Internal Calibrant for an Exact Mass Measurement in Electrospray Ionization Mass Spectrometry

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An exact mass measurement in electrospray ionization mass spectrometry (ESI-MS) has been applied to determine the molecular composition of some large organic molecules.¹ However, a general and suitable internal calibrant has not yet been reported. Although polyglycols and polyethyleneglycols have been considered to be promising calibrants, it is generally difficult to obtain the desired ion intensity and mass number using these polymers. Almost all calibrants have been used as independently measured external standards to avoid any mixing up with the analyte in solution.² Since recent advanced ESI has made it possible to generate various multiply charged molecular ions in the low-mass region³⁻⁵, an exact mass measurement for large organic molecules has become more adaptable.

We directed our attention to some macrocyclic polyethers, crown ether compounds, and consequently found that these exhibited excellent performance as an internal calibrant for an exact mass measurement in ESI-MS. Sixteen different crown ethers (Table 1) were prepared for measurements.

Each crown ether afforded the adducts with an alkali metal ion, such as Na⁺, K⁺, Rb⁺ and Cs⁺, provided in the same manner⁶ and thus the extending variety of calibrants. A total of 64 crown ethers covering a mass range of up to 733u were prepared. These crown ethers exhibited no interference with the analyte appearing in the mass spectrum in the case that the sample solution was mixed up with different kinds of calibrants and their adducts.⁷ A made-to-order response by adjusting the concentration of each compound was obtained.⁸ This is ascribed to the supreme ionization ability of crown ethers in the positive ESI process. These internal calibrants were used for the exact mass measurements of several compounds. The instrumentation and experimental conditions for the ESI-MS are given in Table 2.

The typical spectra in the case of cinchonine (Fig. 1), reserpine (Fig. 2) and angiotensin I (Fig. 3) are shown. A couple of calibrant ion peaks were selected to calibrate the target ion. The target ion peak should be located between these two calibrant ion peaks on the mass spectrum.

As can be seen in Figs. 1 - 3, a deviation in the measured mass number is observed at less than 10 ppm for each compound. This result suggests the excellent capability of these crown ethers used as an internal cal-

Table 1 Exact mass number (amu) of crown ethers

Na:199.0946 K :215.0686 Rb:261.0167 Cs:309.0103	Na:243.1208 K :259.0948 Rb:305.0429 Cs:353.0365	Na:287.1471 K :303.1201 Rb:349.0691 Cs:397.0627	Na:291.1208 K :307.0948 Rb:353.0429 Cs:401.0365

Na:335.1471 K :351.1210 Rb:397.0691 Cs:445.0627	Na:383.1471 K :399.1210 Rb:445.0691 Cs:493.0627	Na:395.2410 K :411.2149 Rb:457.1630 Cs:505.1566	Na:465.2729 K :481.2469 Rb:527.1950 Cs:575.1886

Na:471.1995 K :487.1734 Rb:533.1215 Cs:581.1152	Na:483.1784 K :499.1523 Rb:545.1004 Cs:593.0940	Na:483.2934 K :499.2673 Rb:545.2154 Cs:593.2091	Na:495.2723 K :511.2462 Rb:557.1943 Cs:605.1879

Na:535.2097 K :551.1836 Rb:597.1317 Cs:645.1253	Na:559.2519 K :575.2259 Rb:621.1739 Cs:669.1676	Na:571.2308 K :587.2047 Rb:633.1528 Cs:681.1465	Na:623.2621 K :639.2360 Rb:685.1841 Cs:733.1778

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Na: [M+Na]⁺, K: [M+K]⁺, Rb: [M+Rb]⁺, Cs: [M+Cs]⁺.

Table 2 Instrumentation and experimental conditions for ESI-MS

Mass spectrometer	JMS-700T JEOL
Capillary voltage	2.8 kV
Capillary current	300 – 700 nA
Orifice voltage	10 V
Resolution	5000
Solvent	MeOH
Flow rate	17 μ l/min
Sample concentration	0.1 mmol/l
Crown ether concentration	0.01 mmol/l

Fig. 1 HR ESI-MS spectrum of cinchonine.

ibrant in ESI-MS. Although the range covered by these experiments is up to 733u so far, any desired ion peaks will be able to be created in combination with the modified crown ethers. This method is also available for exact mass measurements in other ionization techniques, such as fast atom bombardment (FAB).

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Fig. 2 HR ESI-MS spectrum of reserpine.

Fig. 3 HR ESI-MS spectrum of angiotensin I.