its neutral form and MH^+ is the protonated analyte.

$$M + H_2O \rightleftharpoons MH^+ + OH^- \quad (S\text{-}1)$$

$$K_b = \frac{[MH^+][OH^-]}{[M]} \quad (S\text{-}2)$$

To calculate theoretical values of $[MH^+]$ from eq 1, a value of the thermodynamic equilibrium constant (K_b) for quercetin was calculated from eq 4 by substituting in the empirical value for $[MH^+]$ at a concentration of 5.8×10^{-5} M in the HESI calibration curve. This datapoint was chosen for the calculation of K_b because it gave the best fit of the experimental data. Equation 4 was derived from eq 3 relying on the relationship that $[M] = (C_i - [MH^+])$ (mass balance equation) and $[OH^-] = K_w/[H_3O^+]$ (rearrangement of autoprotolysis reaction for water). In eq 4, K_w is the autoprotolysis constant for water, which has a published value of 1.82×10^{-15} in 40% aqueous acetonitrile.[3] The K_a of formic acid used in the calculations was 3.02×10^{-5} which was reported in the literature as the appropriate value in this same solvent system.[4]

$$K_b = \frac{[MH^+]\left(\frac{K_W}{[H_3O^+]}\right)}{(C_i - [MH^+])} \quad (S\text{-}3)$$

References (Supplemental)

(1) Aleman, C. *J. Mol. Struct.: THEOCHEM* **2000**, *528*. 65-73.
(2) Ehrmann, B. M.; Henriksen, T.; Cech, N. B. *J. Am. Soc. Mass Spectrom.* **2008**, *19*. 719-28.
(3) Kilic, E.; Aslan, N. *Microchim. Acta* **2005**, *151*. 89-92.
(4) Padro, J. M.; Acquaviva, A.; Tascon, M.; Gagliardi, L. G.; Castells, C. B. *Anal. Chim. Acta* **2012**, *725*. 87-94.