

Online supplement 2.

Calibration of exhaled VOCs

In the case of GC-MS analysis, both external and internal calibration can be used. System response can be checked using external calibration, with the aid of liquid standards [1], gas standards using permeation devices [2] or a mixture of certified calibration gases [3]. Method performance with respect to linearity, limit of quantification (LOQ), limit of detection (LOD), repeatability and reproducibility needs to be established.

Batch-to-batch response is normally checked using a pooled QC [4], though this is rather difficult for breath gas analysis. Spiking of breath samples with an isotopically labelled multi-compound gas standard, using *e.g.* deuterated compounds like acetone-d₆, hexane-d₁₄, toluene-d₈ and xylene-d₁₀, covering a broad boiling point range, is preferred. This allows for monitoring any GC-MS machine drifts between breath samples. Following the discovery phase (untargeted analysis), absolute quantitative analysis (targeted analysis) can be performed using a (labelled) standard for every biomarker of interest [4]. Notably, breath samples contain several easily recognizable compounds that can be used as internal standards for daily control of retention time shifts.

References

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