

The Utility of Water Clusters for Mass Axis Calibration Featuring the Platform LCZ Detector

Highlights: Several authors have shown the utility of water clusters for LC/MS mass axis calibration. This Note describes this process on the Waters AllianceTM System Featuring the Platform LCZ Detector and MassLynxTM software. There are several advantages to using water clusters for mass axis calibration. Unlike the typical calibrant, PEG, source contamination is not a problem. When water is used as a calibrant, regular ions are observed at 18 amu intervals, allowing ample points of calibration throughout a wide mass range. Also, water is readily obtainable and easy to prepare.

Experimental Conditions:

Source conditions:

Ionization mode: Positive ion Electrospray (ESI). Figure 1 shows a typical source tune page for the Platform LCZ in MassLynx software. The setup for water cluster mass axis calibration utilizes a high capillary voltage (maximized at 5.0 kV). Similarly, an unusually high Cone Voltage is required to enhance the higher masses. The source temperature should be set to ambient temperature, although the effect of temperature has not been fully investigated.

Needle position:

For optimum sensitivity, the ESI probe should be positioned as far into the source as possible. Side-to-side position should then be adjusted to maximize the signal.

Gas and Liquid Flows:

Referring to Figure 1, the nitrogen flow is turned off. The syringe pump is also turned off. In this configuration, ionization proceeds by classic electrospray, resulting in an extremely stable signal. The water in the capillary and fused silica line provides an adequate reservoir of water to replenish that which is sprayed. However, to initiate the spray, one should first establish typical infusion conditions (i.e. water flow at approximately 5 uL/min, with appropriate gas flows). Once water clusters are observed, turn off the syringe pump and gas supply to continue operation.

Figure 1. Typical tuning parameters. Note the high capillary and cone voltages and that gas flow has been disabled

H Tune - [DEFAULT , ESP+]	
<u>File Ion Mode Gas Bamp Other Stop Window Help</u>	
🖻 🖬 🖄 🗖 🖻 🗞 🌆 🕘 🤶	
Pressures & Flows	Mass Span Gain
C Analyser Vacuum Gas(L/hr)	Tune Peak 1 361.21 5 5
K1.0e-4 mBar	Tune Peak 2 721.42 5 15
OFF	Tune Peak 3 1000.00 800 20
Source Parameters	Tune Peak 4 1000.00 1000 20
Capillary 5.03 4.96 <	
Cone 178 168 <	
Extractor 4 5 <	MS x20
Rf Lens 0.2 <	
Source Block Temp 22 20	
Desolvation Temp 26 20	and the second sec
MS Parameters	
LM Res 14.0 <	
HM Res 14.0 <	
Ion Energy 0.8 <>	
Multiplier 653 650 <	
Acquire	OPERATE
	Figure 1

Figure 2 shows the smoothed and baseline subtracted water cluster mass spectrum. The inset is an expanded view of the region from 2400-3000. A calibration reference file was created, entitled "Water 3000" which contained ions at 90 amu intervals (5 H_2O increments) from m/z 73 to 2954. Figure 3 is a printout of this reference file. Figure 4 is a copy of the calibration report generated using water clusters to calibrate the mass axis.

Comments:

Because of the low [M+1]+ isotope abundances of both hydrogen and oxygen, determination of instrument resolution is best judged by examination of peak width at half height, rather than the traditional "10% of valley" method used in carbon-containing calibrants such as PEG. While calibration up to m/z 3000 is relatively straightforward using this method, optimization of higher masses has not yet been fully investigated







;H2O Cluster	rs	
;H2O=18.0105		
;H3O=19.0183		
incrementing by 5 units		
73.0502	100	
163.1032	100	
253.1562	100	
343.2092	100	
433.2622	100	
523.3152	100	
613.3682	100	
703.4212	100	
793.4698	100	
883.5272	100	
973.5802	100	
1063.6332	100	
1243.7392	100	
1333.7922	100	
1423.8452	100	
1513.8982	100	
1603.9512	100	
1694.0042	100	
1784.0572	100	
1874.1102	100	
1964.1632	100	
2054.2162	100	
2144.2692	100	
2234.3222	100	
2324.3752	100	
2414.4282	100	
2504.4812	100	
2594.5342	100	
2684.5872	100	
2774.6402	100	
2864.6932	100	
2954,7462	100	

Figure 3. printout of "Water 3000" calibration reference file

Figure 4. Calibration report generated using water clusters as calibrant

Three types of mass axis calibrations are available using MassLynx software: static calibration, scanning calibration and scan speed compensation. For normal instrument use, it is recommended that all three types of calibrations are performed so that any mode of data acquisition can be used. In this way, mass ranges and scan speeds can be changed without sacrificing correct mass assignment. During a static calibration, the quadrupole is held at a single mass. During scanning calibration, the instrument is correctly calibrated over a specified mass range. A scan speed compensation calibration accounts for changes in scan speed during data acquisition. For the scan speed compensation to be used correctly, a scanning calibration should also be performed.



 Waters, Millennium, MassLynx and Alliance are trademarks of Waters Corporation.

 Copyright 1998 Waters Corporation.
 Printed in the U. S. A.
 AMD16