A Method to Estimate False Positive and False Negative Rates with Regard to Resolving Power, Precursor Isolation and Complexity of the Matrix for High Resolution Accurate Mass Spectrometry

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Overview

Purpose: Methodology for estimating the false positive and false negative rates in high resolution accurate mass spectrometry on the basis of matrix measurements

Methods: Potential interference counting counts any potential interference during a LC-MS/(MS) for a "hypothetical" quasi-molecular-ion or given transition. For potential false negative estimation the mass spectrometric resolution is taken into account.

Results: Preliminary results show consistent results in different matrices for different compounds with different HRAM methods. Method could also be adopted for guiding method development in order to set the most properly high resolution accurate mass settings on an orbitrap instrument.

Introduction

Due to the gaining popularity of high resolution accurate mass (HRAM) methods there is a multitude of HRAM methods available currently, ranging from single stage HRAM-MS to data independent data acquisition (with and without precursor selection) to triple quadrupole equivalent methods. All these methods claim the improved selectivity, reduced false positive rates (FPR) and false negative rates (FNR) in comparison to the gold standard. But it seems there are uncertainties to what extend the influencing factors change selectivity, FPR's and FNR's, and consequently, which method or instrument setting is appropriate for the particular experiment.

The concept of "potential interference counting" is introduced and applied here in order to systematically asses the influencing factors and help method development finding the correct settings for high resolution mass spectrometers. All interferences counted are calculated from matrix measurements without any spiking and therefore are not real. However they have the potential to interfere with the analytes in a real experiment, since they meet the criteria defined by the model.

Consequently potential interferences calculated by this approach could be eventually used to estimate false positive and false negative rates dependant on the HRAM applied method.

Methods

Sample Preparation

Six different matrices were prepared with a modified QuEChERS method:

Tomato, leek, wheat, honey, black tea, compound animal feed (horsefeed)

Test method(s)

All matrices were measured on a Thermo Scientific™ Q Exactive™ HF at highest resolution setting of 240,000 (FWHM) at m/z 200 assumed any mass spectrometric interference is resolved.

Different methods were applied:

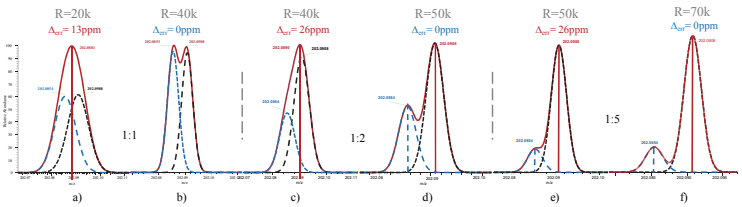
Full MS, wide isolation MS/MS (100Th and 25Th), PRM (1Th)

Data Analysis

An in-house developed software was used to count interferences using the described model. Pot. Fpos and Fneg rates are normalized to the total scan number and are reported as percentage (potential interferences per 100 acquired spectra).

For each compound 5 fragment ions were included into the calculation

FIGURE 1. shows how resolving power and analyte-to-interference ratio affects the mass accuracy (simulation). a) and b) show 1:1 ratios between analyte (blue) and interference (black). A) shows a mass deviation of 13ppm due to the shifted centroid resultin from the non-resolved interference. A minimum resolution of R=40k is required here to resolve both compounds. This effect is increasing with higher ratios shown in c) to f).



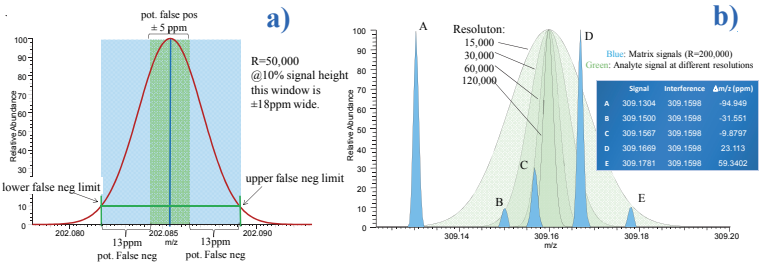
Potential Interference Counting - Model

- Any mass spectrometric interference during the HRAM LC-MS/MS run can potentially cause false positive and false negative results
- *Reasons for false positive results*:
  - a signal (MS or MS2) from a different compound is measured within the tolerance of the mass extraction window (here 5ppm).
  - two unresolved signals resulting in a merged signal, which has the "correct" (false positive) mass.
    - ➢ Unlikely, therefore disregarded here
- *Reasons for false negative results*:
  - A false negative result is observed in MS or MS/MS, if the real mass centroid is shifted outside the extraction window tolerance (here 5ppm)
    - A non-resolved interference can cause this shift.
    - Effect is resolution dependant (Figure 1 and 2)
      - ➢ For interference counting the actual resolution of the method is taken into account

FIGURE 2. depicts the model of potential interference counting.

a) Shows a signal at m/z=202 @ R=50k. ±5ppm are indicated as pot. false positive area (green). All signals occuring in this area are counted as pot. false positives. Peak width at 10% of the signal is 36ppm. All signals within the blue area are counted as pot. false negative interferences (blue)

b) Example of 5 signals (blue, A to E) and how these could be affected by an interference at different resolving power.



Results

Exemplary results are shown in figure 3 (for quasi molecular ions) and in Table 1. and 2. for Aflatoxin as an example compound. Figure 3.a) shows the average potential false positive rate for 10 compounds. Pot. fPos rates are heterogeneous and seem to depend more on the individual compound. Pot. fNeg rates show a clear dependency on the resolution, but are also very heterogeneous (data not shown). An individual data analysis seems to be appropriate. This is shown in Table 1. and 2..

FIGURE 3. Maximum potential false positive (a) and false negative rate (b) of 10 quasi-molecular ios in Full MS mode

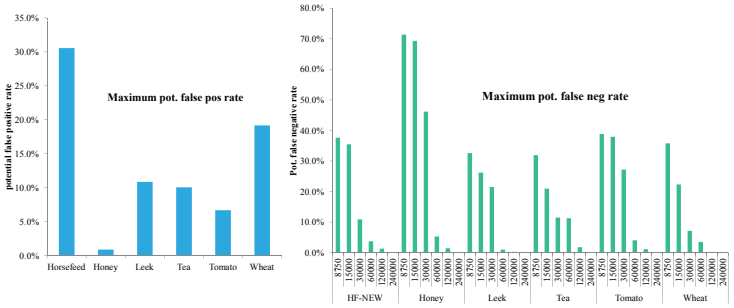


Table 1 shows the individual pot. false pos and neg rate under different conditions in two medium complex matrices (Honey and Tomato). For Honey the full MS resolving power should be set at a minimum of R=60k, while for Tomato 30k seem to be appropriate. Looking at the fragment 285.0755 in targeted mode for both R=30k seem to be sufficient, while the wide isolation increases pot. fPos rates in Honey. Here two alternatives are reasonable. First, picking a different fragment (Table 2 , 241.0498), or secondly narrowing the wide isolation (table 2, right) to 25 Th.

Table 1. Example Aflatoxin B1

			pot. False pos rate	pot. False neg rate	pot. False pos rate	pot. False neg rate	pot. False pos rate	pot. False neg rate
Compound	Set_RES	Matrix	FullMS 313.0707	FullMS 313.0707	Target (1) ==> 285.0755	Target (1) ==> 285.0755	wide Iso (100) ==> 285.0755	wide Iso (100) ==> 285.0755
Aflatoxin B1	8750	Honey	0.0%	47.5%	0.9%	28.9%	7.6%	27.3%
Aflatoxin B1	15000	Honey	0.0%	28.1%	0.9%	5.9%	7.6%	13.4%
Aflatoxin B1	30000	Honey	0.0%	9.1%	0.9%	0.3%	7.6%	1.1%
Aflatoxin B1	60000	Honey	0.0%	0.0%	0.9%	0.3%	7.6%	1.1%
Aflatoxin B1	120000	Honey	0.0%	0.0%	0.9%	0.0%	7.6%	0.0%
Aflatoxin B1	240000	Honey	0.0%	0.0%	0.9%	0.0%	7.6%	0.0%
Aflatoxin B1	8750	Tomato	0.2%	15.5%	0.0%	18.0%	0.1%	34.9%
Aflatoxin B1	15000	Tomato	0.2%	14.7%	0.0%	5.2%	0.1%	10.7%
Aflatoxin B1	30000	Tomato	0.2%	4.8%	0.0%	0.6%	0.1%	0.1%
Aflatoxin B1	60000	Tomato	0.2%	4.7%	0.0%	0.6%	0.1%	0.1%
Aflatoxin B1	120000	Tomato	0.2%	4.7%	0.0%	0.6%	0.1%	0.0%
Aflatoxin B1	240000	Tomato	0.2%	0.0%	0.0%	0.0%	0.1%	0.0%

Table 2. Example Aflatoxin B1. Alternatives to improve pot. false pos rates by picking different fragment (left, or decreasing isolation width to 25 Th (right).

			pot. False pos rate	pot. False neg rate				pot. False pos rate	pot. False neg rate
Compound	Set_RES	Matrix	wide Iso (100) ==> 241.0498	wide Iso (100) ==> 241.0498		Set_RES	Matrix	wide Iso (25) ==> 285.0755	wide Iso (25) ==> 285.0755
Aflatoxin B1	8750	Honey	0.4%	82.9%		8750	Honey	0.2%	26.2%
Aflatoxin B1	15000	Honey	0.4%	27.6%		15000	Honey	0.2%	12.4%
Aflatoxin B1	30000	Honey	0.4%	1.7%		30000	Honey	0.2%	2.7%
Aflatoxin B1	60000	Honey	0.4%	0.8%		60000	Honey	0.2%	2.4%
Aflatoxin B1	120000	Honey	0.4%	0.3%		120000	Honey	0.2%	1.1%
Aflatoxin B1	240000	Honey	0.4%	0.0%		240000	Honey	0.2%	0.0%

Conclusion

- Potential Interference Counting seems feasible to estimate systematically potential interferences causin false positive as well as false negatives without the need for matrix spiking.
  - ➢ Assessment of selectivity of different HRAM methods compared to the gold standard triple quadrupole by means of pot. interference counting
  - ➢ Including the RT as constraint
  - ➢ Interlaboratory study (volunteers welcome!)

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