Budapest

Minor change in the structure major change in the mass spectrum in the example of an *N*-toluene-pyrrolidine derivative

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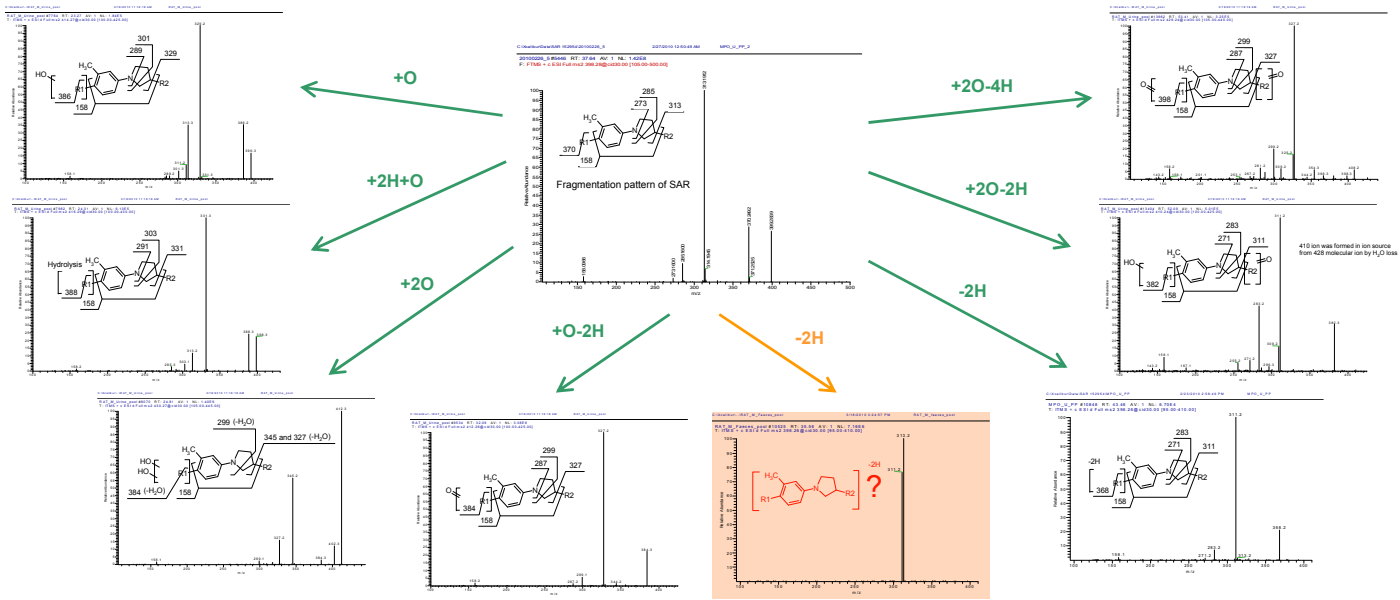
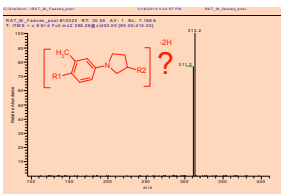
- SAR is a *N*-toluene-pyrrolidine derivative (partly shown in this poster) under development at sanofi-aventis
- The masses of fragments in MS^2 correspond to separate moieties of SAR
- Most of the changes in the parent drug metabolism result in very similar mass spectra
- MS^2 and further MS^n spectra can indicate the parts metabolized, but cannot help in finding the exact structure of either the unchanged drug or most of the metabolites



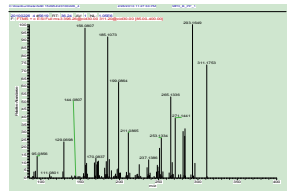
Evidences for being metabolites:

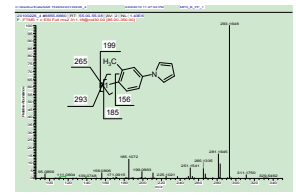
- Radioactivity
- $^{12}C/^{14}C$ isotope pattern (in vitro metabolism study with ^{14}C labelled material)
- Accurate mass of molecular ions and their mass spectrometric fragments



Two different metabolites with this MS^2 spectra

Further fragmentation of this 311 ion resulted a completely different MS^3 spectrum compared to the above 311 ions with the same accurate mass



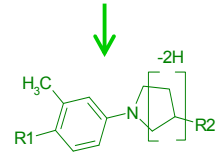


An impurity of SAR

Conclusion
(Benefits of this finding)

- **The structures of two of most important metabolites were elucidated**
- **It is likely that the two metabolites differ only in the positiion of the double bound in the dehydro-pyrrolidine ring ⇒ to be proven by standards**
- **10 other metabolites of all the 59 metabolites of SAR have similar MS fragmentation pattern i.e. two-electron oxidation in the pyrrolidine ring is a major metabolic pathway**
- **Based on the above findings and using other important MS^n fragments the exact or almost the exact structure of some important metabolites (most of them formed by multiple and complex metabolic reactions) of SAR could be elucidated**

The unique MS^3 fragmentation of the 311 MS^2 fragment of the metabolite appeared the same as the MS^2 fragmentation of the impurity of SAR



LC-MS method

HPLC (Waters Alliance 2695)
- Column: Agilent Zorbax SB-Phenyl 4.6*250 mm 5 µm
- Flow rate: 1 mL/min
- Temperature: 40 0C
- Mobil phase A: ammonium acetate 0.5 g and 1 mL formic acid/HPLC water up to 1000 mL
- Mobil Phase B: acetonitrile
- Gradient: 0 min – 10% B, 5 min – 10% B, 42 min - 28.5% B, 55 min 60% B, 55.1 min 80% B, 61 min 80% B, 61.1 min 10% B, 80 min 10% B

Mass spectrometry (LTQ-Orbitrap)
- Ion mode: ESI positive
- Split ratio: 2:8 (LTQ-Orbitrap:Lumaplate)
- Spray voltage: 4 kV
- Capillary temperature: 300 °C
- Mass range: 100-1000 m/z
- Resolution: 15000
- Normalized collision energy: 30-40

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Because health matters