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Accurate Mass Analysis of Phosphoramidites by Electrospray Mass Spectrometry

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ACCURATE MASS ANALYSIS OF PHOSPHORAMIDITES BY ELECTROSPRAY MASS SPECTROMETRY

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□ *A method of accurate mass determination of phosphoramidites is described. The commonly used methanol/water/acid system was replaced by LiCl-containing acetonitrile and the concentrations of LiCl, poly(ethylene glycol), and phosphoramidite samples were optimized.*

Keywords Accurate Mass Determination, Electrospray Mass Spectrometry, Phosphoramidites, Poly(Ethylene Glycol)

INTRODUCTION

Phosphoramidites are the most commonly used building blocks for oligonucleotide synthesis. Ever since the first application was described,^[1] the characterization of these compounds remained elaborate as they are mostly foams or oils even after repeated chromatographic purification. That is why elemental analysis does not provide precise data. Accurate mass analysis could replace elemental analysis and provide very useful characterization information besides H, C, and P-NMR experiments. Unfortunately, phosphoramidites are sensitive to nucleophiles, oxidation, acids, and bases, which renders them incompatible for standard ESI-MS analysis. Furthermore, these compounds often contain substituted trityl moieties, which cause highly intense undesired signals in positive-ion detection mode. These difficulties have been overcome by a method applying LiCl as ion-forming agent and acetonitrile as solvent^[2] instead of acidic aqueous methanol solutions commonly used for routine ESI-MS analysis. In the case of accurate mass analysis, ions formed from commonly used standards usually suppress the signal of the sample, and ready-to-use standards (e.g., Mass Spectrometer Standard Kit, Final

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Test Kit PE SCIEX; P/N:401936) also contain acids with alcohol, thus impeding the direct application of the above method.

The aim of this work was to develop a simple method for the accurate mass determination of phosphoramidites using LiCl to form adduct ions and acetonitrile (ACN) as solvent, to apply polymer standards such as poly(ethylene-glycol) (PEG), and to optimize the sample/standard/LiCl ratio.

RESULTS AND DISCUSSION

The following materials were used for experiments: DMT-T-CE amidite (5'-O-dimethoxytrityl-thymidine-3'-O-(N,N-diisopropyl-O-cyanoethyl phosphoramidite) as sample (Figure 1) in 1, 5, and 10 mM concentrations; PEG-1000 (Sigma-Aldrich-Fluka) as accurate mass standard in 0.1 and 1 mM concentrations; acetonitrile (Acetonitrile UV, B&J Brand[®], High Purity Solvent; 0.001% water) as solvent; and saturated LiCl in acetonitrile as adduct-ion forming agent (diluted 10 ×, 100 ×, and 1000 ×). API QSTAR Pulsar i mass spectrometer (ABI/MDS Sciex) was used to perform analyses. Accurate masses were calculated with the Analyst QS software.

The lowest applicable concentration was 1 mM for standard PEG-1000, both in positive and negative detection mode (signal/noise ratio was relatively small under the above concentrations). The LiCl content was found best if 100 × diluted sat. LiCl/ACN was applied (1000 × dilution resulted in very weak signals, mostly Na⁺ and K⁺ adduct ions. On the other hand, 10 × diluted solution yielded multiple Li⁺ adducts of the PEG-1000 standard.) The optimal phosphoramidite sample concentration was between 5 and 10 in positive and 1 mM in negative detection mode. The best results are shown in Figure 2 (Table 1).

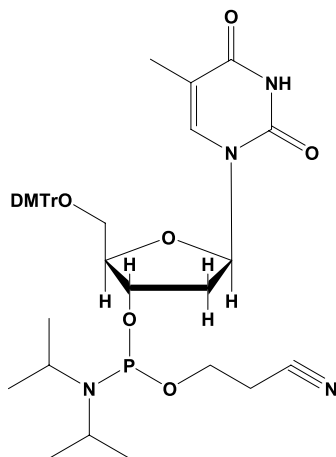


FIGURE 1 DMT-T-CE amidite.

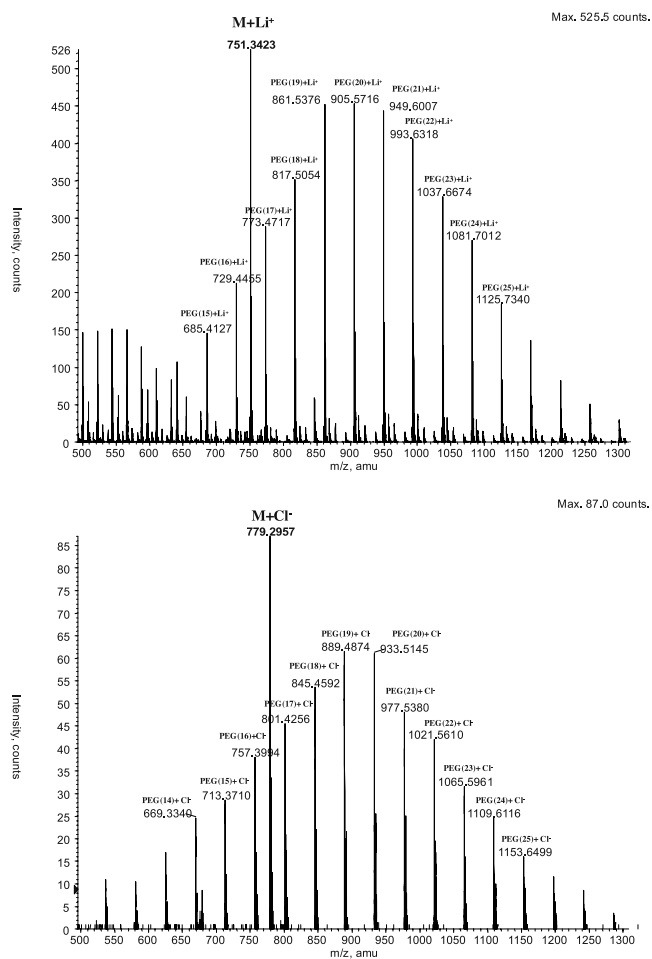


FIGURE 2 Spectra of DMT-T-CE amidite with PEG-1000 standard in LiCl/ACN solution (1 mM PEG-1000, 100 \times diluted sat. LiCl/ACN, 5 mM/1 mM DMT-T-CE-amidite).

We have successfully developed a method for accurate mass measurement of a phosphoramidite compound affording mass accuracy within 5 ppm using LiCl for ion formation and PEG-1000 as standard in ACN solution.

TABLE 1

Molecular formula	Calculated monoisotopic mass	Measured monoisotopic mass ^a	Mass accuracy
$C_{40}H_{49}N_4O_8PLi^+$	751.3443	751.3423	−2.7 ppm
$C_{40}H_{49}N_4O_8PCl^-$	779.2982	779.2957	−3.2 ppm

^aAccurate mass of the analyte compound has been determined by internal calibration using the two nearest monoisotopic peaks of PEG adduct ions.

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