

Fully Unsupervised Automatic Assignment and Annotation of Sum Formulae for Product Ion Peaks, Neutral Losses in MS and Product Ion Spectra

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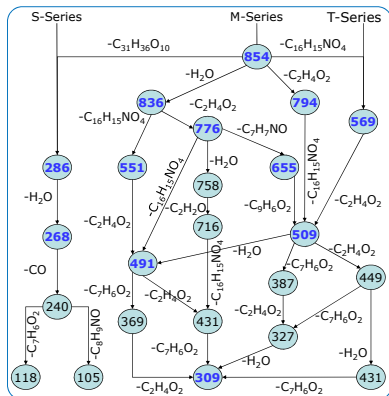
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Introduction

An essential component in the process of characterizing chemical unknowns, is the inspection of collision-induced dissociation (CID) mass spectra from quadrupole/time-of-flight (Q-TOF) instruments. The high resolution and superior mass accuracy is key to establish the elemental composition of molecules. Unfortunately with increasing m/z values the number of possible sum formulae grows exponentially.^{3,5} Several techniques has been developed which uses the information from MS/MS spectra as additional criterion for reducing the number of possible formulae for the precursor ion, by summing up the potential formulae for product ion and neutral loss to establish the identity of the precursor ion.¹⁻⁶ Herein we present a novel software module which implements a different route, which is exemplified with the medium sized compound Paclitaxel (**I**).

Experimental

- Infusion (3 $\mu\text{L}/\text{min}$) of standards (**I**) (~1 ng/ μL)
- Mass spectrometer: micrOTOF-Q™ ESI-Qq-TOF
- Ionization: ESI positive mode
- Scan range 50-1000 m/z
- Mass spectra: MS fullscan, MS/MS, IS-CID-MS³
- MS/MS collision energies: 0-50 eV
- External recalibration of all spectra types on MS full scan spectrum



Scheme 1: Fragmentation pathway of paclitaxel. Blue, bold ions has been subjected to fragmentation (MS/MS and iCID-MS³)

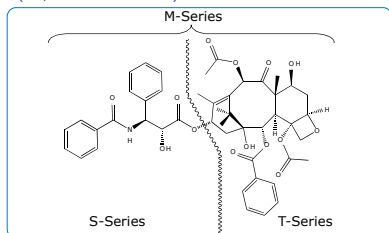


Fig. 1: Paclitaxel (**I**) with indication of origin of fragments.⁶

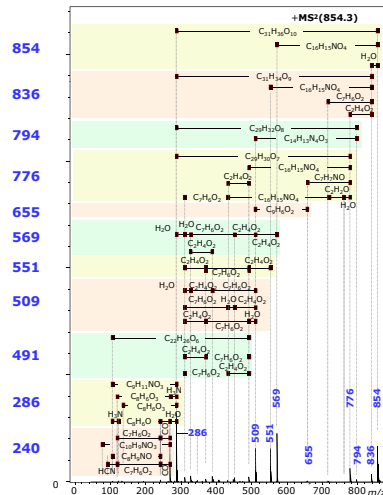


Fig. 2: MS/MS spectrum of **I** with annotation of losses derived from iCID MS³ spectra as indicated on the left hand side. The peaks in the spectrum can be associated mainly to fragments resulting from losses of side chains from the four membered core structure. S- and T series are initiated by breakage of the ester functionality connecting the left hand side chain with the core, whereas the M series starts by losses of water or acetic acid. The T series and the M series eventually join their paths at the common fragment **509** and finally reach fragment **309** which represents the four membered cyclic core structure after loss of nearly all side chains.

| Precursor | m/z meas | m/z calc | err [mDa] | mSigma |
|-------------------------------|----------|----------|-----------|--------|
| 854 C 47 H 52 N 1 O 14 | 854.3390 | 854.3382 | -0.8 | 4.1 |
| Fragment | | | | |
| Fragment | m/z meas | m/z calc | err [mDa] | mSigma |
| 836 C 47 H 50 N 1 O 13 | 836.3280 | 836.3277 | -0.3 | 15.7 |
| 794 C 45 H 48 N 1 O 12 | 794.3174 | 794.3171 | 0.7 | 6.9 |
| 758 C 45 H 44 N 1 O 10 | 758.2949 | 758.2960 | 1.0 | 25.2 |
| 776 C 45 H 46 N 1 O 11 | 776.3067 | 776.3065 | -0.1 | 15.6 |
| 716 C 43 H 42 N 1 O 9 | 716.2835 | 716.2854 | 1.9 | 18.3 |
| 655 C 38 H 39 O 10 | 655.2575 | 655.2538 | -1.6 | 16.3 |
| 569 C 31 H 37 O 10 | 569.2383 | 569.2381 | -0.3 | 1.5 |
| 551 C 31 H 35 O 9 | 551.2279 | 551.2276 | 0.1 | 0.5 |
| 509 C 29 H 33 O 8 | 509.2170 | 509.2170 | 0.0 | 3.2 |
| 491 C 29 H 31 O 7 | 491.2067 | 491.2064 | 2.0 | 11.7 |
| 449 C 27 H 29 O 6 | 449.1963 | 449.1954 | -0.4 | 7.0 |
| 431 C 20 H 27 O 5 | 431.1865 | 431.1853 | -1.2 | 31.6 |
| 387 C 22 H 27 O 6 | 387.1801 | 387.1802 | 0.1 | 11.9 |
| 327 C 20 H 23 O 4 | 327.1590 | 327.1591 | 0.1 | 19.8 |
| 309 C 20 H 21 O 3 | 309.1483 | 309.1485 | 1.1 | 19.8 |
| 286 C 16 H 16 N 1 O 4 | 286.1074 | 286.1074 | 0.0 | 8.1 |
| 268 C 16 H 14 N 1 O 3 | 268.0972 | 268.0968 | 1.2 | 42.9 |
| 240 C 15 H 14 N 1 O 2 | 240.1033 | 240.1019 | -1.4 | 11.4 |
| 118 C 8 H 8 N 1 | 118.0645 | 118.0651 | 0.6 | 3.7 |
| 105 C 7 H 5 O 1 | 105.0351 | 105.0335 | -1.6 | 5.5 |

Table 1: Sum formulae for fragments shown in **scheme 1**. Blue, bold ions have been subjected to MS/MS and iCID-MS³ fragmentation.

References

- 1) Kumar K., et al., *Rapid Commun. Mass Spectrom.* **1992**, *6*, 585-591
- 2) Tenhosaari A., *Anal. Chim. Acta* **1991**, *248*, 71-75
- 3) Grange A., et al., *Rapid Commun. Mass Spectrom.* **2006**, *20*, 89-102
- 4) McDonald L., et al., *Anal. Chem.* **2003**, *75*, 2730-2739
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Algorithm

The elucidation multiple precursor formulae and corresponding product ion formulae is pursued in two steps:

1. Generate all possible formulae for the precursor ion, as well for all product ions, applying restrictive filters mass accuracy, formula boundaries, rings plus double bond equivalents and SigmaFit™ thresholds.
2. Filter out all product ion formulae which are not a subset of the precursor ion formulae. Crosscheck every potential pair with neutral loss candidates. Eliminate all formulae not belonging to a triple consisting of formulae for precursor ion, product ion, and neutral loss.

If higher MSⁿ data are available, they can be used for reducing recursively the sets of possible candidate formulae.

Conclusions

- ESI-Qq-TOF mass spectrometers providing accurate MS, MS/MS and IS-CID-MS³ data are important tools in structure elucidation.
- The isotopic pattern plays an important role in the generation of formulae for MS to MSⁿ spectra.
- The automated generation and consolidation of proposed formulae for observed ions and neutral losses increases the confidence in resulting formulae for precursor and product ions
- This significantly accelerates the interpretation of MS and MS/MS spectra.