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Electrospray Accurate Mass Measurement Using Polyethylenimine as an Internal Reference Standard

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

Introduction

The determination of the elemental formulae of small molecules can be achieved by accurate mass measurement[1]. Accurate mass spectrometry has been summarised elsewhere and guides are available[2,3]. At Swansea, computer-assisted peak-matching accurate mass spectrometry[4] is the preferred method, using polyethylenimine (PEI) as the internal reference standard[5]. PEI is suitable for measurements between m/z 150 and 1200.

Experimental methodology

Chemicals

PEI (low-molecular weight) and ammonium acetate (NH_4Ac) were purchased from Aldrich (Dorset, UK). HPLC grade dichloromethane (DCM), methanol (MeOH) and water (H_2O) were purchased from Fisher Scientific (Loughborough, UK).

Reference preparation

PEI calibration solution was prepared as follows: Stock solution 1µL PEI dissolved in 10ml 90:10 MeOH:5mM NH_4Ac. The reference solution was prepared 80:10:10 MeOH:5mM NH_4Ac:PEI stock solution.

Sample preparation

Samples were received dry, and dissolved in 100µL DCM (or suitable solvent). A small volume (approx. 10µL) was placed into 350µL MeOH.

Mass Spectrometry

Accurate mass measurements were obtained using the Finnigan MAT 900XLT mass spectrometer fitted with an electrospray source and operated with the standard procedures[6]. Xcalibur v1.4 SR1 with High Resolution Mass Spectrometry programs v1.4 software[7] was used to produce lists of elemental formulae.

Results and discussion

The low resolution mass spectrum of PEI is shown in Figure 1. It can be seen that there are a spread of ions over the mass range (150 to 700Da shown); those which are most intense are ammoniated adducts (refer to Table 1); less intense ions are proton or sodium adduct ions. The addition of NH_4Ac to the reference solution causes the preferential formation of $[M+NH_4]^+$ (selected ions labelled, Figure 1) over other adduct ions. The distribution of the PEI ions allows calibration throughout the mass range. Adjustment of the source voltages will alter the peak envelope maximum.

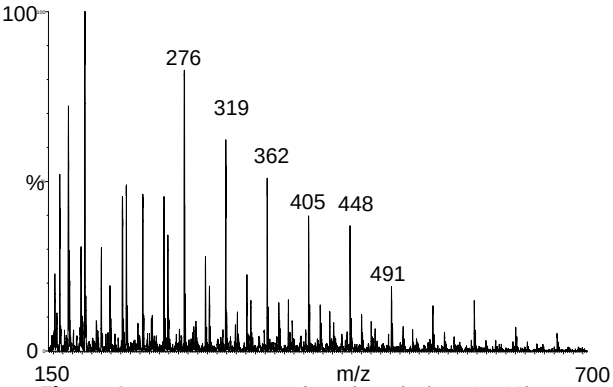

Figure 1: *Mass spectrum of PEI ions (m/z 150-700)*

Table 1: Exact masses* of polyethylenimine ($[M+NH_4]^+$, 100-1000Da)

147.1604	362.3714	577.5824	792.7512
190.2026	405.4136	620.6246	835.5307
233.2448	448.4558	663.6668	878.8778
276.2870	491.4980	706.7090	921.9200
319.3292	534.5402	749.7512	964.9622

Typical single measurements of an analyte by this method are accurate to within 2ppm. An example is shown in Figure 2; proposed molecular formula $C_{11}H_{13}O_2I$, exact mass m/z 322.0298, observed mass m/z 322.0300. The "LIST" software returned the matches shown in Table 2 (assumed to be the pseudo-molecular ion $[M+NH_4]^+$).

Table 2: List of possible matches for ion m/z 322.0300, $[M+H]^+$

m/z	Delta (ppm)	RDB	Composition
322.0298	0.2	3.5	$C_{11}H_{17}O_2N_1I_1$
322.0285	1.5	4.0	$C_9H_{15}O_1N_4I_1$

It is important to note that accurate mass calculations in mass spectrometry should account for the mass of the electron. For positive-ion mass spectrometry an electron is lost (so 0.55mDa must be subtracted from the neutral calculated mass), for negative-ion mass

spectrometry an electron is gained (so 0.55mDa must be added to the neutral calculated mass).

Table 3: Parameters set in "LIST" software

Parameter	Min.	Max.
C	0	50
H	0	80
O	0	10
N	0	10
I	0	2
Double bonds/ ring equiv.	-2	100
Tolerance window	±5ppm	
Charge	+1	

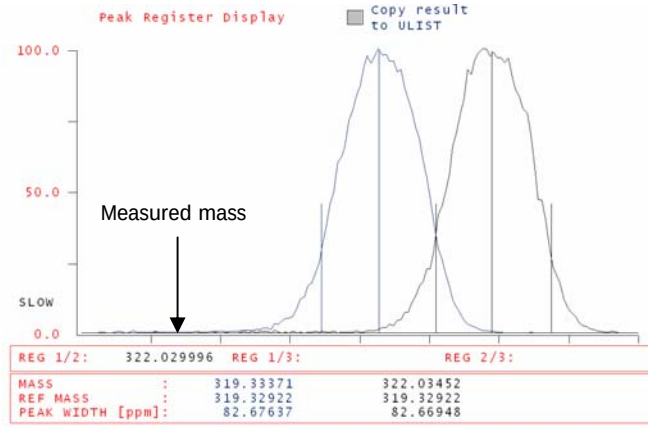

Figure 2: *Peak match display showing accurate mass measurement.*

For samples of unknown composition, peak matching is performed at least five times and the results averaged to reduce the uncertainty in the measurement. Accuracy by this method is usually better than 1ppm.

Conclusions

PEI is used very successfully for the routine accurate mass measurement of low molecular weight compounds by electrospray ionisation. The combination of high sensitivity (through peak matching), electrospray ionisation and PEI as a reference compound has enabled a method of acquiring accurate mass measurements which is versatile, fast (less than 1 minute per sample) and only requires one daily calibration, which holds valid for the whole mass range. PEI gives an intense and reproducible ion current; suppression of analyte signal does not usually occur; the compound is stable at room temperature over many months and does not give the strong memory effects typical of many other polymers used for similar purposes.

References

1 Beynon, J. H., High Resolution Mass Spectrometry of Organic Materials. *Adv. Mass Spectrom*. **1959,** 328–354.

2 Bristow, A. W. T.; Webb, K. S., Intercomparison Study on Accurate Mass Measurement of Small Molecules in Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2003,** *14,* 1086–1098.

3 http://www.bmss.org.uk/Docs/VIMMS_guide.pdf

4 D'Agostino, P. A.; Hancock, J. R.; Provost, L. R.; Semchuk, P. D.; Hodges, R. S. High-Resolution Electrospray Mass Spectrometry with a Magnetic-Sector Instrument Accurate Mass Measurement and Peptide Sequencing. *Rapid Commun. Mass Spectrom.* **1995,** *9,* 597–603.

5 A.B. Coddington and H.G. Ramjit A Novel Reference Compound for Electrospray and FAB was Developed for Non-routine LC/MS Studies. *Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics, Dallas, TX,* **1999**

6 http://www.swansea.ac.uk/nmssc/Instop.htm

7 Xcalibur High Resolution MS Programs Version 1.4, Thermo Electron, San Jose, CA

Acknowledgements

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