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RCM

Letter to the Editor

To the Editor-in-Chief
Sir,

Simple two-point calibration of hybrid quadrupole time-of-flight instruments using a synthetic lipid standard

Introduced a few years ago, hybrid quadrupole time-of-flight (TOF) mass spectrometers have already made significant impact in the field of structural characterization of biomolecules by acquiring tandem mass spectra with high speed, resolution, mass accuracy and sensitivity provided by parallel detection of all fragment ions (reviewed in Ref. 1). The high mass accuracy of quadrupole TOF instruments can be achieved by using a simple two-point calibration of the TOF analyzer. It is sufficient to select the masses of two reference peaks that have *m/z* values which either flank the desired range of measured masses, or are separated from each other by a few hundred Thomson (Th). Although saturation of the MCP detector should be avoided, reference peaks should be abundant and preferably should have similar intensities so that their shapes and the positions of centroided peaks are not affected by ion statistics.

Standard mixtures recommended by manufacturers of quadrupole time-of-flight mass spectrometers for two-point calibration of TOF analyzers in positive ion mode typically use a heavy metal cation (like Cs^+) as the first reference peak and a cation of a protonated peptide with MW > 800 Da as the second reference peak. Such mixtures are well suited for conventional electrospray ionization with a typical infusion flow rate at 1–10 μL/min and a spraying voltage of several kilovolts. Under these conditions, peptides that do not contain basic amino acid residues are mostly detected as abundant singly charged ions. However, if the instrument is fitted with a nanoelectrospray ion source,[2] peptides containing more than 4–5 amino acid residues are generally detected as doubly and/or triply charged species regardless of their amino acid composition, with the singly charged ion represented by only a minor peak. In such cases, the *m/z* value of the doubly charged ion is too close to that of the first reference peak to achieve a calibration valid in the entire *m/z* region of interest. Furthermore, the low *m/z* region of the spectrum is densely populated with intense background ions, which can affect the shapes of reference peaks (Fig. 1(A)). Calibrating the instrument in tandem mass spectrometry (MS/MS) mode using masses of peptide fragments

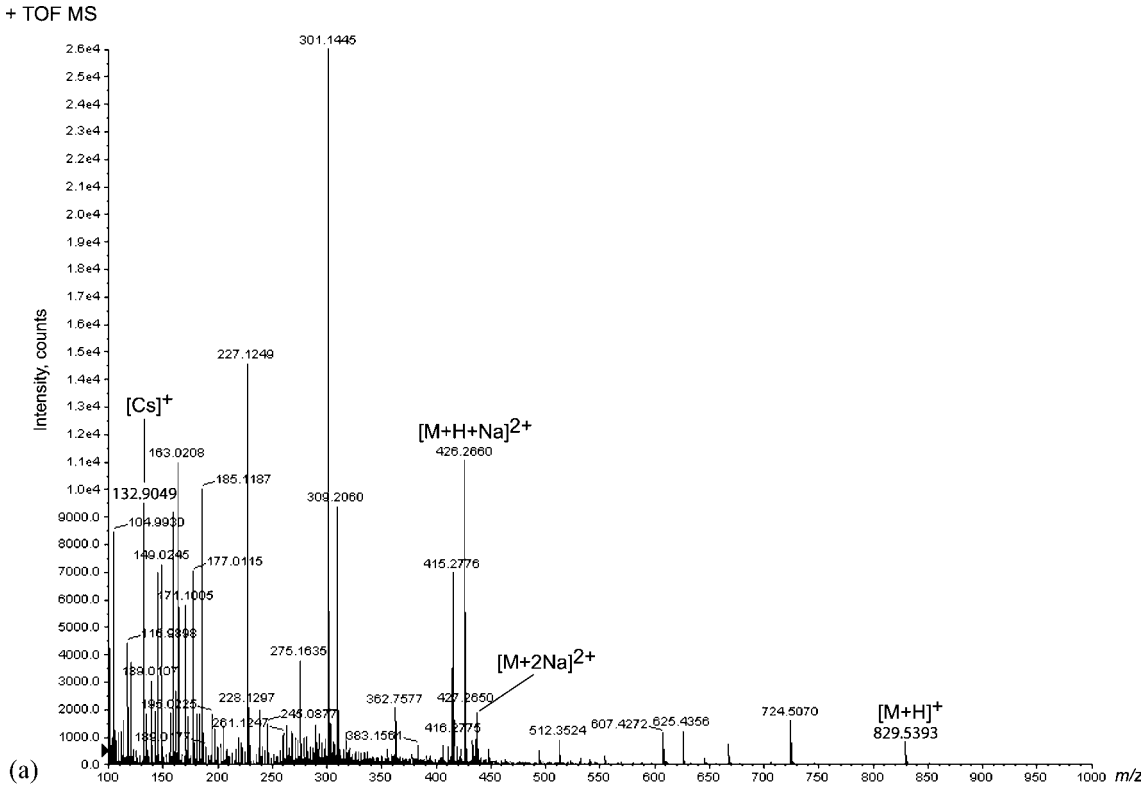

Figure 1. (A) TOF mass spectrum of a conventional calibration mixture (20 pmol/μL CsI, 2 pmol/μL peptide ALILTLVS, Bachem cat # H-9985 in 50% methanol and 0.1% acetic acid) acquired using a QSTAR Pulsar *i* mass spectrometer (MDS Sciex, Canada) fitted with a nanoelectrospray ion source. (B) MS/MS spectrum of DGPC. Reference peaks: a fragment ion of the head group ($C_5O_4H_{15}NP$, *m/z* calc. 184.0733) and the intact molecular ion ($C_{56}O_8H_{113}NP$, *m/z* calc. 958.8198) are labeled.

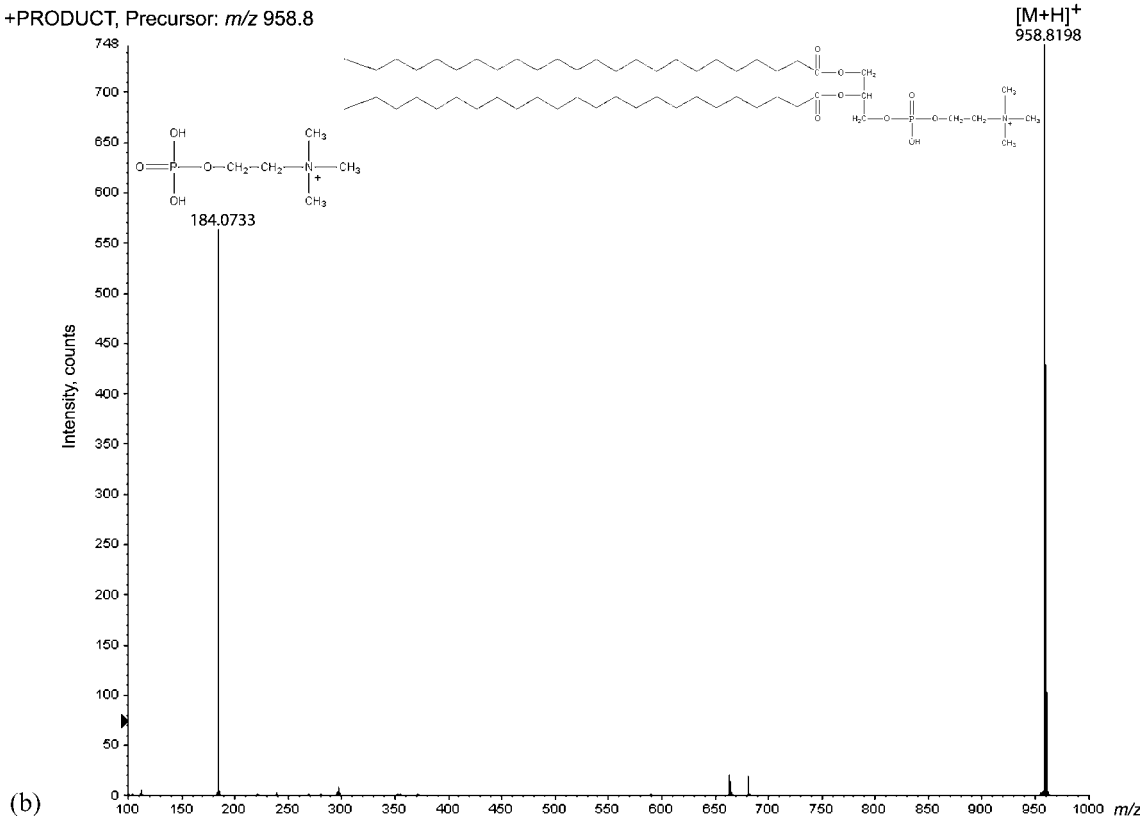

Figure 1. *Continued.*

might also be rather inconvenient, as in the range of *m/z* 150–300 many fragments with overlapping masses are typically formed.[3] At the same time using peaks of immonium ions should be avoided because low RF settings of the RF-only quadrupole Q2 (the collision cell) perturb focusing of the ion beam. It is also difficult to optimize the collision energy in such a way that low and high *m/z* reference peaks are both abundant and, at the same time, their intensities are balanced. These problems do not seriously affect peptide sequencing, although they may affect precision of the instrument calibration.

We have found it particularly convenient to use a synthetic lipid phosphatidylcholine (PC) standard for calibrating TOF analyzers. Regardless of the employed electrospray ion source (conventional or nanospray), PCs are always detected as singly charged species. PCs are easy to fragment and the characteristic fragment of the head group that contains a tertiary amino group is obtained in high yield (see Fig. 1(B)). Fragmentation mechanisms for phospholipids have been reviewed.[4] For calibration we typically use a 250 fmol/μL solution of 1,2-dilignoceroyl-*sn*-glycero-3-phosphocholine (DGPC) (Avanti Polar Lipids, cat #850373) in methanol/chloroform (2:1 v/v), containing 5 mM ammonium acetate. The solution is stable for months at −20 °C. To avoid possible overlap of the low *m/z* reference peak with background peaks we calibrate the instrument in MS/MS mode. The first analytical quadrupole (Q1) is operated under low-resolution settings that enable unperturbed transmission of the entire isotopic cluster of the precursor. Peaks corresponding to the intact precursor and the head group fragment are used as the high *m/z* and low *m/z* references, respectively (Fig. 1(B)). Application of medium collision energy (38 eV) allows us to balance the intensities of the two peaks.

The same standard solution can also be applied to calibrate the instrument in negative ion mode. DGPC is detected as a singly charged adduct with an acetate anion ($C_{58}H_{115}O_{10}NP$, *m/z* calc. 1016.8264). Upon collisional fragmentation of the adduct abundant peaks of demethylated DGPC ($C_{55}H_{109}O_8NP$, *m/z* calc. 942.7896) and of the acyl anion of lignoceric acid ($C_{24}H_{47}O_2$, *m/z* calc. 367.3581) are yielded and can be further used as references as described above.

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