

# Statistical evaluation of the benefit of combined use of accurate mass and isotopic pattern

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## Introduction

Quality of sum formula generations depends on: a) preciseness of the mass determination, b) use of the isotopic pattern information and c) accuracy of the isotopic pattern measurement. The accurate mass and the isotopic pattern provide two complementary dimensions, allowing a sum formula generation for compounds of masses up to ~500 Da. If molecular mass is higher, additional information has to be used. Exact mass alone is not sufficient here anymore since the required mass accuracy would be in the range of below 200 ppb.

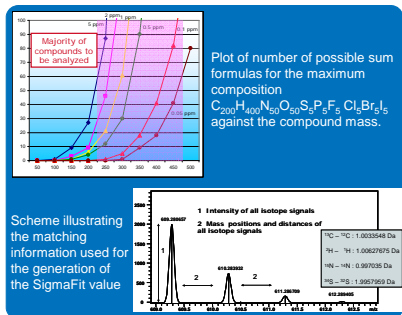


Fig. 1 Potential sum formula calculation and SigmaFit generation

## Methods

For the statistical evaluation we analyzed 125 known compounds of masses between 100 and 1000 Da by ESI-TOF-MS using external calibration. Sum formulas for these compounds were generated by the algorithm SmartFormula using the exact mass as well as the isotopic pattern information. We evaluated the achieved accuracy as well as the rankings based on mass accuracy as well as on the SigmaFit value provided by the algorithm, which takes the isotopic pattern information into account. We also evaluated for larger compounds the additional use of exact mass and isotopic pattern on the MS/MS fragments, which must be a subset of the sum formula of the intact molecule.

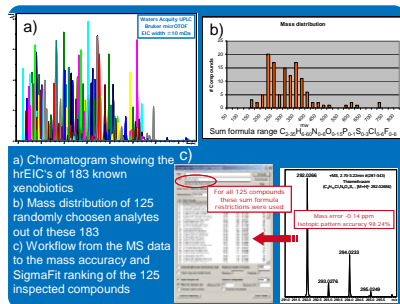


Fig. 2 Scheme of the experiment to obtain the statistical data

## Results

The compounds which were analyzed had elemental compositions ranging from 2-35 carbon atoms, 6-60 hydrogen atoms, 0-6 nitrogens, 0-15 oxygens, 0 or 1 phosphorus, up to 3 sulfur atoms and up to 6 chlorine or fluorine atoms covering a large range of different compound classes. The evaluated compounds were all measured within the instrument specification of 5 ppm mass accuracy with external calibration. The absolute error was usually better than +/- 1 mDa. Therefore, every sum formula within this error range had to be regarded as equally likely. We observed that the additional use of the full isotopic pattern information provided significantly better results.

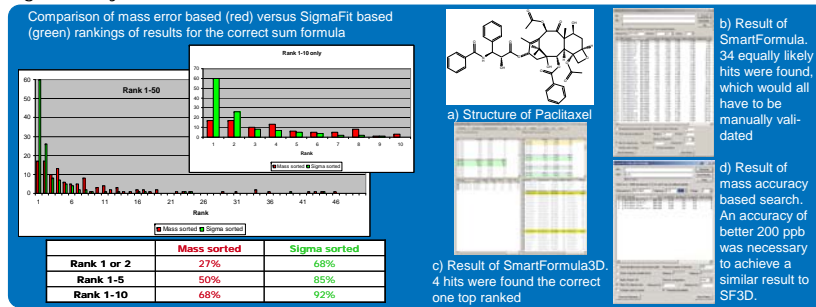


Fig. 3 Results of the statistical evaluation for 125 randomly selected compounds out of 183

While mass accuracy alone showed rank number 1 or two for the correct sum formula for only 27%, the use of the isotopic pattern increased this value to the 68%.

For the ranks 1-5 these values are 50 and 85%, respectively. In addition, we analyzed which mass accuracy would be required to obtain the selectivity, which arises from the combined use of accurate mass and isotopic pattern information on MS and MS/MS, thus basically four independent analytical dimensions. For the compound Paclitaxel with a mass of 854 Da, we obtained 4 sum formula suggestions using this so-called SmartFormula3D approach.

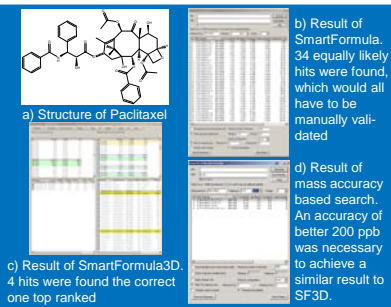


Fig. 4 Results of the evaluation for a high mass compound using SmartFormula3D

## Summary

To achieve similar results based on the MS mass accuracy alone, a mass accuracy of 200 ppb would be required. While it is possible to achieve such a mass accuracy by using internal calibrations in current instrumentation once in a while, one has to achieve this on a constant reliable base in everyday routine to be able to apply it for such an application. This is currently not possible on any instrument and thus the combined use of the four dimensions provides a higher selectivity than any instrument can routinely deliver today based on mass accuracy alone.

## Conclusions

A mass accuracy of 2 ppm alone is not sufficient to unambiguously create sum formulas from compounds.

- the isotopic pattern as an additional criterion delivers considerably better results
- MSMS provides additional dimensions
- these additional dimensions provide information which relates to a mass accuracy corresponding to significantly below 200 ppb