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### AUTOMATED FT-MS<sup>n</sup>

VICTOR FURSEY, CHRISTIAN BERG, SUNIA AFZAAL, PAUL SPEIR, GARY KRUPPA  
AND FRANK LAUKIEN

Bruker Daltonics Inc., Manning Park, Billerica, MA 01821, U.S.A.

The rapid and reliable screening of samples like peptides, natural products and combinatorial libraries by mass spectrometry has become important for an increasing number of drug discovery and analytical laboratories. In addition to very accurate molecular weight assignment, additional structural information with < 2 ppm mass accuracy is often required. This level of mass accuracy in MS<sup>n</sup> permits unambiguous identification and substructure determination based on reliable elemental composition analyses of both the molecular ion and its MS/MS and MS/MS/MS products. Furthermore, for high throughput applications, such as HPLC/FTMS and automated (i.e. 96/384-well plate) flow injection FTMS, multiple stages of MS must be acquired in an unattended manner. In other cases where an important drug candidate or interesting molecule has already been identified, throughput may be less important, but as much structural information as possible with accurate mass is required. For these purposes we have developed FTMS methods for automated MS/MS and MS<sup>3</sup>, using macros written in Tcl/Tk (Tool Command Language/Tool Kit) which can be activated within the XMASS<sup>®</sup> acquisition software of the APEX II.

All experiments were carried out on an APEX II 47e (Bruker Daltonics, Billerica, MA) Fourier transform mass spectrometer (FTMS) equipped with a 4.7 Tesla shielded superconducting magnet, a patented Infinity<sup>™</sup> cell<sup>1</sup> and a computer-controlled ESI source. Ionization was performed by pumped flow nano-electrospray (167 nL/min) of a 16 micromolar solution containing reserpine in a solvent system of water/methanol/acidic acid (49/49/2%). Isolation of parent ions is achieved using correlated swept frequency excitation.<sup>2</sup> Sustained off-resonance irradiation (SORI) was used for the MS<sup>n</sup> collisional activation.

The MS<sup>3</sup> automation macro runs with a graphical user interface (GUI) for automated selection of the most abundant ions (above a defined relative intensity threshold, *Min. Int [%]*) for the MS<sup>2</sup> and MS<sup>3</sup> experiments. The software includes provisions to filter the automatically selected list of peaks for isotopic redundancies. The parameters used for performing SORI-CID (i.e. attenuation of V<sub>p-p</sub> of the excitation RF and irradiation time) are automatically selected from the tuning file used to optimize conditions. The data was externally calibrated using fragment ions generated from the MS/MS of human angiotensin I.

Data from the MS<sup>2</sup> and MS<sup>3</sup> experiments is shown in Figure 1. The most abundant [M+H]<sup>+</sup> ion of reserpine at m/z=609 was automatically selected (selection criteria set to > 99%) from the primary mass spectrum (Figure 1 a)) for the MS<sup>2</sup> experiment shown in Figure 1 b). The selection criteria for MS<sup>3</sup> experiments were set to 70% leading to the automatic selection of the two most abundant fragment ions at m/z 448 and 397 for further analysis. Results from the MS<sup>3</sup> experiments on these two ions are shown in Figure 1 c) and d). Average mass accuracy calculated for fragment ions observed for all MS<sup>2</sup> and MS<sup>3</sup> experiments on m/z= 609, 448 and 397 was 1.3 ppm with an average standard deviation of 0.9 ppm.

We also present initial results on the use of an automated MS/MS macro on a complex tryptic digest of a 142 kDa enzyme.

<sup>1</sup> P. Caravatti, M. Allemann *Organic Mass Spectrometry* **26** (1991) 514-518

<sup>2</sup> L.J. De Konig, N. M.M. Nibbering, S. L. Van Orden, F.H. Laukien *Int. Journal of Mass Spectrometry and Ion Processes* **165/166** (1997) 209-219.