

USE OF A NOVEL EXACT MASS ISOTOPIC MATCHING ALGORITHM AND LDI FOR RAPID IDENTIFICATION OF DYES AND PIGMENTS

Joanne Mather¹, Jeff Goshawk¹, Ramesh Rao¹, Ronan O'Malley¹, Daniel McMillan¹, Neil Oldham²
¹Waters MS Technologies Centre, Manchester, UK: ²Oxford University, Chemistry Department, Oxford, UK

INTRODUCTION

The advancement of MS technologies in recent years has led to the use of exact mass measurement becoming widespread in qualitative applications. In many cases this allows the routine confirmation of identity of known compounds and identification of unknowns by calculation of theoretical elemental composition from the exact masses measured. The number of elemental compositions depends on the mass accuracy and mass calibration of the instrument, mass calibration is typically accomplished by addition of a 'lock mass' calibrant or including an internal calibrant in the sample of interest. Here we describe a novel isotopic matching algorithm which gives greater confidence when assigning elemental composition to exact mass spectra, in this case the LDI spectra of dyes.

This proprietary isotopic matching algorithm can be applied to any exact mass data (GC-TOF, LC-TOF, Q-TOF) to give extra confidence in qualitative results.

A variety of compounds were prepared and analysed, including phthalocyanines, porphyrins, cyclopentadienes, coronene, rubrene, coumarin 6 and truxenone. These compounds are used in a variety of industries and applications, including laser and fluorescent dyes and catalysts. Some are also found in nature, for example porphyrins are pigments found in animals and plants and are involved in the formation of many important substances in the body including hemoglobin.

METHODS

The data was generated using a MALDI (Matrix Assisted Laser Desorption Ionisation) Time-of-Flight (TOF) MS instrument. Exact mass measurement is usually performed using an internal calibrant, preparation of which is time consuming as the concentration is significant, and in addition in some cases the calibrant can react with the compound(s) of interest giving erroneous results. The dyes described were run using no internal calibrant, using the isotopic matching algorithm to overcome any errors in mass calibration and exact mass measurement.

Typically, when using a MALDI TOF MS instrument, a matrix is required to aid ionisation, in this case the sample was spotted directly onto a stainless steel MALDI sample plate without the addition of matrix, significantly reducing the time taken to prepare samples.

Selected samples were also analysed with the use of DIOS (*Desorption-Ionization On Silicon*) target plates to assess the use of these for poly-aromatic samples. DIOS is performed on a plate containing porous silicon spots. The porous silicon provides the conduit for sample ablation by absorbing and transferring energy from the mass spectrometer laser to the analyte. As DIOS is a matrix free analysis procedure any background usually attributed to matrix ions are removed, thus permitting the analysis of low molecular weight and low concentration samples.

The new software, presented herein contains a scoring algorithm called i-FIT, which compares the measured isotopic ratios to those of the suggested elemental composition. The i-FIT is a measure of the likelihood that a collection of peaks in the spectrum matches a theoretical isotope model. The value reported is the log-likelihood (using a model based on a χ^2 distribution). Calculation is weighted for intensity and mass accuracy; the closer to zero the better the fit.

RESULTS & DISCUSSION

On submitting the exact masses into any typical elemental composition calculator, it is possible to get several possible hits within the range of the mass measurement accuracy of the instrument used.

A LDI exact mass spectrum of Rubrene ($C_{42}H_{28}$, CAS No. 517-51-1) - structure in Figure 5 - was obtained and the exact mass of the most intense ion in the highest mass isotopic cluster seen submitted to the elemental composition calculator with the new isotopic matching algorithm.

Figure 1a shows the mass spectrum of the compound over the region containing the highest mass isotopic cluster and the elemental composition results. It can be seen that ranking the results by ppm difference the true ($C_{42}H_{28}$) elemental composition was ranked 10th (-1.5ppm error).

Figure 1b shows the results ranked by the calculated isotope matching 'i-FIT' parameter. It can be seen that ranking the results by 'i-FIT' the correct composition was ranked 1st.

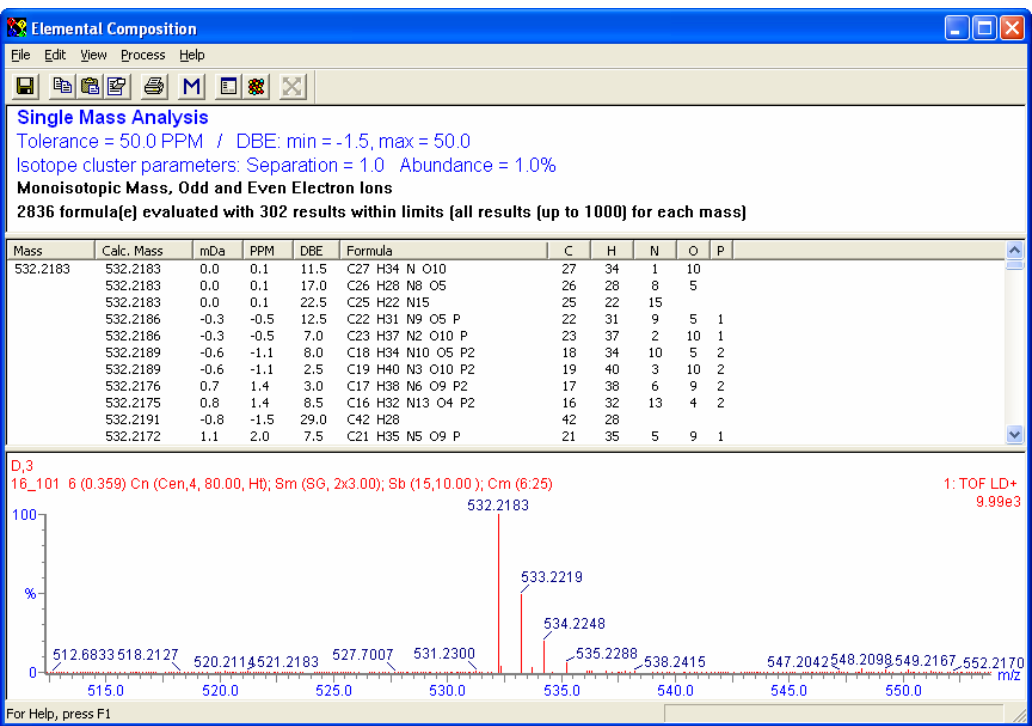


Figure 1a. Spectrum and elemental composition calculations for Rubrene ranked by ppm difference

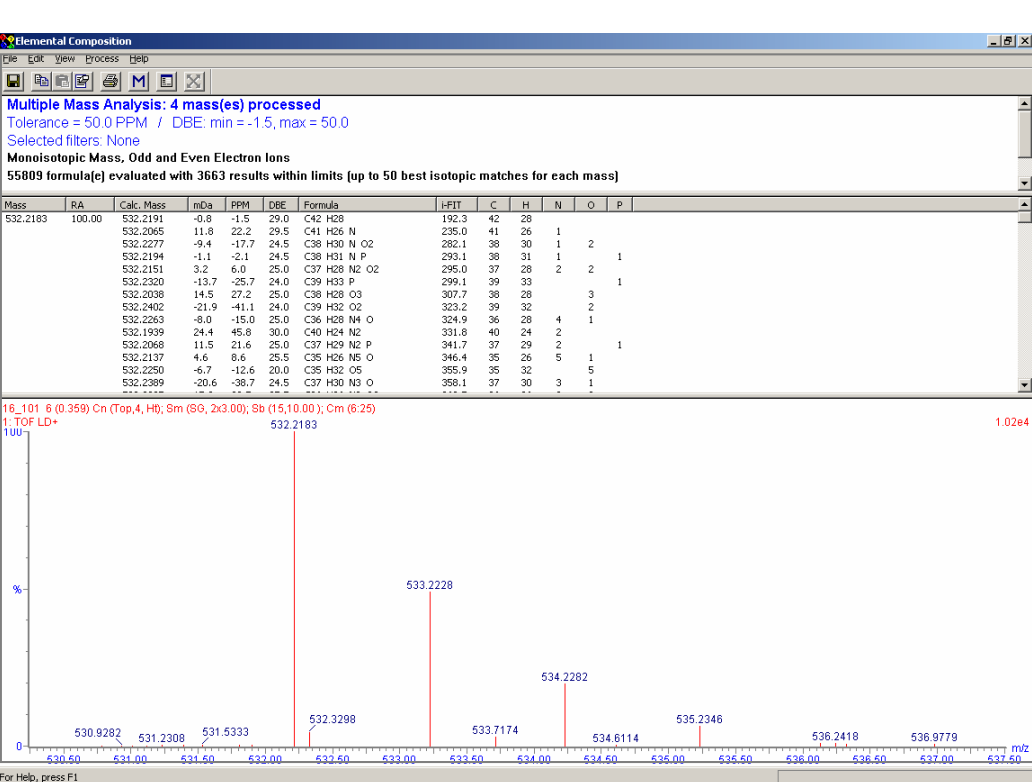


Figure 1b. Spectrum and elemental composition calculations for Rubrene ranked by 'i-FIT' isotope match

A LDI exact mass spectrum of Truxenone ($C_{27}H_{12}O_3$, CAS No. 4430-15-3) - structure in Figure 5 - was obtained and the exact mass of the most intense ion in the highest mass isotopic cluster seen submitted to the elemental composition calculator with the new isotopic matching algorithm.

Figure 2a shows the mass spectrum of the compound over the region containing the highest mass isotopic cluster and the elemental composition results. It can be seen that ranking the results by 'score' the true elemental composition ($C_{27}H_{12}O_3$) was ranked 5th.

Figure 2b shows the results ranked by the calculated isotope matching 'i-FIT' parameter. It can be seen that ranking the results by 'i-FIT' the correct composition was ranked 1st.

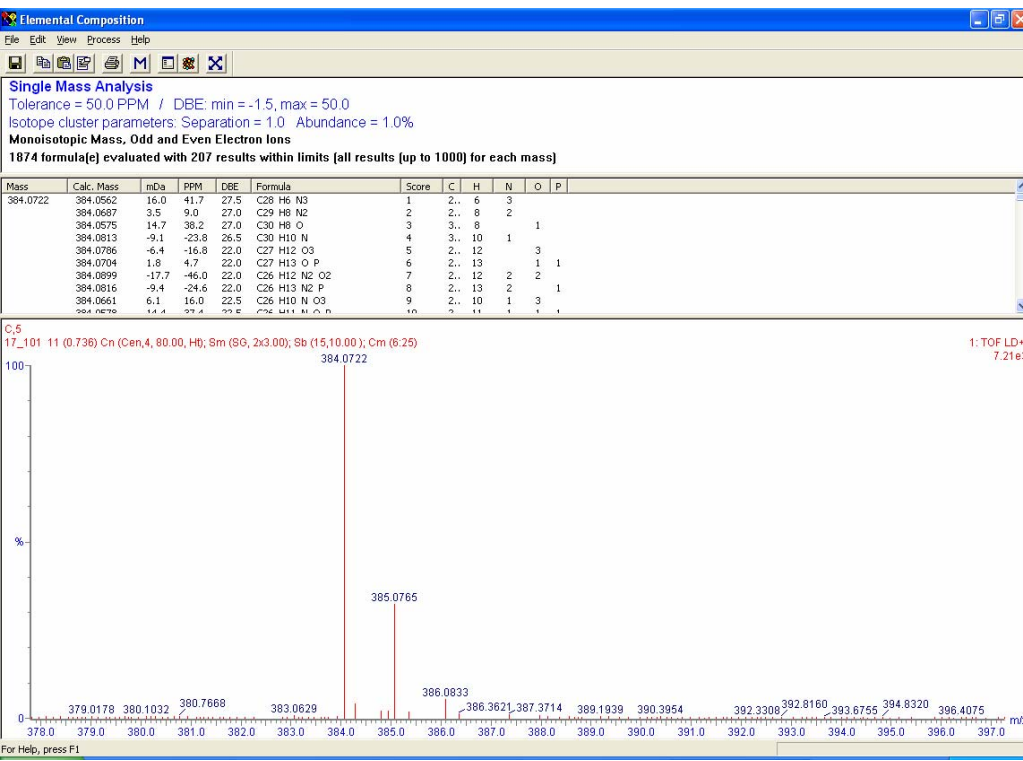


Figure 2a. Spectrum and elemental composition calculations for Truxenone ranked by score.

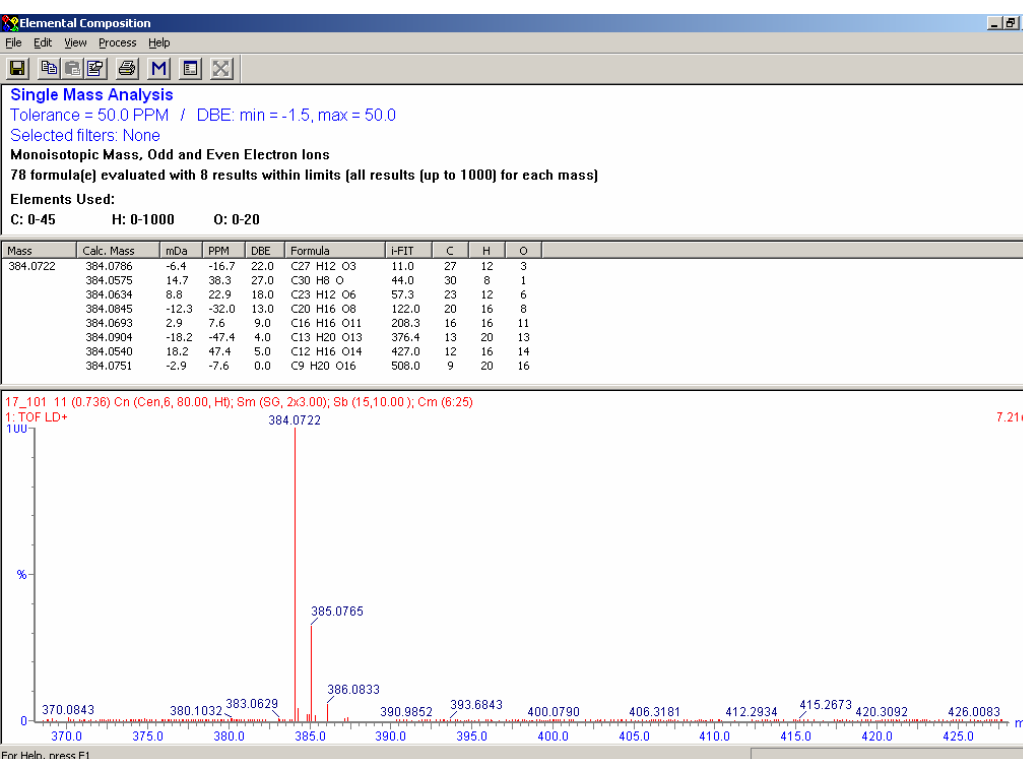


Figure 2b. Spectrum and elemental composition calculations for Truxenone ranked by 'i-FIT' isotope match

A LDI exact mass spectrum of copper (III) phthalocyanine ($C_{32}H_{16}N_8Cu$, CAS No. 147-14-8) - structure in Figure 5 - was obtained and the exact mass of the most intense ion in the highest mass isotopic cluster seen submitted to the elemental composition calculator with the new isotopic matching algorithm.

Figure 3a shows the mass spectrum of the compound over the region containing the highest mass isotopic cluster and the elemental composition results. It can be seen that ranking the results by ppm difference the correct composition ($\text{C}_{32}\text{H}_{16}\text{N}_6\text{Cu}$) was ranked 6th even though the mass measurement error was only -0.3ppm (-0.2mDa).

Figure 3b shows the results ranked by the calculated isotope matching 'i-FIT' parameter. It can be seen that ranking the results by 'i-FIT' the correct composition was ranked 1st.

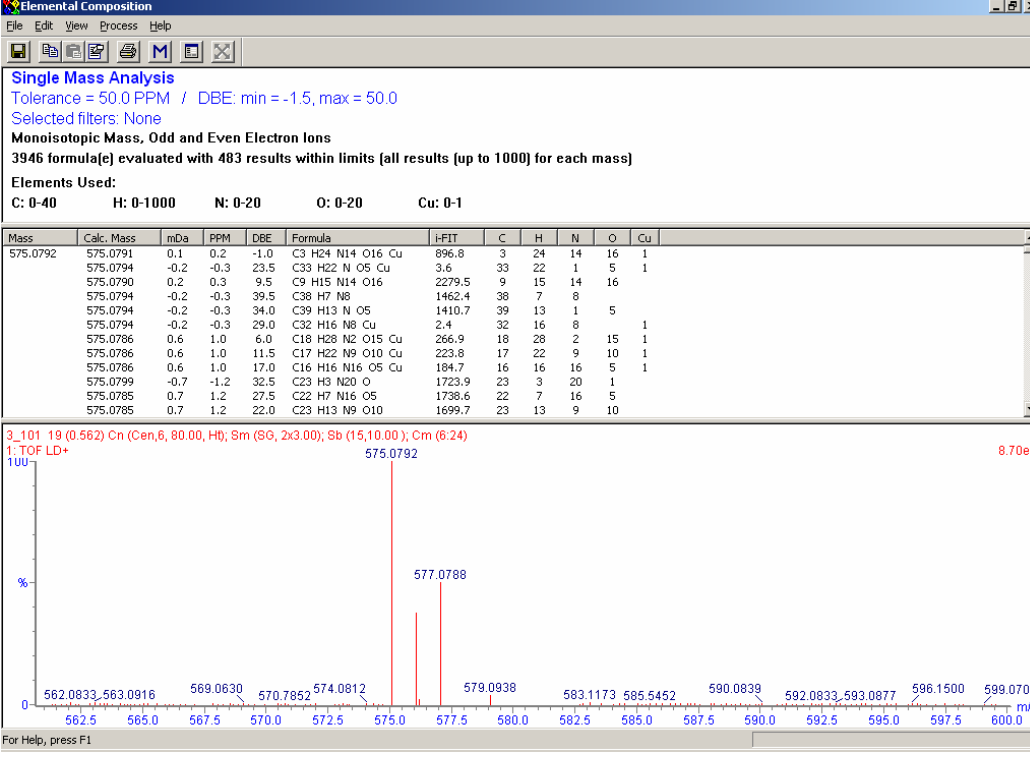


Figure 3a. Spectrum and elemental composition calculations for Copper (II) Phtalocyanine ranked by ppm difference

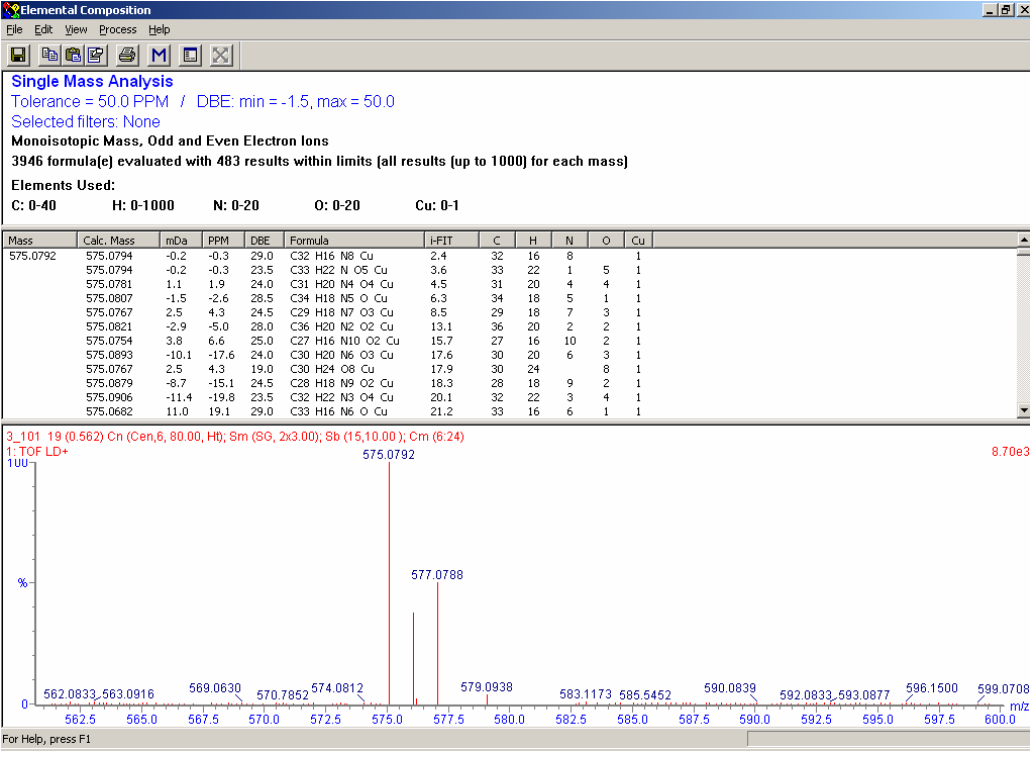


Figure 3b. Spectrum and elemental composition calculations for Copper (II) Phtalocyanine ranked by 'i-FIT' isotope match

As an extension of the work on dyes and pigments the ionization of some selected compounds was investigated using the matrix-free DIOS (*Desorption-Ionization On Silicon*) plates. DIOS is performed on a plate containing porous silicon spots, providing the conduit for sample ablation by absorbing and transferring energy from the mass spectrometer laser to the analyte. As DIOS is a matrix free analysis procedure any background usually attributed to matrix ions are removed, thus permitting the analysis of low molecular weight and low concentration samples.

Figure 4a shows the theoretical isotope model (upper) and LDI spectrum (lower) for the polyaromatic compound $C_{74}H_{93}BrO_4$ using a conventional stainless steel MALDI target plate. Figure 4b shows the spectrum from the compound on a DIOS target plate (upper) and on a stainless steel target plate (lower).

It can be seen clearly that the DIOS plate enables ionisation to allow molecular weight information to be obtained without the use of a matrix.

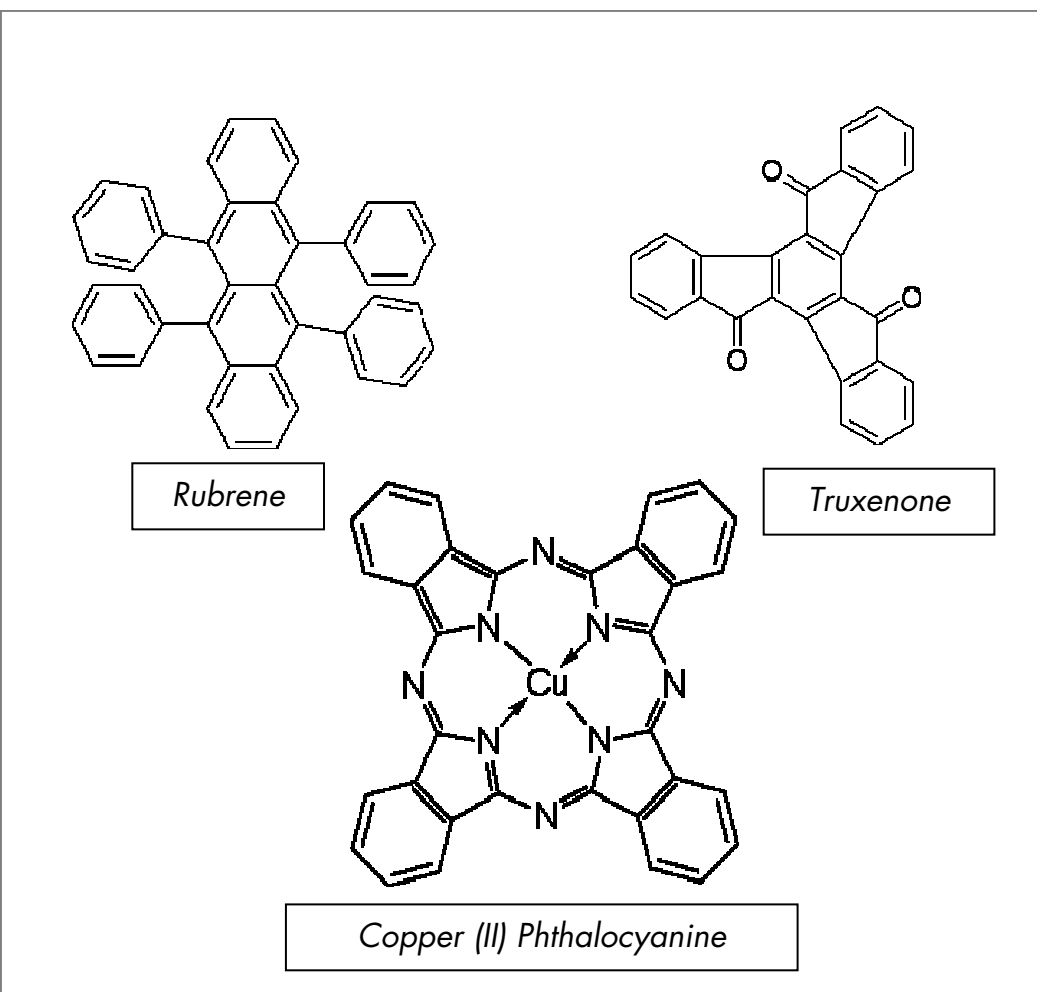


Figure 5. Structures of compounds for isotopic matching

CONCLUSIONS

As exact mass measurement has become a routine technique, the need for tools to aid compound identification and confirmation has increased. As the number of possible elemental compositions is dependent on the mass accuracy and mass calibration of the instrument, the novel isotopic matching algorithm 'i-FIT' gives greater confidence when assigning elemental composition to exact mass spectra

This proprietary isotopic matching algorithm can be applied to any exact mass data (GC-TOF, LC-TOF, Q-TOF). There is currently major interest in the use of TOF technology and the application of library search routines for targeted screening for a variety of types of compounds, from pesticides to drugs of abuse. Library searching routines can often give multiple hits, particularly for structurally similar compounds. In these instances isotopic matching can be used to narrow down the candidate lists to aid identification.

REFERENCES

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