SCIENTIFIC ANALYSIS INSTRUMENTS

Application Note

# LaserToF LT3 Plus

## Sequencing Tryptic Peptides Using Sulphonated Derivatives

### Introduction

Post Source Decay (PSD) can be used in modern MALDI-TOF instrumentation to select, fragment and so sequence a peptide. If the instrument uses a harmonic field reflectron, the MS/MS technique can be performed seamlessly (sPSD), without adjustment of the reflectron voltages and without the need for stitching in software. This leads to rapid sPSD acquisition with improved calibration.

Fragmentation requires the free movement of a proton along the peptide backbone. Tryptic peptides are difficult to fragment because their C-terminal lysine or arginine strongly binds the proton. A new and novel derivitization places a sulphonated group at the peptides N-terminal. This acidic N-terminal derivative forms a zwitterion with the Lys/Arg C-terminal at the pH of the matrix. Subsequent protonation by MALDI provides a labile proton for fragmentation. As a result, fragmentation via proton mobilisation is improved. Examples of this so called chemically activated fragmentation (CAF) are described.

### Materials and Methods

Tryptic digests of cytochrome C, creatine phosphokinase and alcohol dehydrogenase were sulfonated. Candidate peptides from their maps were partially sequenced by sPSD.

Sulphonation was achieved by one of two methods. In the first, the sulphonation of cytochrome C digest was achieved using the reagent in the CAF-MALDI sequencing kit available commerically (Amersham BioSciences). The protocol used was that described in the kit. The second reagent was 2-sulfobenzoic acid cyclic anhydride. The protocol used was that developed by Keough et al (1999) with some minor modifications (details available on request).

### Results

Figure 1 shows the sPSD mass spectrum of a derivatised peptide which was believed to correspond to the amino acid sequence TGPNLHFGR from a tryptic digest of

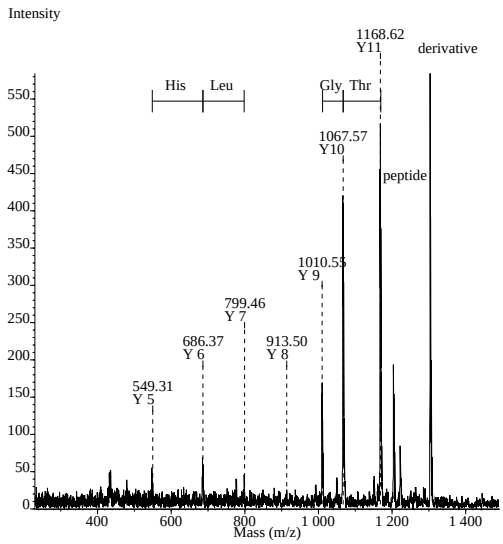


**Figure 1: PSD of sulphonated peptide selected from a tryptic peptide of a cytochrome C digest**

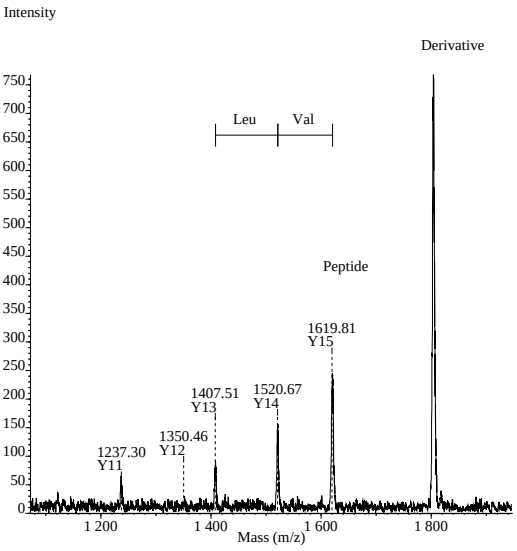


**Figure 2: sPSD of a tryptic peptide from ADH suspected as being VLGIDGGEKEELFR**

SCIENTIFIC ANALYSIS INSTRUMENTS

cytochrome C. The PSD of such sulphonated tryptic peptides gives a simplified Y only ion spectrum. The series confirms the peptide to be TGPNLHFGR. Note the absence of a peak for the proline N-terminal y-ion, as is usual for these peptides.

Figure 2 shows the sPSD mass spectrum of a derivatised ADH tryptic digest using the alternative CAF reagent 2-sulfobenzoic acid cyclic anhydride. The results show a partial sequence which is adequate for the purposes of confirmatory identification.

Figure 3 shows sPSD mass spectrum of a tryptic peptide from creatine phosphokinase suspected as being DLFDPIIQDR using the CAF reagent 2-sulfobenzoic acid cyclic anhydride. Again a partial sequence is given which is sufficient to identify the parent proteins.

## Conclusions

1) Sulphonication of pepides leads to simplified Y-ion spectra.

2) Seamless PSD of sulphonated tryptic peptides gives rapid confirmatory sequence information of tryptic peptides without the need for time consuming stitching of spectra.

3) A definative CAF reagent is available commerically, but in some cases the reagent 2-sulfobenzoic acid cyclic anhydride may prove adequate for confirmatory purposes.

## References


*Dongre A R, Jones J L, Somogyi A and Wysocki V H. (1996) J. Am. Chem. Soc. 118, 8365-8374*

*McCormack A L, Somogyi A, Dongre A R, Wysocki V H (1993) Anal. Chem. 65, 2859-2872*

*Keough T, Youngquist R S and Lacey M P (1999) Proc. Natl. Acad. Sci. USA Vol 96, pp. 7131-7136*

*Keough T, Lacey M P and Youngquist R S (2000) Rapid Communications in Mass Spectrometry 14, 2348-2356*

*Keough T, Lacey M P and Youngquist R S (2002) Rapid Commun. Mass Spectrom. 16: 1003-1015.*


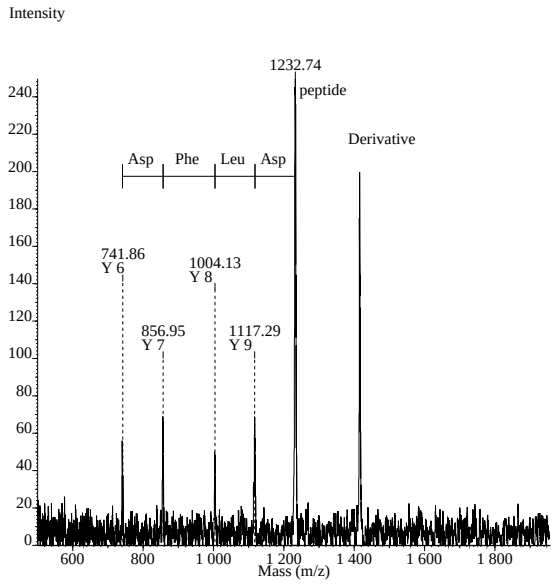


**Figure 3: sPSD of a creatine phosphokinase tryptic peptide suspected as being DLFDPIIQDR.**

**Local Representative**


Scientific Analysis Instruments Ltd
Hadfield House, Hadfield Street
Manchester M16 9FE
Tel: + 44 (0) 161 874 2460
Fax: + 44 (0) 161 874 2461
E-mail:sales@saiman.co.uk
http://www.saiman.co.uk



Ref. No.LTAN3/02.10