Supplementary material

A Zwitterionic Hydrophilic Interaction Liquid Chromatographic Photo Diode Array Method as a Tool to Investigate Oxalic Acid in Bees: Comparison with Mass Spectrometric Methods

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GC-MS system and operating conditions

The GC-MS analysis was performed on a Chromtech Evolution 3 MS/MS triple quadrupole mass spectrometer (Bad Camberg, Germany) built on an Agilent 5975 B inert XL EI/CI MSD system (Agilent Technologies, Santa Clara, CA, USA). Samples were injected with a Gerstel MPS-2 autosampler using a 10 μL syringe. Separations were performed on a HP-5ms UI, length 30m, ID 0.25mm, film thick. 0.25 μm (J&W Folsom, USA). Helium was used as the carrier gas at a flow rate of 1.4 mL min^{-1}. The column oven temperature program started from 45°C at which was held for 1 min, increased to 250°C at a rate 5°C min^{-1}, where it stayed for additional 5 min. The mass spectrometer was operated in EI mode using the full scan data acquisition mode. The transfer line, manifold and source of ionization temperatures were 300, 40 and 230°C. The total GC analysis time was 47 min.

Figure S1. HPLC-UV chromatogram of a blank honeybee sample extract

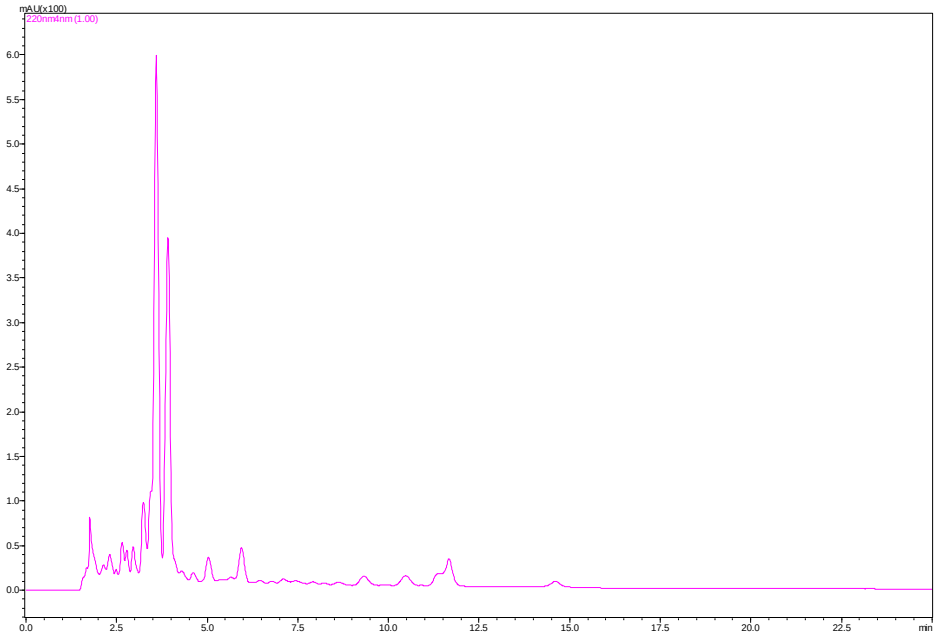

Figure S2. Magnified HILIC-UV chromatogram of a blank honeybee sample

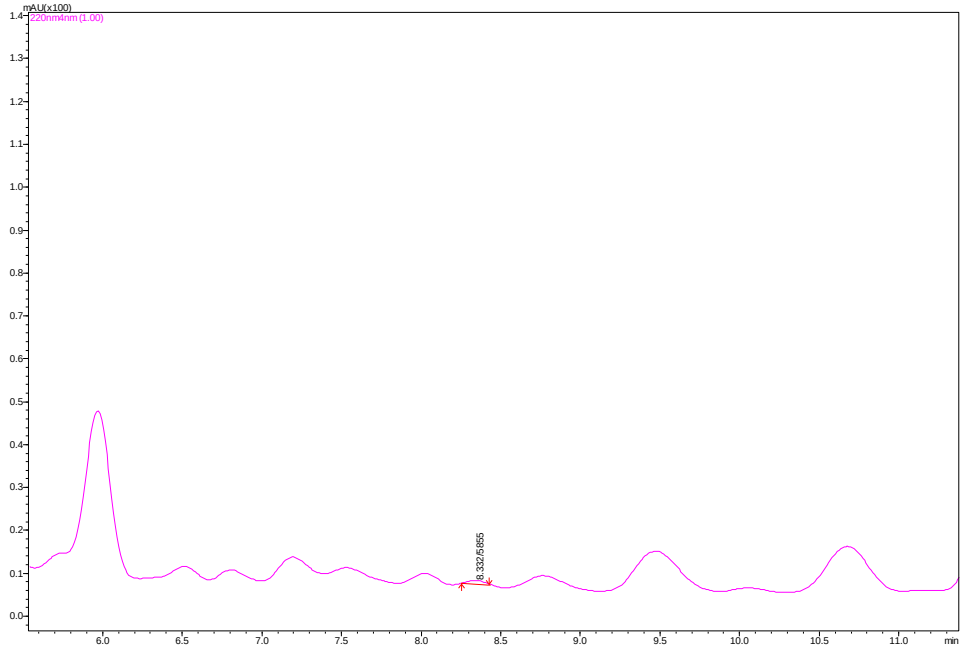

Figure S3. HPLC-UV chromatogram of a standard solution of oxalic acid at 20 µg/mL.

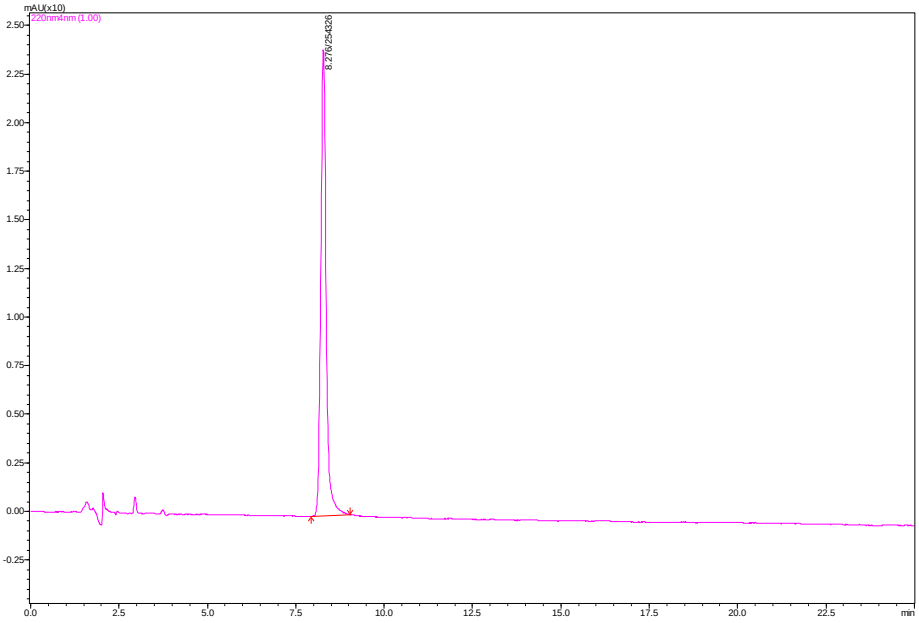

Figure S4. Peak purity curve obtained for a positive bee sample in OA

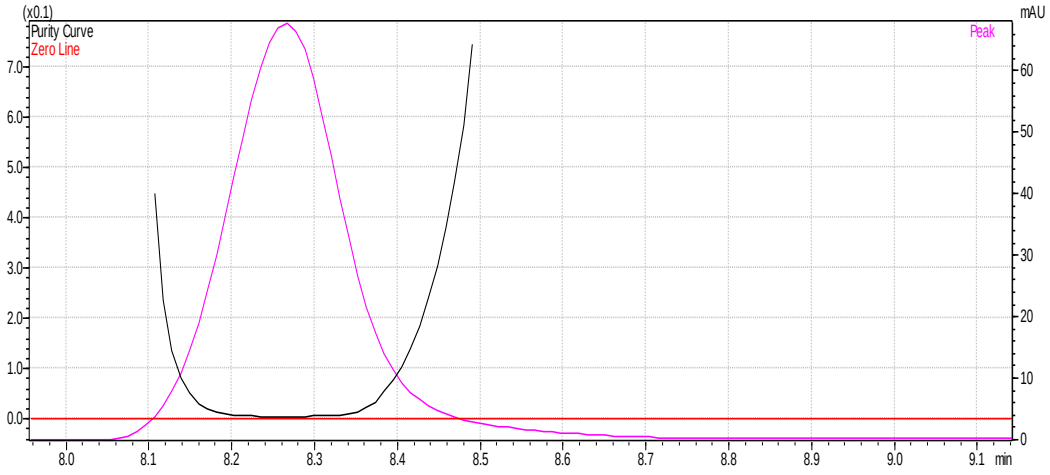

Figure S5. TIC, MRM chromatograms of a OA positive bee sample.

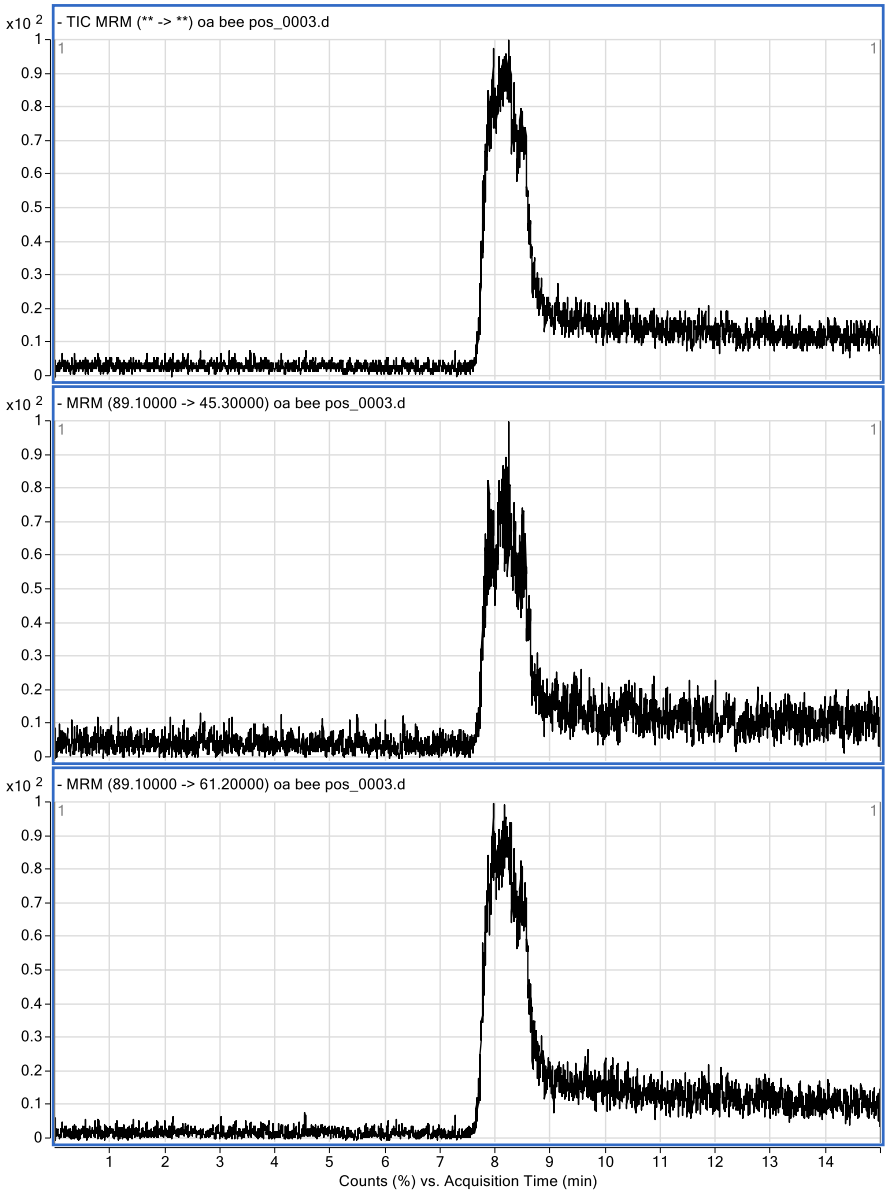

Figure S6. TIC, MRM chromatograms of a control bee sample.

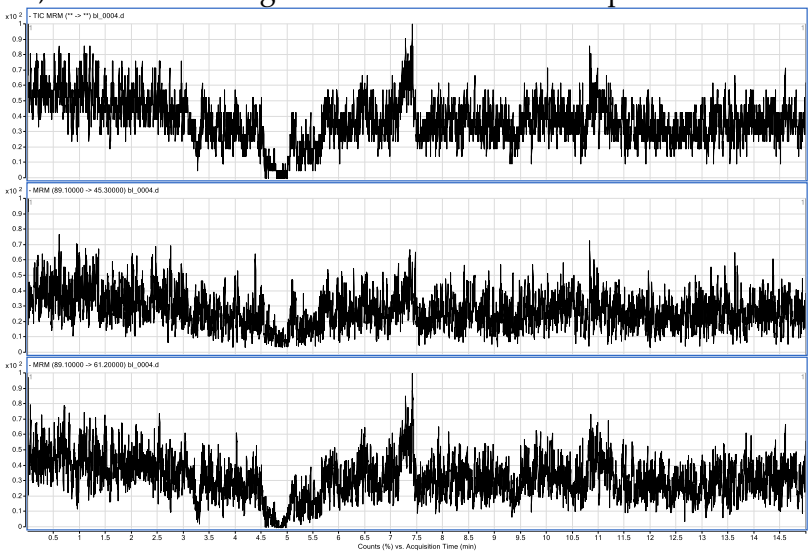

Table S1. Chromatographic parameters and MRM transitions for oxalic acid

Compound	Retention time (min)	Precursor ion (m/z)	Product ions (m/z)	Dwell time	Fragmentor voltage	CE (eV)
Oxalic acid	8.2	89.1	61.2[a]	15	34	10
			45.3[b]	15	34	34

[a]Quantitation ion, [b]Confirmation ion

Figure S7. Full scan GC-MS total ion chromatogram of derivatized OA standard in ACN at 1 µg/mL.

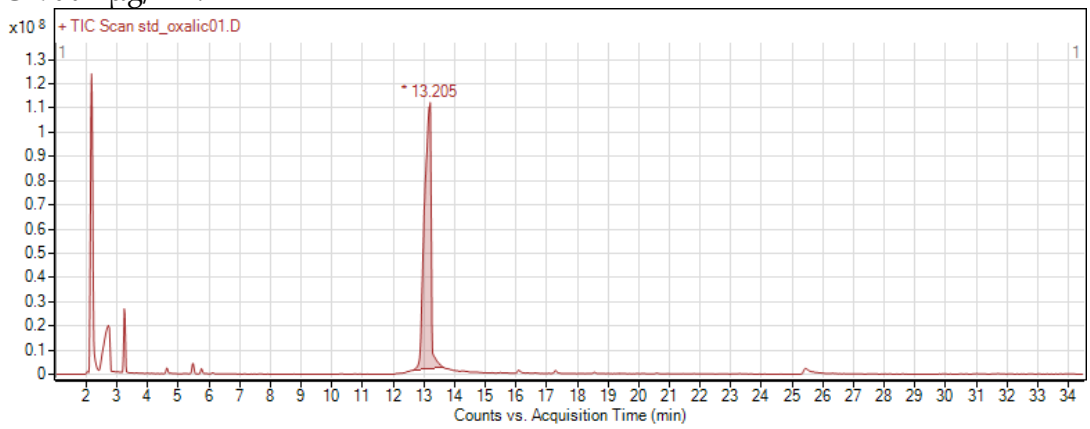

Figure S8. HILIC-UV chromatogram of a standard mixture of OA and LA at 10 µg/mL (using phosphate buffer).

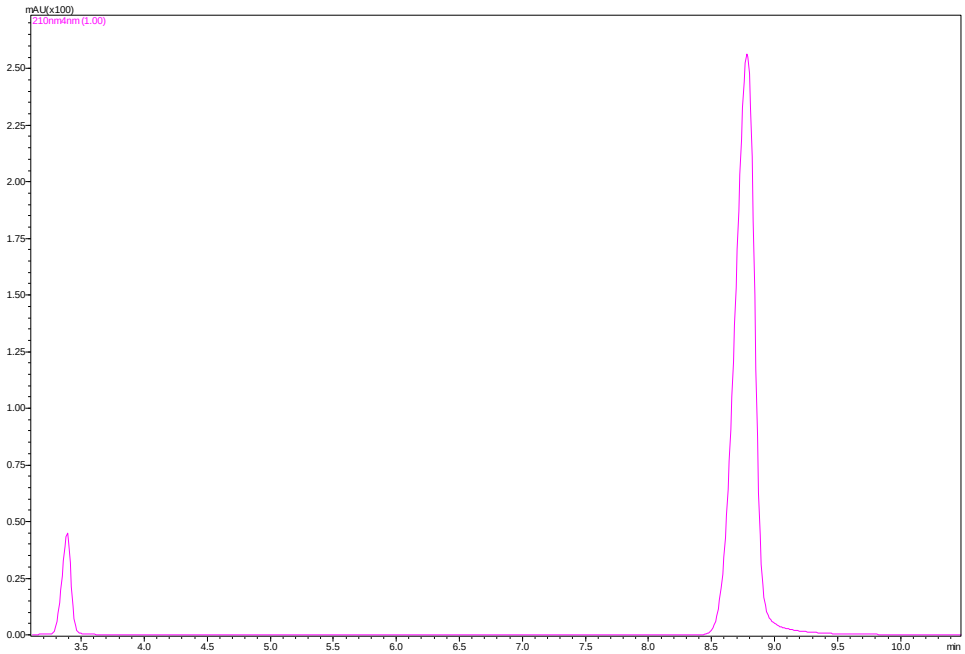

Figure S9. HILIC-PDA chromatogram of LA and OA in standards solution mix (using ammonium acetate buffer)

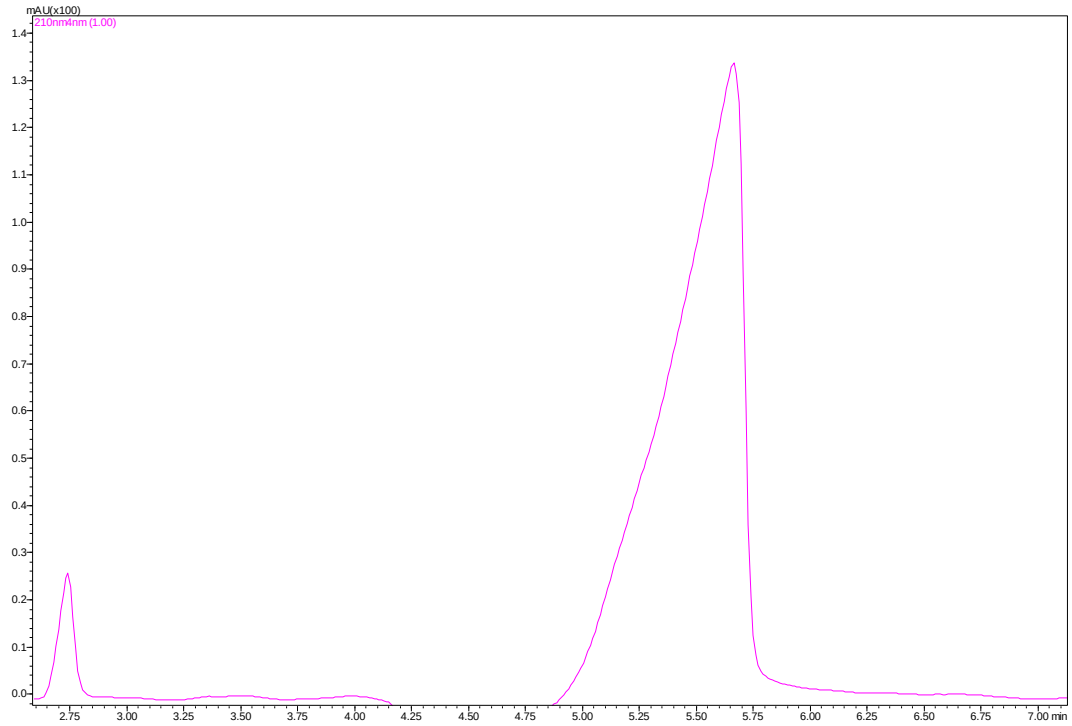

Figure S10. HILIC-ESI/MS SIM chromatogram of LA and OA in standards solution mix (using ammonium acetate buffer)