Supplementary Material

Adjustment of matrix effects in analysis of 36 secondary metabolites of microbial and plant origin in indoor floor dust using liquid chromatography-tandem mass spectrometry

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Key words: Indoor Floor dust; Matrix effect adjustment; Microbial secondary metabolite (MSM); Recovery; Validation

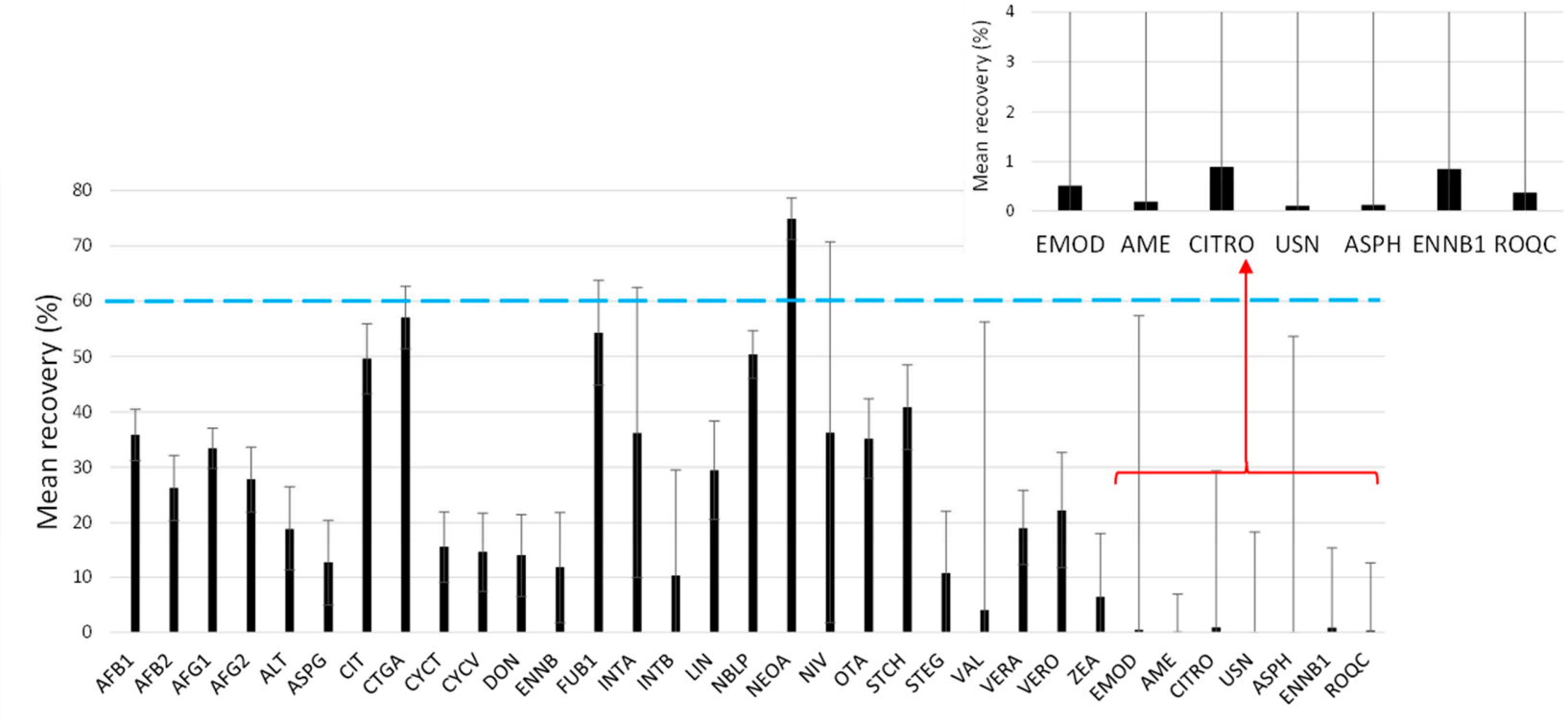

Figure S1. Mean percent recovery rates and standard deviations of SMs (insert: < 1% mean percent recoveries) when matrix effects were unadjusted in the initial experiment. Blue line shows acceptable recovery threshold (≥ 60%).

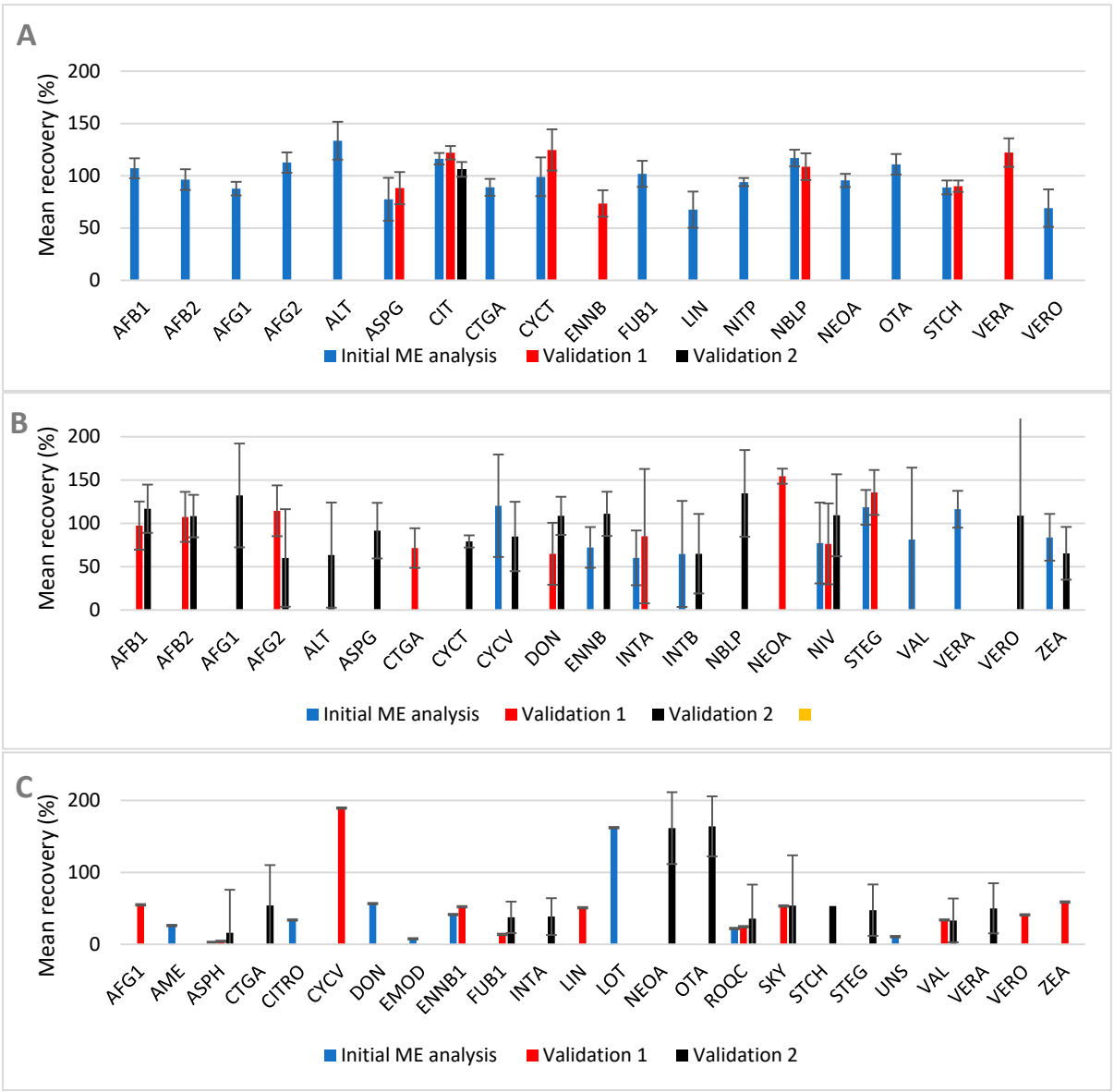

Figure S2. Mean percent recovery rates and standard deviations of SMs from the three experiments when matrix effects were adjusted with the best performing ISTDs. A: metabolites for which recoveries were in 60–140% with <20% CV; B: recoveries in 60–140% but % CV > 20; and C: recoveries <60 or > 140%. ME = matrix effects

Table S1. Proposed mechanisms, results and scenarios of matrix effects

Matrix effect occurrence site	Mechanism of Matrix Effect	Result of Matrix Effect
Mass spectrometer	Coeluting sample matrix components with same transitions masking or enhancing signals of target analyte [1, 2]	Matrix-induced enhanced or suppressed signal; difficult integration conditions such as peak shape distortion; elevated background; poor and inconsistent signal reproducibility between replicate injections [2]; wider peaks on tailing or fronting sides of background or matrix peaks
High voltage capillary	Viscous materials co-eluted with sample matrix components adversely hinder effective nebulization of mobile phase by a combination of pneumatic force (from high velocity nitrogen gas) and electrospray voltage (electric field)	Diminished efficiency in both nebulization and Coulomb explosion of liquid droplet, depending on matrix component concentration
High voltage capillary and liquid droplet	Both electrolyte salts and non-conducting inorganic matrix components such as carbonates/sulfates in high concentrations alter both the drying rate and electrical conductivity of the mobile phase exiting the high voltage capillary as well as liquid droplets [2, 3]. For matrix based extracts, this will ultimately have a significant effect on the dispersion of solute free ions in the gas phase, influencing the chemical composition and abundance of desolvated analyte ions in the gas phase [4]	Inefficient drying and subsequent low ionization of target analytes due to a combination of the shift in effective drying gas flow, drying temperature and the low conductivity of liquid droplets
Liquid droplet	Coeluting sample matrix components compete for limited charge space on the surface of the nebulized liquid droplet [2, 5]	Diminished charge capture by analytes of interest due to more charging capture by matrix components. Lower proportion of target ions ejection from surface of charged liquid droplet [6]
Liquid droplet	Coeluting matrix components may alter the surface tension holding the liquid droplet together, thereby altering the degree of Coulomb force (force of	Formation of gas phase ions in matrix extracts occurs at different rates and efficiency as with matrix-free analytical standard: (i) in case of lower surface tension, surfactant-like matrix

	repulsion between the like charges) required to overcome the surface tension (Rayleigh limit) [7].	components rapidly collapse the liquid droplets as they can no longer easily contain the Coulombic force [7, 8]; and (ii) in case of higher surface tension resulting from matrix components, larger and less frequent Coulombic explosions are generated, which disperses the sample across a much larger area beyond the MS sampling cone, causing analyte loss [7, 8].
Liquid droplet	Partitioning of target analytes strongly onto non-volatile matrix components. Non-volatile components may reduce the electrical conductivity of the nebulized liquid droplets, resulting in reduction in the ability of the charged droplets to generate enough electrostatic stress (Coulomb force [7]) on the surface on the liquid droplet required to generate a coulombic explosion (burst of the liquid droplet) as well as generate coulombic fission events to generate smaller liquid droplets.	Inefficient ionization of target analytes due to low coulombic explosions and coulombic fission.
Liquid droplet and gas phase	Before undergoing Coulomb fission (according to Fenn's model of ion formation), large nebulized liquid droplets contain several different analytes of different shapes and sizes [7]. As a result of matrix components in the large liquid droplet , there is more competition for proton/adduct transfer between the analyte of interest and the coeluting analytes. This competition may be carried over to the gas phase.	Diminished efficiency of proton/adduct acceptance/release by the analyte of interest

References

1. Tisler, S., D. I. Pattison and J. H. Christensen. "Correction of matrix effects for reliable non-target screening lc–esi–ms analysis of wastewater." *Analytical Chemistry* 93 (2021): 8432-41. 10.1021/acs.analchem.1c00357. https://doi.org/10.1021/acs.analchem.1c00357.
2. Nasiri, A., R. Jahani, S. Mokhtari, H. Yazdanpanah, B. Daraei, M. Faizi and F. Kobarfard. "Overview, consequences, and strategies for overcoming matrix effects in lc-ms analysis; a critical review." *Analyst* (2021):
3. Page, J. S., R. T. Kelly, K. Tang and R. D. Smith. "Ionization and transmission efficiency in an electrospray ionization–mass spectrometry interface." *Journal of the American Society for Mass Spectrometry* 18 (2007): 1582-90. https://doi.org/10.1016/j.jasms.2007.05.018. https://www.sciencedirect.com/science/article/pii/S1044030507004357.
4. Nguyen, S. and J. B. Fenn. "Gas-phase ions of solute species from charged droplets of solutions." *Proc Natl Acad Sci U S A* 104 (2007): 1111-7. 10.1073/pnas.0609969104.
5. Skillman, B. and S. Kerrigan. "Identification of suvorexant in blood using lc–ms-ms: Important considerations for matrix effects and quantitative interferences in targeted assays." *Journal of Analytical Toxicology* 44 (2019): 245-55. 10.1093/jat/bkz083. https://doi.org/10.1093/jat/bkz083.
6. Fenn, J. B. "Ion formation from charged droplets: Roles of geometry, energy, and time." *Journal of the American Society for Mass Spectrometry* 4 (1993): 524-35. 10.1016/1044-0305(93)85014-O. https://doi.org/10.1016/1044-0305(93)85014-O.
7. Banerjee, S. and S. Mazumdar. "Electrospray ionization mass spectrometry: A technique to access the information beyond the molecular weight of the analyte." *International Journal of Analytical Chemistry* 2012 (2012): 282574. 10.1155/2012/282574. https://doi.org/10.1155/2012/282574.
8. Kirjavainen, P. V., M. Täubel, A. M. Karvonen, M. Sulyok, P. Tiittanen, R. Krska, A. Hyvärinen and J. Pekkanen. "Microbial secondary metabolites in homes in association with moisture damage and asthma." *Indoor Air* 26 (2016): 448-56. 10.1111/ina.12213.