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An electrospray mass spectrometric method for accurate mass determination of highly acid-sensitive phosphoramidites

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An accurate mass determination method utilizing electrospray ionization mass spectrometry is described for analysis of several different types of phosphoramidites that are extremely acid-sensitive compounds. An earlier method, which applied a LiCl/acetonitrile system, was extended for this special application by using polymeric standards including poly(ethylene glycol) (PEG), poly(ethylene glycol) dimethyl ether (PDE) and poly(propylene glycol) (PPG). Concentrations of standards, samples and LiCl were optimized and potential impurities that affect the analyses were also investigated. Copyright © 2008 John Wiley & Sons, Ltd.

In the early 1980s, 2′-deoxynucleoside phosphoramidites as monomer units revolutionized nucleic acid synthesis.[1,2] These 2′-deoxynucleoside derivatives are relatively stable but after mild acidic activation become highly reactive for the condensation reaction that forms the sugar-phosphate backbone of nucleic acids. Following this early research,[3] several phosphoramidite derivatives have been synthesized for different purposes (e.g. fluorescent dye or biotin-containing molecules, 3′- and 5′-amino or thiol modifiers, sugar or nucleobases with diverse protecting groups).[4–12] However, chemical characterization of these compounds remains problematic. Currently, ^{1}H, ^{13}C and ^{31}P NMR are used to provide important data but a complete characterization, including elemental analysis, is not possible as phosphoramidites are isolated as foams or oils. Accurate mass determination could replace elemental analysis but the commonly used mild ionization techniques (MALDI, FAB, LSIMS, ESI) apply acidic media or matrices that are incompatible with the acid-labile phosphoramidites. Additionally, many phosphoramidites contain acid labile moieties such as substituted trityl protecting groups which provide strong, undesired signals in the positive detection mode. A method using electrospray ionization mass spectrometry (ESI-MS) has been used with LiCl in acetonitrile instead of an acidic aqueous methanol solution in order to characterize phosphoramidites in both positive and negative mode.[13] However, for accurate mass determination, which requires an internal standard, there is an additional problem. This is because the internal standard and the analyte molecule mutually perturb one another's ionization in the ion source. Application of a dual ESI spray ion source can reduce the analyte-reference competition during the formation of ions but this procedure also requires constant optimization of the reference standards and analyte concentrations.[14,15] Additionally, these two channel sources are not widely available.

Recently, a method has been described by Fujitake *et al*. for measuring the accurate mass of phosphoramidites using liquid secondary ion mass spectrometry (LSIMS) with a double focusing mass spectrometer and a novel triethanolamine-NaCl matrix system.[16] These workers analyzed nucleoside phosphoramidites but only in the positive ion detection mode. Although they could measure several phosphoramidites with very high accuracy, the relative intensities of the sample peaks compared to the undesired, substituted trityl peaks were only 0.4–20.7%, which results in lower sensitivity and reproducibility.

Our aim was to develop a one-channel ESI-MS procedure in combination with different polymeric standards such as poly(ethylene glycol) dimethyl ether (PDE), poly(ethylene glycol) (PEG), and poly(propylene glycol) (PPG)[15,17,18] as a simple method for accurate mass determination of various phosphoramidites. In our preliminary experiments, as reported in a poster session,[19] 5′-*O*-dimethoxytrityl-2′-deoxythymidine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite, a PEG standard, and LiCl in acetonitrile were used to optimize the sample/standard/LiCl ratio. Based on these promising results, we investigated many additional phosphoramidites and polymer standards in order to explore the general use of this procedure. These results are summarized in this manuscript.

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EXPERIMENTAL

Materials

LiCl (puriss), PEG (average MW: 600 and 1000), PDE (average MW: 600 and 1000), PPG (average MW: 725 and 1000) and acetonitrile (Acetonitrile UV, B&J Brand®, High Purity Solvent, 0.001% water) were purchased from Sigma-Aldrich-Fluka. The phosphoramidite samples as well as the methylphosphinoamidite (Fig. 1, compound **11**) were ordered from Glen Research and Chemgenes Corporations and the two 2′-deoxynucleoside-3′-*O*-(diisopropylamino) phosphino acetic acid 1,1-dimethylcyanoethyl esters (Fig. 1, compounds **12** and **13**) were purchased from Metasense Technologies.

The following stock solutions were prepared: saturated LiCl solution in acetonitrile; 10× and 100× diluted solutions (10 × d and 100 × d, respectively) of saturated LiCl in acetonitrile; 100, 10 and 1 mM phosphoramidites in acetonitrile; 100, 10 and 1 mM PEG, PDE and PPG standards in acetonitrile.

Instrumentation

All mass spectrometric analyses were performed on an API Q-STAR pulsar i (ABI/MDS SCIEX) instrument in TOF-MS mode using a standard ion-spray source with a fused-silica capillary and the following instrument parameters: ion source voltage in positive (3800 V) and negative (−3800 V) modes; declustering potential 1 was 40 V and −45 V; declustering potential 2 was 10 V and −10 V; focusing potential 265 V and −265 V; focusing rod offset was 20 V and −20 V; ion source gas (N_2) was 20 L/h; curtain gas (N_2) was 40 L/h; grid was 15 V and −20 V. Accumulation time: 1 s; scan range: m/z 300–1400.

General method for accurate mass measurement of phosphoramidites

Volumes of 10 μL of 10 × d LiCl, 10 μL of 10 mM PEG, PDE or PPG, 10 μL of 100 mM phosphoramidite and 70 μL of acetonitrile were mixed, injected using a syringe pump and the accurate mass was measured in positive and negative detection modes. When the intensity or the ratio of the intensities of analyte/reference was not acceptable, another 100 μL mixture of LiCl, standard, and sample in acetonitrile was prepared with appropriate changes in sample concentrations. For mixtures having high polymer signals, the maximum final phosphoramidite concentration was increased to 50 mM; in contrast, when the polymer signal was of low intensity, the phosphoramidite concentration was decreased to 0.02 mM. The optimal, final concentrations (i.e. where the polymeric standard and phosphoramidite signal intensities were similar) in both positive and negative mode are shown in Table 1. Theoretical masses for reference standard molecules and phosphoramidite samples were calculated using the Analyst™ QS software package using the built-in isotopic distribution calculator. Data of the phosphoramidites are presented in Table 1. Internal calibration of spectra and a determination of the accurate mass of the analyte phosphoramidites were performed using the two nearest monoisotopic reference standard peaks relative to the sample peaks.

RESULTS AND DISCUSSION

Observations leading to the LiCl/acetonitrile system containing polymeric standards

Our aim was to develop a simple and easily applicable, general method for measuring accurate mass of phosphoramidites by ESI-MS. As all ions that form in the ion source are competitors and perturb one another's ionization, suppression of undesired peaks (e.g. trityls and other decomposition side-products) compared to the sample peak is important because suppression of these peaks enhances formation of the internal standard and sample ions which increases the sensitivity of the method. These variables can be easily managed with ESI using LiCl instead of acids. The application of ESI has a further advantage compared to other ionization techniques as this ion source is presently one of the most popular, convenient and widely used.

Our initial experiments focused on the LiCl/acetonitrile system and PPG, a ready-to-use standard (Mass Spectrometer Standard Kit, Final Test Kit PE SCIEX; P/N: 401936) which is commonly used with non-acid labile compounds. As these standard mixtures contain volatile acids such as acetic acid and formic acid, we could not detect any signal from the phosphoramidite samples, presumably because these acids degraded the analyte. We then turned to an investigation of non-acidic mixtures containing PEG, PPG and PDE. These polymeric standards are cheap, easily available, stable and soluble in non-protic organic solvents such as acetonitrile or tetrahydrofuran. They also form ions in both positive and negative mode with metal and halogen ions and thus yield ion ladders having 44 (PEG, PDE) or 58 (PPG) mass differences. These advantages make sample preparation, measurement and mass calibration very convenient. PDE has the added advantage of not containing hydroxyl groups which could be important for nucleophile-sensitive samples.

Optimization of LiCl content and standard concentration

Preliminary experiments were completed using saturated LiCl in acetonitrile as a stock solution with a PPG standard and compound **4** (10 mM) in acetonitrile (structures are presented in Fig. 1). The optimal LiCl content was found to be the 100 times diluted (100 × d) mixture of a saturated LiCl solution. When solutions with less LiCl were applied, the signal intensities for both sample and standards decreased. Near these low Li^+ concentrations, Na^+ and K^+ adducts could also be seen which creates a more complex spectrum. With higher concentrations of LiCl, the reference molecules formed not only $[M+Li]^+$ ions, but also $[M+2Li]^{2+}$ and $[M+3Li]^{3+}$ multiply charged ions which, in positive detection mode, leads to overlapping peak series (Fig. 2).

Although these doubly and triply charged reference ion peak series could also be used for calibration, we tried to decrease the intensity of multiple charged standard peaks by applying the lowest possible salt concentration in order to obtain simple and clear spectra. Using the 100 × d LiCl solution, all three standards (PEG, PDE, PPG in acetonitrile) gave excellent spectra with $[M+Li]^+$ and $[M+Cl]^-$ ion series

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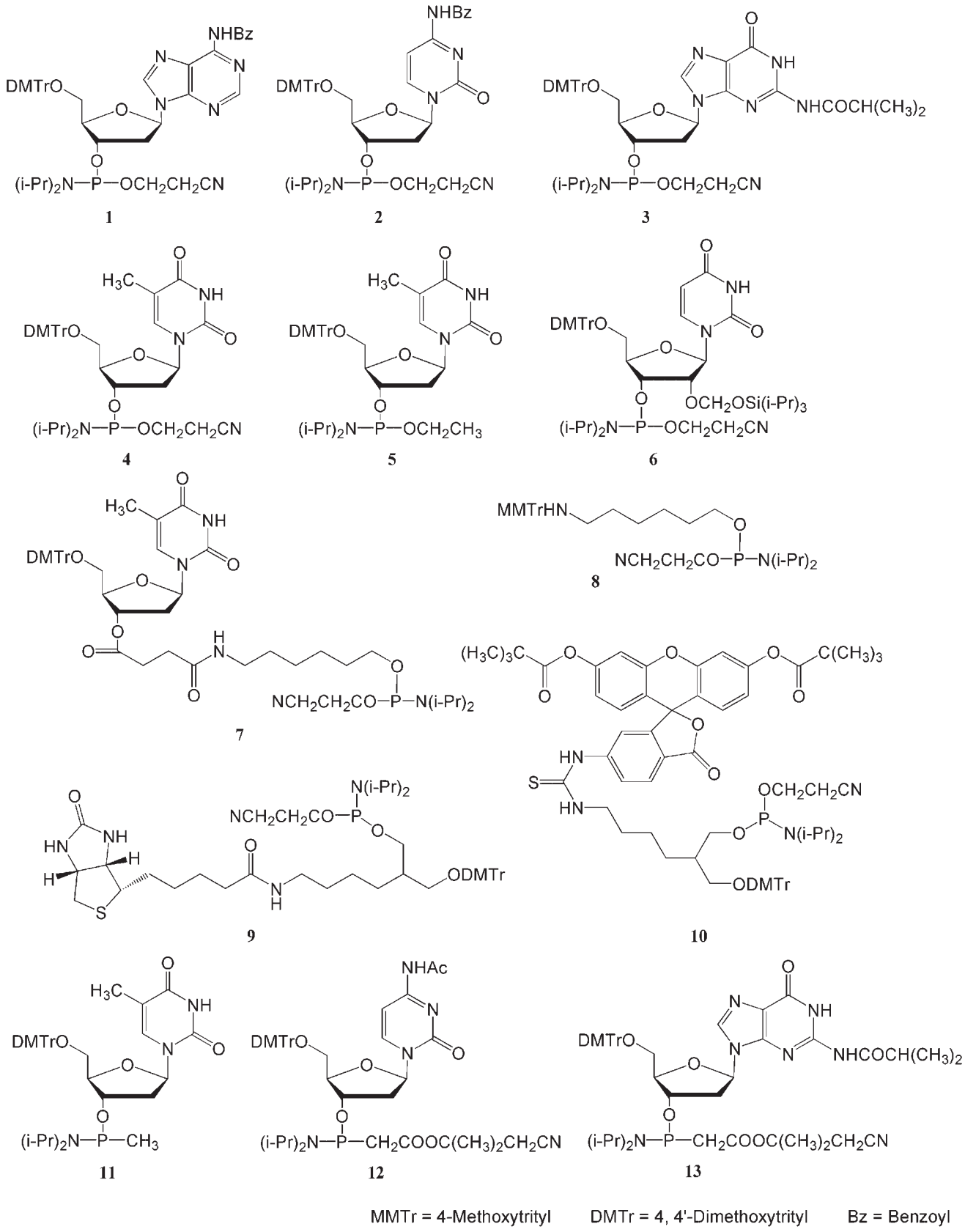

Figure 1. Structures of phosphoramidite compounds **1–13**.

in positive and negative ion mode, respectively. For these reference standards, 0.2–1 mM was found to be optimal (the range examined was 0.05–10 mM). At lower concentrations the signal-to-noise ratio was close to 1 which leads to low resolution and accuracy. The intensities of these reference peaks were also relatively low at 0.05–0.2 mM. On the other hand, concentrations higher than 1 mM did not provide improved results.

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Table 1. Optimal sample and reference concentrations, calculated, measured masses and accuracies for accurate mass analyses of compound **1–13** phosphoramidites

		Concentration (mM)			Masses		
No.	Standard	Sample	Standard	Ions	Calculated	Measured	ΔM (ppm)
1	PEG	1	1	$[M+Li]^+$	864.3821	864.3853	3.7
	PDE	10	1			864.3779	−4.9
	PPG	10	1			864.3824	0.3
	PEG	1	1	$[M+Cl]^-$	892.3359	892.3365	0.7
	PDE	—	—			[b]	—
	PPG	10	1			892.3363	0.4
2	PEG	10	1	$[M+Li]^+$	840.3709	840.3731	2.6
	PDE	10	1			840.3744	4.2
	PPG	20	1			840.3698	−1.3
	PEG	10	1	$[M+Cl]^-$	868.3247	868.3254	0.8
	PDE	—	—			[b]	—
	PPG	10	1			868.3265	2.1
3	PEG	10	1	$[M+Li]^+$	846.3926	846.3926	0
	PDE	10	1			846.3925	−0.1
	PPG	5	1			846.3936	1.2
	PEG	0.05	1	$[M+Cl]^-$	874.3465	874.3455	−1.1
	PDE	0.05	1			874.3478	1.5
	PPG	10	1			874.3463	−0.2
4	PEG	1	1	$[M+Li]^+$	751.3443	751.3438	−0.7
	PDE	10	1			751.3442	−0.1
	PPG	10	1			751.3448	0.7
	PEG	0.2	1	$[M+Cl]^-$	779.2982	779.2972	−1.3
	PDE	0.1	1			779.2978	−0.5
	PPG	1	1			779.2980	−0.3
5	PEG	30	1	$[M+Li]^+$	726.3490	726.3499	1.2
	PDE	10	1			726.3474	−2.2
	PPG	10	1			726.3496	0.8
	PEG	2	1	$[M+Cl]^-$	754.3029	754.3020	−1.2
	PDE	—	—			[b]	—
	PPG	10	1			754.3034	0.7
6	PEG	10	1	$[M+Li]^+$	939.4676	939.4675	−0.1
	PDE	10	1			939.4685	1.0
	PPG	10	1			939.4687	1.2
	PEG	0.2	1	$[M+Cl]^-$	967.4214	967.4225	1.1
	PDE	0.1	1			967.4243	3.0
	PPG	10	1			967.4213	−0.1
7	PEG	10	1	$[M+Li]^+$	950.4652	950.4627	−2.6
	PDE	10	1			950.4625	−2.8
	PPG	10	1			950.4665	1.4
	PEG	0.2	1	$[M+Cl]^-$	978.419	978.4184	−0.6
	PDE	0.2	1			978.4186	−0.4
	PPG	5	1			978.4184	−0.6
8	PEG	10	0.2	$[M+Li]^+$	596.3588	596.3585	−0.5
	PDE	10	1			596.3611	3.9
	PPG	10	0.2			596.3570	−3.0
	PEG	10	1	$[M+Cl]^-$	624.3127	624.3109	−2.9
	PDE	—	—			[b]	—
	PPG	10	1			624.3135	1.3
9	PEG	10	1	$[M+Li]^+$	882.4576	882.4592	1.8
	PDE	10	1			882.4591	1.7
	PPG	10	1			882.4558	2.0
	PEG	2	1	$[M+Cl]^-$	910.4114	910.4096	−2.0
	PDE	1	1			910.4110	−0.4
	PPG	1	1			910.4081	−3.6
10	PEG	—	—	$[M+Li]^+$	1213.5308	[a]	—
	PDE	50	0.5			1213.5304	−0.3
	PPG	50	0.5			1213.5267	−3.4
	PEG	—	—	$[M+Cl]^-$	1241.4846	[a]	—
	PDE	0.05	1			1241.4845	−0.1
	PPG	0.5	1			1241.4819	2.2

(*Continues*)

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Table 1. (Continued)

No.	Standard	Concentration (mM)		Ions	Masses		ΔM (ppm)
		Sample	Standard		Calculated	Measured	
11	PEG	10	1	$[M+Li]^+$	696.3385	696.3373	−2.3
	PDE	10	1			696.3415	4.3
	PPG	10	1			696.3392	1.0
	PEG	10	1	$[M+Cl]^-$	724.2923	724.2898	−3.5
	PDE	—	—			[b]	—
	PPG	10	1			724.2891	−4.4
12	PEG	10	1	$[M+Li]^+$	848.3971	848.3956	−1.8
	PDE	10	1			848.3974	0.4
	PPG	10	1			848.3949	−2.6
	PEG	1	1	$[M+Cl]^-$	876.3509	876.3513	0.5
	PDE	0.2	1			876.3533	2.7
	PPG	0.5	1			876.3536	3.1
13	PEG	5	1	$[M+Li]^+$	916.4345	916.4306	−4.3
	PDE	10	1			916.4344	−0.1
	PPG	10	1			916.4340	−0.5
	PEG	0.5	1	$[M+Cl]^-$	944.3884	944.3867	−1.8
	PDE	0.02	1			944.3884	0
	PPG	0.5	1			944.3839	−4.8

Each row represents an analysis showing the type of standard, the optimal concentration applied, and the calculated and measured masses with the accuracy.

[a]In the case of compound **10**, using PEG standard, a reference peak overlapped with the analyte peak; therefore, the accurate mass could not be determined.

[b]Signal intensities were too low to determine the accurate masses.

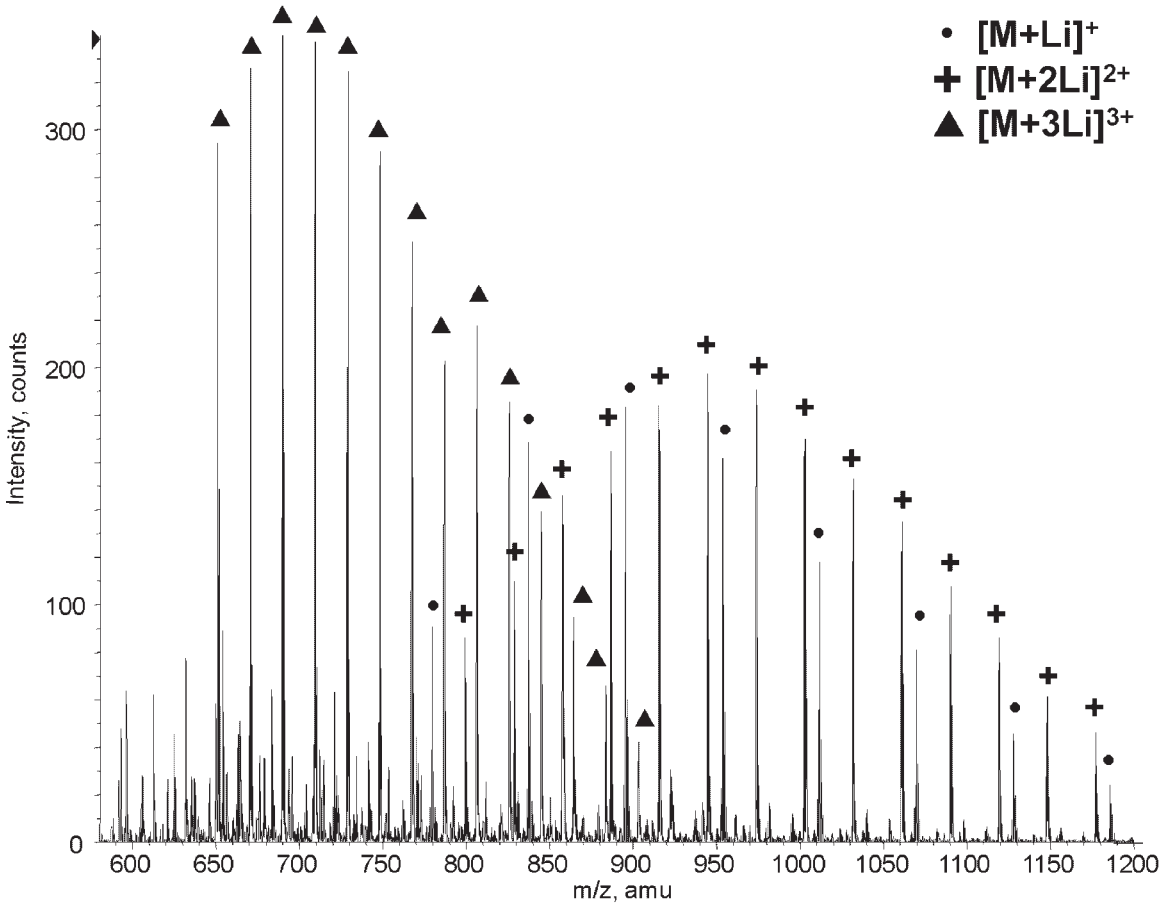

Figure 2. Spectrum of PPG reference standard with LiCl in acetonitrile; C(PPG): 1 mM; LiCl-content: 10 × d.

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ESI-MS measurement of phosphoramidite samples

Ten nucleoside and 2′-deoxynucleoside phosphoramidites and three non-nucleoside phosphoramidites (5′-amino modifier, biotin and fluorescein containing phosphoramidites, compounds **8**, **9**, **10**) were chosen in order to test the applicability of the ESI-MS approach to the determination of accurate mass (Fig. 1). The nucleosides included 2-cyanoethyl phosphoramidites of 2′-deoxyadenosine, 2′-deoxycytidine, 2′-deoxyguanosine, 2′-deoxythymidine, 2′-*O*-triisopropylsilyloxymethyluridine, and the 3′-*O*-succinyl hexamide 2′-deoxythymidine (compounds **1**–**4**, **6**, **7**). Also examined were a 3′-*O*-ethylphosphoramidite of 2′-deoxythymidine (compound **5**) and three phosphinoamidites containing oxidation-sensitive P–C bonds (one methylphosphinoamidite and two 3′-*O*-(diisopropylamino) phosphinoacetic acid 1,1-dimethylcyanoethyl esters of 2′-deoxynucleosides, compounds **11**, **12** and **13**). LiCl previously optimized at 100 × d was used as the final solution in all cases. The polymeric reference standards were 1 mM (0.2 mM was applied when sample intensities were too low even when using higher sample concentrations). Starting concentrations of phosphoramidites were 10 mM. When the intensities of polymeric standard and analyte signals were not in the same range, the sample concentration was modified to 0.05–50 mM. Optimal concentrations were found by 1–3 injections for both positive and negative ionization modes. Optimal conditions were also found for PEG, PDE and PPG. In a few cases, when the PDE reference standard was applied, the signal intensities were too low to determine the accurate masses even with several sample concentrations. For compound **10**, the analyte peak overlapped with the reference signal for PEG as the

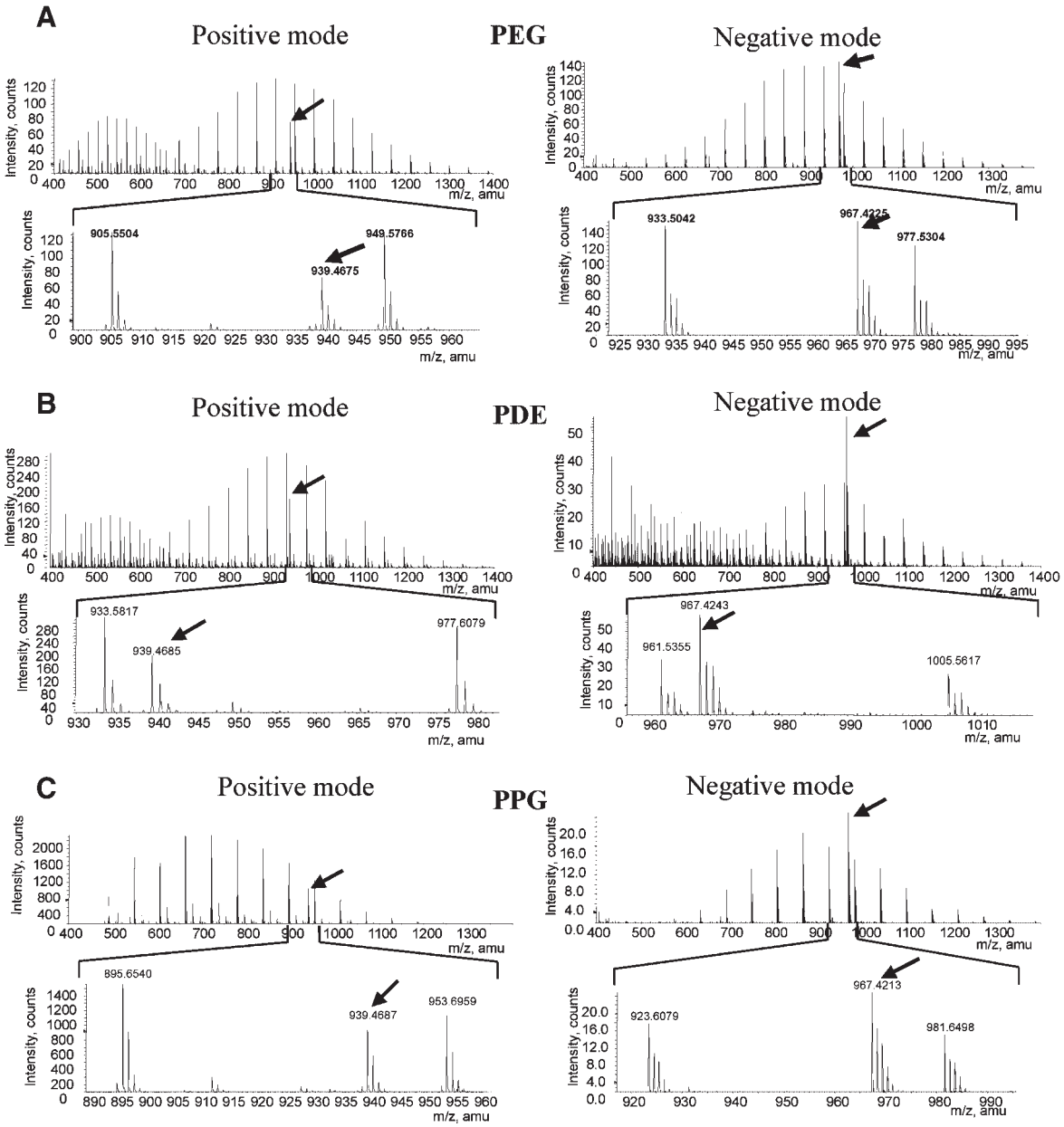

Figure 3. Mass spectra of compound **6**. Reference standards: PEG (A), PDE (B); and PPG (C). Arrows show the peak of the analyte.

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molecular weights (MWs) are almost identical. Generally, the range of sample MWs were 589 to 1206; therefore, reference polymer mixtures having 1000 as the average MW were used. In the case of compound **8** (MW: 589), the reference standard MWs were 600 or 725. Typical spectra can be seen in Fig. 3 with compound **6** as analyte. The two nearest reference peaks were used for mass calibration. The best concentrations of each phophoramidite with different standards, the theoretical and measured molecular weights, and accuracies are shown in Table 1.

During optimization of analyte and polymeric reference concentrations, our aim was to find an approximate 1:1 ratio for the analyte/reference signal intensity. In most cases the ratio was between 1:0.5 and 1:2, which is suitable for accurate mass determination. Occasionally, ratios of 5:1 to 1:5 were observed. Optimal sample concentrations were dependent on the applied reference standard and the ionization mode (Table 1). For PPG very similar sample concentrations were found as optimal for both the positive and negative mode. In contrast with PEG in the negative mode, approximately five to ten times lower concentration was necessary when compared to the positive detection mode. In order to obtain comparable signal intensities with the PDE standard, lower concentrations (10–50×) were used in the negative ion mode but, in the positive mode, concentrations were similar to those used for PPG and PEG. These results suggest that the formation of $[M+Cl]^-$ ions from PEG and PDE standards was suppressed which did not occur with PPG. On the other hand, in the positive mode, no significant differences were observed in signals among these reference standards. Although there were small differences between the optimal concentrations for the samples, no relation was found between the phosphoramidite structures and the optimized concentrations.

Table 2. Reproducibility studies using compound **4** with PEG reference standard

Ionization mode	Measured *m/z*	ΔM (ppm)	Average error (ppm)	SD (ppm)
Positive	751.3420	−3.1	1.8	1.21
	751.3424	−2.5		
	751.3426	−2.3		
	751.3441	−0.3		
	751.3438	−0.7		
Negative	779.2969	−1.7	1.3	1.51
	779.2997	1.9		
	779.299	1.0		
	779.2989	0.9		
	779.2974	−1.0		

SD: standard deviation.

As we used a quadrupole time-of-flight (Q-TOF) instrument, we did not expect the same high accuracy and reproducibility as observed when using a double focusing sector instrument. However, the mass accuracies varied by less then 5 ppm and mostly below 3 ppm when compared to the calculated masses. The average mass error and standard deviation were 1.7 and 2.2 ppm, respectively. There were no significant differences in standard error between the three references and also the positive and negative detection modes. To test the reproducibility of this procedure,

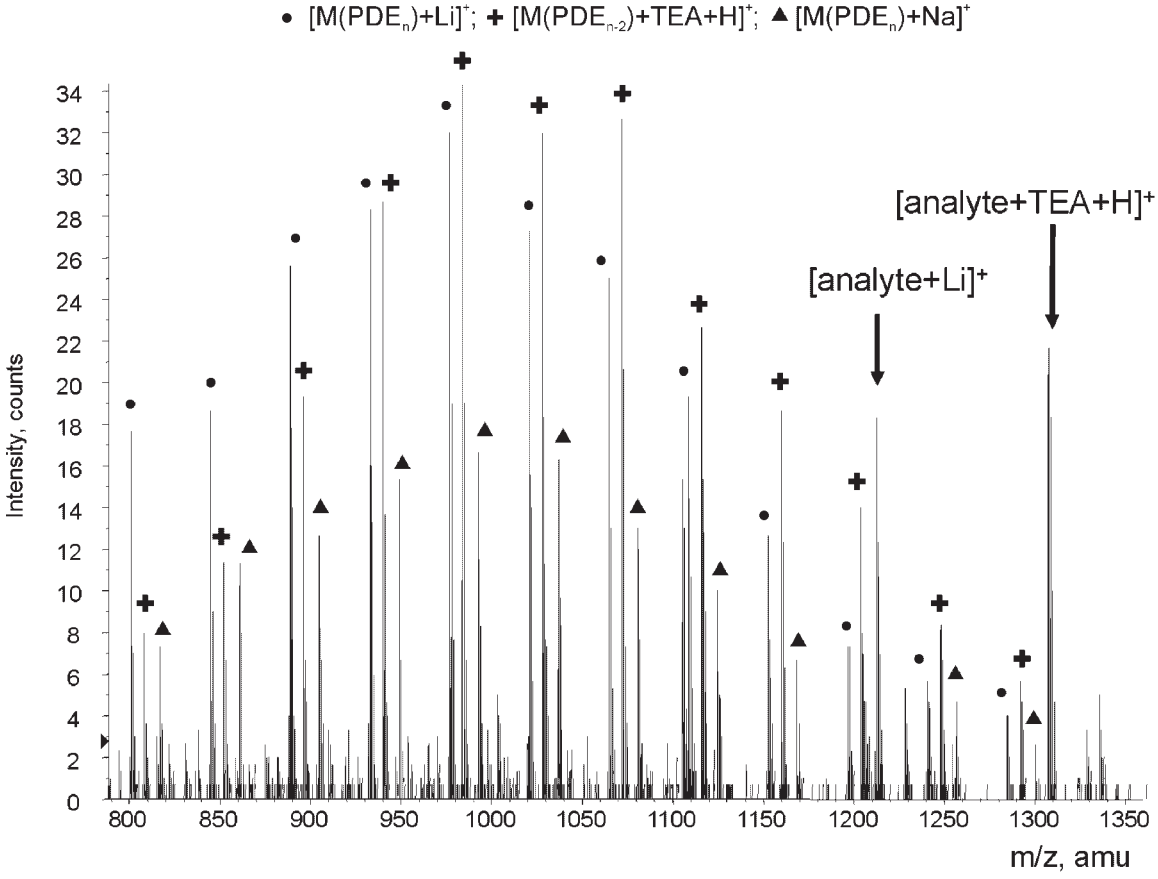

Figure 4. Effect of TEA and Na^+ impurities (analyte: compound **10**; reference standard: PDE).

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compound **4** was injected and measured consecutively five times using the PEG standard and previously optimized concentrations in the positive and negative detection modes. Data in Table 2 show relatively good reproducibility. The average errors and standard deviations were less than 2 ppm in both positive and negative ion modes.

Other observed effects

Because we applied the lowest possible LiCl concentrations, $[M+Na]^+$ ions were sometimes detected near $[M+Li]^+$ ions in the positive mode, mainly when molecular sieves were used to dry solvents. Noteworthy, the use of molecular sieves for drying saturated LiCl/acetonitrile solutions eliminated almost completely the $[M+Li]^+$ adduct from the spectra. Presumably this was due to the ion-exchange properties of molecular sieves. Therefore, other drying agents and procedures were used for drying solvents (e.g. acetonitrile distillation from phosphorus pentoxide).

Another interesting adduct-ion series contained protonated triethylamine (TEA) instead of Li^+. $[M+TEA+H]^+$ ions were observed in some samples in the positive detection mode for not only the analyte peak, but also the polymeric reference signals. These ghost-peak signals sometimes had comparable intensities to $[M+Li]^+$ peaks (Fig. 4). The mass difference between $[TEA+H]^+$ and Li^+ is 95, but the difference between the two nearest reference peaks is 44 and 58 for PEG/PDE or PPG, respectively. Therefore, the observed *m/z* differences compared to the nearest reference peaks were +7 and +37 for PEG/PDE and PPG, respectively. The main reason for TEA in the samples is that this amine is usually applied in the eluent during the chromatographic purification process in order to avoid decomposition of acid-sensitive compounds on silica gel. After evaporation, traces of TEA may remain in the product. It can be avoided by using pyridine instead of TEA to neutralize the chromatography column before purification of the phosphoramidite.

Applying Li^+ as an ion-forming agent in the positive mode has another advantage. The natural Li isotopic distribution is 8% of ^6Li and 92% of ^7Li, thus it can be used as a marker for Li-containing peaks in analysis of the mass spectra. Among the elements found in most organic compounds, only boron has a similar 'm – 1' isotope peak (with 25% intensity) when compared to the most intense isotope signal. Therefore, mass spectrometric analysis of simple organic compounds, including phosphoramidites (which do not contain boron or any transition metal elements), by determining Li^+-containing peaks is very easy.

CONCLUSIONS

Based on the LiCl/acetonitrile method, a simple accurate mass determination procedure was developed for analyzing highly acid-sensitive phosphoramidites. Three different polymeric reference standards (PEG, PDE and PPG) were applied and the LiCl/sample/reference concentrations were optimized for thirteen different phosphoramidites. These phosphoramidites were selected so as to contain many different structural elements including nucleosides with ribose or 2′-deoxyribose and having various protecting groups on nucleobases and sugars, several phosphorous modifications (ethyloxy, cyanoethoxy, methylphosphinoamidite, acetic acid phosphinoamidites) and also fluorescent dye or biotin moieties. In all cases, the analyses were performed successfully for both positive and negative detection modes with comparable reference and analyte signals as Li^+ and Cl^- adducts. The accuracy of the measurement had a standard deviation usually less than 3 ppm with excellent reproducibility (±2.2 ppm). Potentially challenging effects, as observed during the analyses, were also investigated. For example, TEA was found as an interfering impurity in the samples. We could see no significant differences in the sensitivity and accuracy among the compounds, which means the success of the analyses did not depend on the phosphoramidite structure. All of these results suggest that this method is generally applicable, convenient and a powerful tool for analyzing acid-sensitive phosphoramidites.

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REFERENCES

1. Caruthers MH, Barone AD, Beaucage SL, Dodds DR, Fisher EF, McBride LJ, Matteucci M, Stabinsky Z, Tang JY. *Methods Enzymol.* 1987; **154**: 287.
2. Caruthers MH, Beaton G, Wu JV, Wiesler W. *Methods Enzymol.* 1992; **211**: 3.
3. Beaucage SL, Caruthers MH. *Tetrahedron Lett.* 1981; **22**: 1859. DOI: 10.1016/S0040_4039(01)90461_7.
4. Venkatesan N, Kim SJ, Kim BH. *Curr. Med. Chem.* 2003; **10**: 1973; DOI: 10.2174/0929867033456909.
5. Beigelman L, Matulic-Adamic J, Karpeisky A, Haeberli P, Sweedler D. *Methods Enzymol.* 2000; **317**: 39.
6. Connolly BA, Rider P. *Nucleic Acids Res.* 1985; **13**: 4485. DOI: 10.1093/nar/13.12.4485.
7. Zuckermann R, Corey D, Schultz P. *Nucleic Acids Res.* 1987; 5305. DOI: 10.1093/nar/15.13.5305.
8. Sproat BS, Beijer B, Rider P, Neuner P. *Nucleic Acids Res.* 1987; **15**: 4837. DOI: 10.1093/nar/15.12.4837.
9. Dreyer GB, Dervan PB. *Proc. Natl. Acad. Sci. USA* 1985; **82**: 968. DOI: 10.1073/pnas.82.4.968.
10. Durand M, Chevrie K, Chassignol M, Thuong NT, Maurizot JC. *Nucleic Acids Res.* 1990; **18**: 6353. DOI: 10.1093/nar/18.21.6353.
11. Ruth JL, Morgan C, Pasko A. *DNA: J. Mol. Cell. Biol.* 1985; **4**: 93.
12. Takeshita M, Chang CN, Johnson F, Will S, Grollman AP. *J. Biol. Chem.* 1987; **262**: 10171.
13. Kele Z, Kupihár Z, Kovács L, Janáky T, Szabó PT. *J. Mass Spectrom.* 1999; **34**: 1317. DOI: 10.1002/(SICI)1096_9888(199912)34:12<1317::AID_JMS886>3.0.CO;2_S.
14. Jiang LF, Moini M. *Anal. Chem.* 2000; **72**: 20. DOI: 10.1021/ac990777e.
15. Takahashi Y, Fujimaki S, Kobayashi T, Morita T, Higuchi T. *Rapid Commun. Mass Spectrom.* 2000; **14**: 947. DOI: 10.1002/(SICI)1097_0231(20000615)14:11<947::AID_RCM970>3.0.CO;2_N.
16. Fujitake M, Harusawa S, Araki L, Yamaguchi M, Lilley DMJ, Zhao ZY, Kurihara T. *Tetrahedron* 2005; **61**: 4689. DOI: 10.1016/j.tet.2005.02.036.
17. Paul G, Winnik W, Hughes N, Schweingruber H, Heller R, Schoen A. *Rapid Commun. Mass Spectrom.* 2003; **17**: 561. DOI: 10.1002/rcm.952.
18. Cody RB, Tamura J, Musselman BD. *Anal. Chem.* 1992; **64**: 1561. DOI: 10.1021/ac00038a012.
19. Kupihár Z, Timár Z, Dellinger DJ, Caruthers MH. Accurate mass analysis of phosphoramidites by electrospray mass spectrometry. *16th Int. Round Table Nucleosides, Nucleotides and Nucleic Acids 2004*; P81, 12-16 September 2004, Minneapolis, Minnesota, USA