Appendix D Calibration Reference Information

D.1 Overview

Calibration reference files consist of two columns of numbers separated by any number of spaces or tab characters. The first column contains the reference peak masses, and the second column contains the reference peak intensities.

The reference files listed in this appendix have all ion intensities set to 100%. Actual ion intensities are not, in fact, all 100%, but the calibration software does not take account of the ion intensities and this is therefore a convenient way of storing the reference files in the required format. However, if required, realistic intensity values can be entered to improve the appearance of the reference spectra.

Most samples can be purchased from the Sigma chemical company. To order, contact Sigma at http://www.sigma.sial.com. This site contains a list of worldwide Sigma offices, many with local toll-free numbers.

D.2 Editing a Calibration Reference File

Calibration reference files can be created or edited using any Windows text editor. To read a reference file into the Notepad text editor:

1. In the Tune window, select Calibration > Calibrate Instrument to open the Calibration dialog box.
2. Select the required reference file from the drop-down list box.
3. Click  or select Edit > Reference File to open the reference file in Notepad.

To save the reference file after editing, select File > Save to save the file under the current name, or select File > Save As to save as a new reference file with a new name.

Textual information or comments can be stored in the reference file. Lines that are textual information or comments must start with the semicolon (;) character.

D.3 Positive Ion Calibration Reference Files

The following table lists typical reference files that can be used for positive ion calibration.

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
PEGNH4	Ammoniated polyethylene glycol		50 to 2000	General calibration
NAICS	Sodium iodide/cesium iodide mixture		20 to 4000	General, ES+ calibration
UBQ	Bovine ubiquitin [U6253]	8564.85	650 to 1500	General
HBA	Human α globin [H753]	15126.36	700 to 1500	Hb analysis
SOD	Superoxide dismutase [S2515]	15591.35	900 to 1500	Hb (internal calibration)
HBB	Human β globin [H7379]	15867.22	800 to 1500	Hb analysis
MYO	Horse heart myoglobin [M1882]	16951.48	700 to 1600	General
PEGH1000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000		80 to 1000	ES+ and APCI+ calibration
PEGH2000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000+1450		80 to 2000	ES+ calibration
NAIRB	Sodium iodide/rubidium iodide mixture		20 to 4000	ES+ calibration

D.3.1 Polyethylene Glycol

PEG + $NH4^+$

Reference File: PEGHNH4.REF

Calculated m/z Value				
89.06	459.28	872.54	1268.78	1665.01
133.09	503.31	916.57	1312.80	1709.04
177.11	564.36	960.60	1356.83	1753.07
221.14	608.39	1004.62	1400.86	1797.09
239.15	652.41	1048.65	1444.88	1841.12
283.18	696.44	1092.67	1488.91	1885.15
327.20	740.46	1136.70	1532.94	1929.17
371.23	784.49	1180.73	1576.96	1973.20
415.25	828.52	1224.75	1620.99	2017.22

D.3.2 Sodium Iodide and Cesium Iodide Mixture

Reference File: NAICS.REF

Calculated m/z Value				
22.9898	772.4610	1671.8264	2571.1918	3470.5572
132.9054	922.3552	1821.7206	2721.0861	3620.4515
172.8840	1072.2494	1971.6149	2870.9803	3770.3457
322.7782	1222.1437	2121.5091	3020.8745	3920.2400
472.6725	1372.0379	2271.4033	3170.7688	
622.5667	1521.9321	2421.2976	3320.6630	

D.3.3 Sodium Iodide and Rubidium Iodide Mixture

Reference File: NAIRB.REF

Calculated m/z Value				
22.9898	772.4610	1671.8264	2571.1918	3470.5572
84.9118	922.3552	1821.7206	2721.0861	3620.4515
172.8840	1072.2494	1971.6149	2870.9803	3770.3457
322.7782	1222.1437	2121.5091	3020.8745	3920.2400
472.6725	1372.0379	2271.4033	3170.7688	
622.5667	1521.9321	2421.2976	3320.6630	

D.4 Negative Ion Calibration Reference Files

Note: *A positive ion calibration is sufficient for most applications. A specific negative ion calibration is not required when using the Quattro Premier XE.*

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
NAINEG	Sodium iodide/cesium iodide (or rubidium iodide) mixture		200 to 3900	ES- calibration
SUGNEG	Sugar mixture of maltose [M5885], raffinose [R0250], maltotetraose [M8253], and corn syrup [M3639]		100 to 1500	Low mass range
MYONEG	Horse heart myoglobin [M1882]	16951.48	700 to 2400	General

You can use rubidium iodide to obtain a peak at m/z 85 ($^{85}Rb^{+}$) with an intensity of about 10% of the base peak at m/z 173. Rubidium iodide has the advantage that no rubidium clusters that can complicate the spectrum are formed. Note that rubidium has two isotopes (^{85}Rb and ^{87}Rb) in the ratio 2.59:1, giving peaks at m/z 85 and 87.

Use reference file NAIRB.REF.

D.5 Preparing Reference Sample Solutions

D.5.1 Preparing the PEGNH4 Reference Sample Solution

Add 1 ng of each of the following to 50:50 acetonitrile/10 mM aqueous ammonium acetate:

- PEG 200
- PEG 400
- PEG 600
- PEG 1000

D.5.2 Preparing the Sodium Iodide and Cesium Iodide Mixture Sample Solution

Mix a 2 ng/µL solution of sodium iodide with a 50 ng/µL solution of cesium iodide.