Quantitative Non-Targeted Analysis: From Data to Decisions

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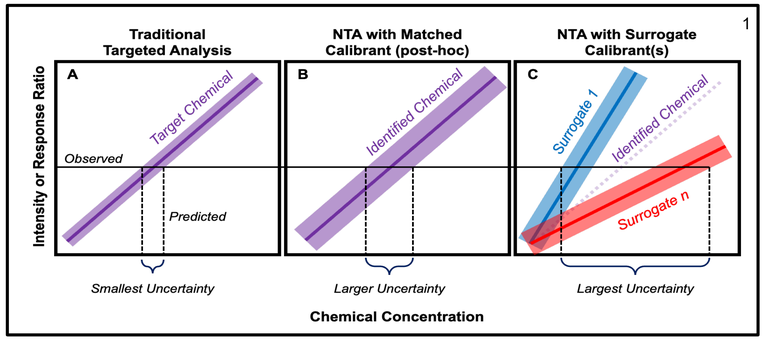
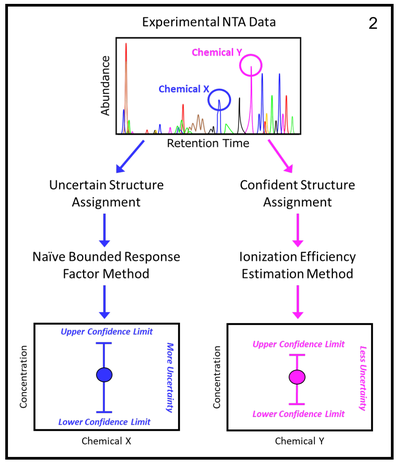
Introduction

- Traditional targeted analysis requires standards for methods development
- Targeted analysis using standards facilitates robust compound quantitation
- NTA studies can acquire standards for confirmation and post-hoc quantitation
- Post-hoc analyte quantitation is subject to increased estimation error
- True quantitative NTA (qNTA) does not utilize structure-matched standards
- qNTA relies on calibration information from one or more surrogate analytes
- Estimation error is larger with qNTA than with post-hoc quantitative analysis
- Strategies are needed to estimate and minimize qNTA estimation error



Concept



- Every HRMS measurement of every compound yields an empirical response factor (RF=abundance/concentration)
- The RF is assumed stable when operating within the linear dynamic range
- The RF is never perfectly stable
- An RF from any surrogate can be used to estimate the concentration of any analyte
- A distribution of RFs across many compounds can be used to estimate the uncertainty about individual concentration predictions
- Models that predict ionization efficiency may be able to reduce quantitative estimation uncertainty

Methods

Data

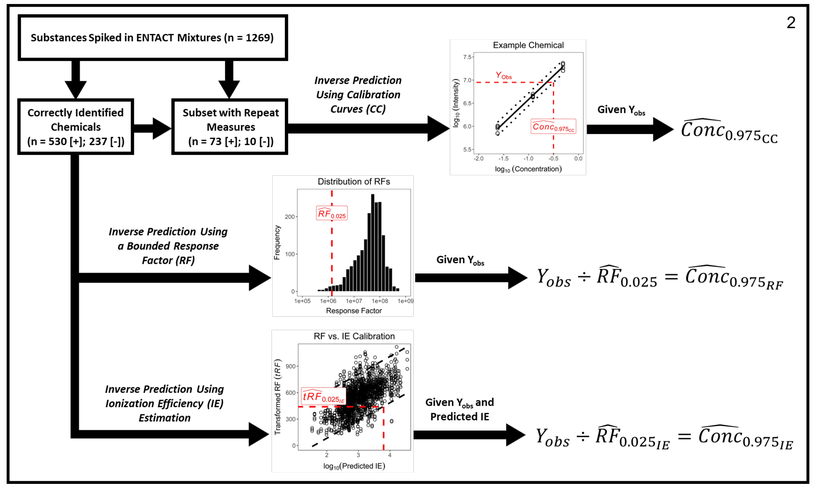
- NTA data were from EPA's Non-Targeted Analysis Collaborative Trial (ENTACT)
- All data were collected and processed using semi-automated techniques
- Full dataset included 530 chemicals for ESI+ mode and 237 chemicals for ESI- mode
- Each chemical in the full dataset was measured at multiple dilutions
- A chemical subset was measured at multiple dilutions in multiple samples

Modeling

- Inverse concentration prediction was performed using three methods:
  - ***Traditional calibration curve method:***
    - Only for chemical subset measured in multiple samples
    - Performed using log-log regression with 95% prediction intervals
  - ***Bounded response factor (RF) method:***
    - Naïve method that does not consider chemical structure
    - Requires non-parametric estimation of RF 2.5th and 97.5th percentiles
  - ***Ionization efficiency (IE) estimation method:***
    - Uses chemical structures and predicted IE values to restrict possible RF values
    - Requires data transformations and linear mixed-effects modeling

Evaluation

- Performed hierarchical bootstrap sampling with five-fold cross validation
- Upper confidence limit estimates of concentration used for evaluation
- Error quotient (EQ) is the upper confidence limit / true concentration



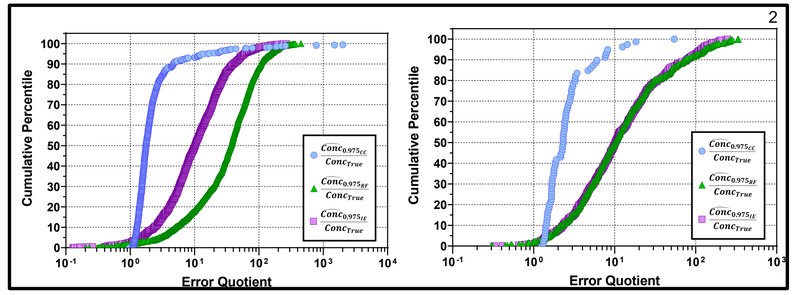
Results

ESI+ Mode Data:

- Calibration curve method:
  - 95% of EQs ≤ 16
  - 50% of EQs ≤ 2
- Bounded response factor method:
  - 95% of EQs ≤ 152
  - 50% of EQs ≤ 37
- Ionization efficiency estimation method:
  - 95% of EQs ≤ 60
  - 50% of EQs ≤ 10

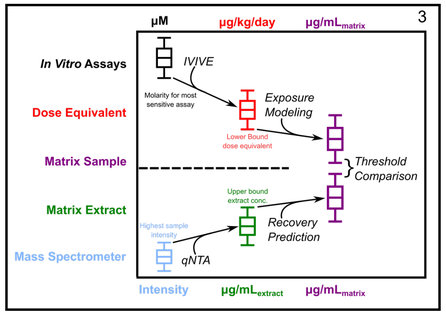
ESI- Mode Data:

- Calibration curve method:
  - 95% of EQs ≤ 8
  - 50% of EQs ≤ 2
- Bounded response factor method:
  - 95% of EQs ≤ 128
  - 50% of EQs ≤ 10
- Ionization efficiency estimation method:
  - 95% of EQs ≤ 117
  - 50% of EQs ≤ 10



Conclusions & Next Steps

- Multiple viable methods for estimating uncertainty in qNTA predictions
- The magnitude of estimation error reflects random and between-chemical effects
- IE prediction models can help reduce estimation error
- Future models must additionally consider extraction & matrix effects
- Upper-bound qNTA estimates require hazard-based context for risk-based interpretation



References

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[2] Groff et al. Anal. Bioanal. Chem. *Accepted*
[3] McCord et al. Environ. Int. 2022. doi:10.1016/j.envint.2021.107011