

EPSRC National Mass Spectrometry Centre, Swansea NMSSC Application Note No 2

Accurate mass measurement of positive radical ions by MALDI-TOFMS using porphyrin based calibration standards

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<http://www.swan.ac.uk/nmssc/>

Introduction

Accurate mass measurement increases the certainty of analyte identification, and general background literature and operational guidelines are available.^{1,2} At the NMSSC, computer-assisted peak matching on a magnetic sector instrument with an electrospray (ESI) source³ 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. A method for the accurate mass measurement of positive radical ions by MALDI-TOFMS was developed recently in our laboratory.⁴ Greater accuracy is achieved when radical ions are calibrated with radical ion standards, hence tetra-substituted porphyrin calibration standards have been developed (see Figure 1). Sequential increase of the alkyl chain length, plus occasional phenyl groups, provides standards that cover the practical mass range of ≤ 1000 Da. A limited service is currently available and the NMSSC will be able to offer a full service from Spring 2007.

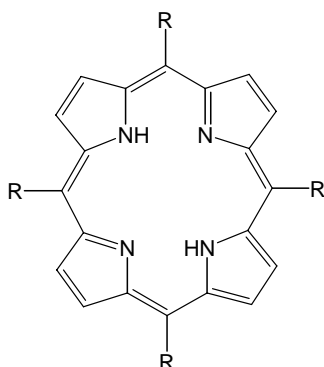


Figure 1. 17 porphyrin calibration standards; R = H, methyl to hexyl, phenyl, 4-methylphenyl to 4-hexylphenyl, biphenyl, 4-(4-methylphenyl)phenyl and 4-(4-ethylphenyl)phenyl.

Experimental methodology

Chemicals

All samples (1-5) were submitted for analysis to the EPSRC National Mass Spectrometry Service Centre (NMSSC) as part of the normal operation of the Centre. DCTB matrix and standard no. 8 (R = phenyl) were purchased from Fluka (Dorset, UK). Standard no. 9 (R = 4-methylphenyl) was purchased from Sigma-

Aldrich (Dorset, UK). Standards no. 10 (R = 4-ethylphenyl) and 11 (R = 4-isopropylphenyl) were synthesised by literature methods.^{5,6} HPLC grade dichloromethane (DCM) was purchased from Fischer Scientific (Loughborough, UK).

MALDI Sample Preparation

DCTB matrix solution was prepared to a concentration of 20 mg mL⁻¹ in DCM. Sample and calibration standard solutions were prepared to an approximate concentration of 1 mg mL⁻¹ in DCM. In a plastic, snap-top lid sample vial, 1 μ L each of sample and bracketing standard solutions were vortex-mixed with 49 μ L of matrix solution. 0.5 μ L of the final mixture was spotted (see **TIP!** in NMSSC Application Note No. 1) onto 5 wells on a sample plate (gold-plated, deep-welled plates are advantageous for organic solvent based mixtures) and allowed to dry, leaving an opaque crystal layer.

Mass Spectrometry

MALDI-TOFMS spectra were acquired using an Applied Biosystems Voyager DE-STR spectrometer (Framingham, MA, USA), which is equipped with a nitrogen laser ($\lambda = 337$ nm). The instrument was operated in positive ion, reflectron mode. The accelerating voltage was 20 kV, while the grid voltage was maintained at 65.5 %. The delay time was 100 ns and the laser fluence was optimised for each sample. The laser was fired at a frequency of 3 Hz and spectra were accumulated in multiples of 25 laser shots, with 50 shots in total. Post-acquisition calibration was applied using Data Explorer V4.0 software supplied by Applied Biosystems.

Results and discussion

MALDI-TOFMS data were calibrated using a two-point, linear method. Each sample was bracketed by the porphyrin standards closest in mass, as shown for 1 in Figure 2. Due to inherent inaccuracies with MALDI-TOFMS, several results per sample were acquired. Data was acquired manually and calibrated afterwards, which means results cannot be operator led. 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 obtaining good peak shapes and, therefore, mass accuracy. Ionisation efficiencies must be considered, and mass accuracy is often worse when the sample peak is the least intense.

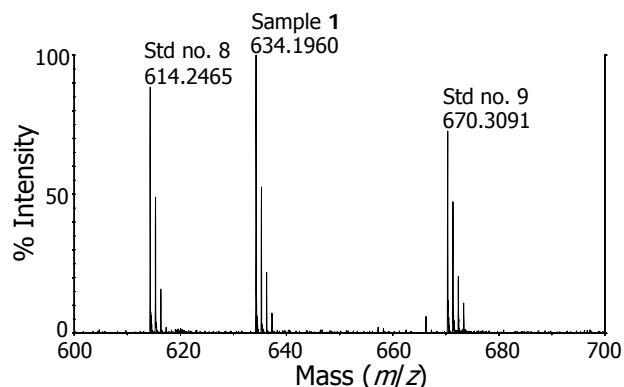


Figure 2. Example of accurate mass measurement data acquired for sample 1, calibrated with bracketing porphyrin standards no. 8 and 9.

Data for samples 1-5 is presented in Table 1. Samples 4-5 offer a further challenge, as they contain elements with multiple isotopes of significant abundance. The lowest mass isotopic peak is always due to a single species, but can sometimes be of too low intensity to be measured accurately. However, the most intense isotopic peak is a mixture of isotopologues that cannot be resolved by MALDI-TOFMS, and it is the weighted average mass of these isotopologues that is measured. Hence, there is an error between the measured mass and exact monoisotopic mass, and this must be considered when using the result to generate a list of possible elemental formulae.

Sample (Exact mass)	Porphyrin Standards	Result (Error ppm)	Mean Error (ppm)
1 C ₃₈ H ₃₀ N ₆ S ₂ (634.1968)	8 and 9	-1.6 0.3 3.8 -1.3 -0.5	0.1
2 C ₄₅ H ₂₉ N ₄ O ₃ F ₃ S (762.1907)	10 and 11	0.7 -0.7 2.6 2.1 -2.2	0.5
3 C ₄₂ H ₄₁ N ₅ O ₂ (647.3255)	8 and 9	2.2 1.7 0.2 2.8	1.7
4 C ₅₈ H ₅₆ B ₂ (774.4563) (¹¹ B)	10 and 11	-1.9 -1.5 0.8 -1.7	-0.8
5 C ₃₃ H ₄₁ O ₂ PF _e Pd (662.1223) (⁵⁶ Fe, ¹⁰⁶ Pd)	8 and 9	1.2 1.2 -0.2 1.1	0.8

Table 1. Accurate mass measurement results in terms of the error between exact and measured masses for samples 1-5.

Conclusions

Porphyrin-based calibration standards have been used for the accurate mass measurement of positive radical ions by MALDI-TOFMS. Mean mass accuracy errors ranged from -0.8 to 1.7 ppm and all individual measurements had ± 5 ppm accuracy. Extra care was taken when accurately measuring ions which did not comprise the lowest mass isotopes of all elements in the species. Here we have presented a proven methodology, which can be applied to samples that require a MALDI source or when MALDI is the only technique available.

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