

Porphyrin Calibration Standards for Accurate Mass Measurement of Radical Ions by MALDI-TOFMS

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Overview

- Recent research has focussed on the use of MALDI-TOFMS to characterise organometallic/coordination/highly conjugated compounds.
- 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) matrix is very effective for these compounds, with radical molecular ions observed.
- Accurate mass measurement increases the certainty of analyte identification, but a MALDI-TOFMS method needed to be developed.
- Recent investigations showed that greater accuracy is achieved when radical ions are calibrated with radical ion standards, and that a general benchmark of ± 5 ppm accuracy could be realised.
- Porphyrin-based standards have been identified and accurate mass measurements were made for five analytes; mean mass accuracy errors ranged from 0.1–1.7 ppm and all measurements had ± 5 ppm accuracy.
- This technique is especially useful for instances when only MALDI ionises an analyte or when a MALDI is the only ionisation mode available.

Introduction

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOFMS) is a very powerful analytical technique, which can be applied to a wide range of sample classes, including organometallic, coordination, and highly conjugated compounds. Such samples constitute a considerable proportion of those received at the NMSSC and are, in general, observed as radical ions; they have lost (or gained) an electron. Previous work has shown the matrix DCTB to be highly effective.¹

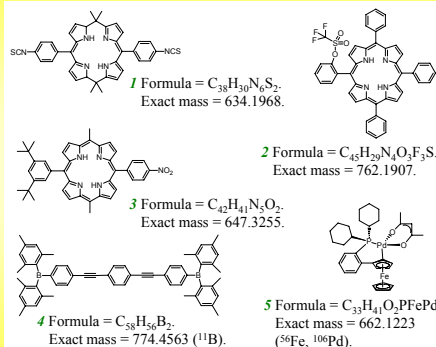
Accurate mass measurement increases the certainty of analyte identification, and background literature and general operational guidelines are available.^{2,3} At the NMSSC, computer-assisted peak matching on a magnetic sector instrument with an electrospray (ESI) source⁴ is preferred for the wide range of samples received. Alternative sources are used where ESI is not appropriate, and while these systems generally give excellent results, some samples specifically require MALDI ionisation.

However, a method needed to be developed for accurate mass measurement of radical ions by MALDI-TOFMS. Initial studies showed that greater accuracy is achieved when radical ions are calibrated with radical ion standards.⁵ Tetra-substituted porphyrins were identified as potential calibration standards and used in this study to measure five compounds that had previously been measured by peak matching. Here we present a proven methodology, which can be applied to samples that require a MALDI source or when MALDI is the only technique available.

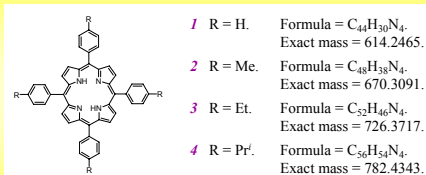
Materials and Instrumentation

- DCTB, 5,10,15,20-tetraphenylporphyrin (Standard 1), boron trifluoride diethyl etherate, and tetrachloro-*p*-benzoquinone were purchased from Fluka (U.K.).
- HPLC-grade dichloromethane was purchased from Fisher (U.K.).
- 5,10,15,20-tetra-4-methylphenylporphyrin (Standard 2), 4-ethylbenzaldehyde, and 4-isopropylbenzaldehyde were purchased from Sigma-Aldrich (U.K.).
- Pyrrrole was purchased from Acros (U.K.).
- Samples were submitted to the NMSSC by:
 - S. C. Jha and R. W. Boyle, University of Hull, U.K.
 - P. J. Scaife and A. N. Cammidge, University of East Anglia, U.K.
 - C. Pariani and A. C. Bennison, University of Newcastle, U.K.
 - J. C. Collings and T. B. Marder, University of Durham, U.K.
 - J. Amin and C. J. Richards, Queen Mary, University of London, U.K.
- MALDI-TOFMS measurements were acquired with a Voyager DE-STR instrument (Applied Biosystems, U.S.A.). Data were acquired using positive ion, reflectron mode. Delay time = 100 ns, acceleration voltage = 20 kV, and grid voltage = 65.5%. Every acquisition was an accumulation of 2×25 laser shots, and the laser power was optimised for each.

Samples 1-5

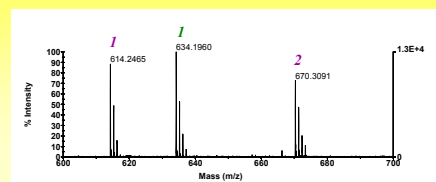


Calibration Standards 1-4



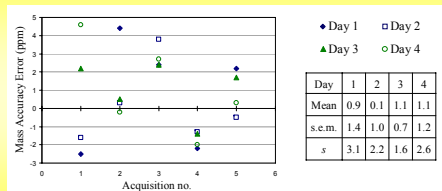
*Standards 3 and 4 were synthesised according to literature methods.^{6,7}

Accurate Mass Measurement



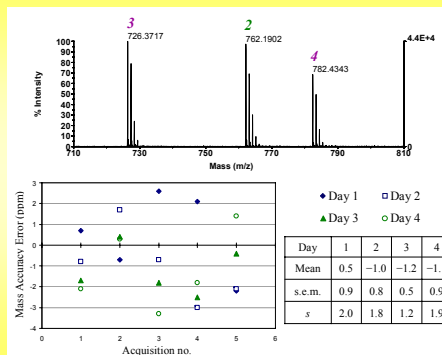
- Sample 1 was calibrated by bracketing Standards 1 and 2.
- Post-acquisition calibration was applied using Data Explorer V4.0 software supplied by Applied Biosystems.
- No data processing was performed, as this can adversely affect peak shapes.
- The three peaks used in a measurement should all have comparable ion intensity; too weak and the peak shape is poor, too strong and detector echoes form a high-mass shoulder, altering the centroid.
- The correct sample/standards/matrix ratio was crucial to good peak shapes and, therefore, mass accuracy, and ionisation efficiencies must be considered.
- Mass accuracy is often worse when the sample peak is the least intense.

Results for 1



*Five measurements were made on four separate days.

Results for 2

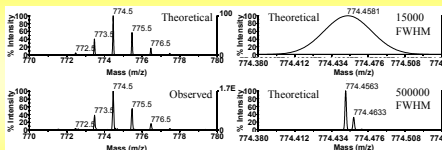


Results for 3

Day	1	2	3	4
Mass Accuracy Error (ppm)	2.2	1.7	0.2	2.8
Mean	1.7			
s.e.m.	0.6			
s	1.1			

- Sample 3 was calibrated by bracketing Standards 1 and 2.
- One measurement was made on four separate days.

Results for 4



- Initial characterisation provided a good isotope distribution match.
- Intensity of the m/z 772.5 ion was insufficient for accurate measurement.

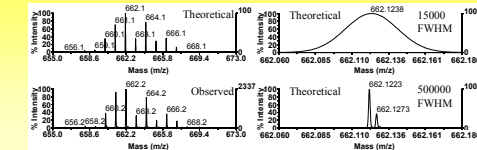
Results for 4 continued

- The resolution of the instrument is insufficient to observe the monoisotopic ion.
- The average centroid (774.4581) of the monoisotopic ion (774.4563) and its nearest isotopologues must be measured.
- 774.4581 – 774.4563 = 2.3 ppm, which must be considered when calculating a list of elemental formulae from the accurate mass measurement.

Day	1	2	3	4
Mass Accuracy Error (ppm)	-1.9	-0.5	0.8	-1.7
Mean	-0.8			
s.e.m.	0.6			
s	1.2			

- Sample 4 was calibrated by bracketing Standards 3 and 4.
- One measurement was made on four separate days.

Results for 5



- Initial characterisation provided a good isotope distribution match.
- Intensity of the m/z 656.2 ion was insufficient for accurate measurement.
- The average centroid (662.1238) of the monoisotopic ion (662.1223) and its nearest isotopologues must be measured.
- 662.1238 – 662.1223 = 2.3 ppm, which must be considered when calculating a list of elemental formulae from the accurate mass measurement.

- Sample 5 was calibrated by bracketing Standards 1 and 2.
- One measurement was made on four separate days.

Conclusions

- Porphyrin-based calibration standards have been used for the accurate mass measurement of radical ions by MALDI-TOFMS.
- Mean mass accuracy errors ranged from 0.1–1.7 ppm and all individual measurements had ± 5 ppm accuracy.
- Extra care must be taken when accurately measuring ions which do not comprise the lowest mass isotopes of all elements in the species.

References

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