

Nano lockspray for enhanced mass measurement accuracy in proteomics studies

Alan Millar, James Langridge, Therese McKenna, Steve Pringle, Robert Bateman, Kevin Giles, John Hoyes and Phil Young,

Waters Corporation, Floats Road, Manchester, United Kingdom.

Nanoscale LC-MS/MS performed on a hybrid quadrupole orthogonal acceleration time of flight (Q-ToF) mass spectrometer is an established technique for high sensitivity identification and characterisation of proteins. Typically these experiments employ LC columns which have an internal diameters of 75 μm , operating at flow rates of approximately 200 nL/min. Whilst this set-up offers the optimum sensitivity it does not allow the post-column addition of an internal reference ion, as this would detrimentally effect the resolution of the LC separation, resulting in peak broadening. The use of an internal reference is required to provide reliable sub 5ppm mass measurement accuracy. Here we report the use of a nanoflow lock spray interface to routinely provide enhanced mass measurement in the analysis of protein digests, and report on the advantages in database search results and *de novo* sequencing capabilities that this affords.

In this paper we describe a novel electrospray interface that allows routine exact mass measurement to be performed at nanoscale flow rates. The interface consists of a dual sprayer arrangement, with each spray sampled individually by the mass spectrometer by means of an electronically controlled baffle plate. Sampling of the reference spray can be performed at a user-defined interval.

Separation of tryptic digest samples was performed using a capillary HPLC system operating at nanoliter per min flow rates coupled to a Q-ToF mass spectrometer. Samples were injected onto a C18 trapping cartridge whilst they are desalted. The peptides are then eluted from the C18 pre-column, at 200 nL/min, onto a 75 μM ID x 10 cm C18 analytical column for separation and elution into the mass spectrometer.

Tryptic digest mixtures of known standard proteins were initially used to assess the mass measurement capabilities over extended periods of time. Mass measurement in both the MS and MS/MS modes provided data with mass measurements better than 5 ppm RMS. The improvement in databank search results using this mass accuracy will be discussed with specific examples where the enhanced mass measurement allows removal of ambiguous protein identifications from the protein identification list.
