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High-throughput biopolymer desalting by solid-phase extraction prior to mass spectrometric analysis

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Abstract

In the last 10 years mass spectrometry (MS) has become an important method for analysis of peptides, proteins and DNA. It was recently utilized for accurate high-throughput protein identification, sequencing and DNA genotyping. The presence of non-volatile buffers compromises sensitivity and accuracy of MS biopolymer analysis; it is essential to remove sample contaminants prior to analysis. We have developed a fast and efficient method for desalting of DNA oligonucleotides and peptides using 96-well solid-phase extraction plates packed with 5 mg of Waters Oasis® HLB sorbent (Waters, Milford, MA, USA). This reversed-phase sorbent retains the biopolymer analytes, while non-retained inorganic ions are washed out with pure deionized water. DNA oligonucleotides or peptides are eluted using a small amount (20–100 μl) of acetonitrile–water (70:30, v/v) solution. The SPE desalting performance meets the requirements for MS applications such as protein digest analysis and DNA genotyping.

Keywords: Sample preparation; Desalting; Oligonucleotides; Peptides

1. Introduction

Mass spectrometry (MS) has become an indispensable tool for analysis of biopolymers such as DNA oligonucleotides, peptides and proteins. Both electrospray ionization (ESI) MS and matrix-assisted laser ionization/desorption time-of-flight (MALDI-TOF) MS techniques have been most commonly used for protein identification [1–5] in biological research. Typically, the proteins are separated using two-dimensional gels, extracted from the gel, and digested by protease. The resulting peptide mixture is analyzed by MS, and obtained molecular masses are compared with the theoretical fragmentation pattern of known proteins published in specialized databases. The acquisition of high quality MS spectra dramatically increases the chances for unambiguous protein identification. MS is also used for de novo protein and oligonucleotide sequencing [6,7–10].

In the last 5 years MS has become increasingly utilized for finding and identifying genetic variances in DNA [11–15]. The understanding of genetic variances is a key to rational drug development, since the therapeutic effect, metabolism and side effects of drugs are related to an individual's genetic makeup. It is expected that rapid advance in the field of pharmacogenomics, genetic variances discovery, and genotyping diagnostics ensures the development of new diagnostic procedures and therapeutic products. They will enable selective drug prescription to the specific groups of patients, for whom they are safe and effective [16].

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Genetic variances can be detected either on a DNA/RNA or protein level. Advanced biochemical tools for handling and amplification of DNA, such as polymerase chain reaction (PCR), as well as advances in high-throughput DNA sequencing make the search for variances in DNA structure more convenient than a complex analysis of proteome.

The most frequent genetic variation is a single nucleotide change in DNA structure known as a single nucleotide polymorphism (SNP). There has been a growing interest in the development of technologies for detection and discovery of SNPs [17–19]. A well-constructed SNP map will find application in many fields including molecular diagnostics, clinical genetic testing, population genetics, and drug development.

Many techniques for SNP detection utilize chip-based oligonucleotide arrays [20–22], flow cytometry of tagged beads [23,24], homogenous fluorescent assays [25–29], separation techniques such as capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) [30,31], as well as MS [12,14,15,32–35]. Although genotyping using oligonucleotide arrays offers the benefit of extremely high throughput, the genotype recognition by allele specific DNA hybridization is prone to false positive signals [17]. None of the currently available methods meets the requirements for routine genotyping: high throughput at low cost, and high accuracy.

The advantage of MS is its high accuracy. An unambiguous genotype assignment is made possible by accurate determination of the DNA probe molecular mass [12,14,15,32–35]. Although MS genotyping is performed in serial fashion (analysis of one sample requires several seconds), current progress in instrumentation development and possibility of sample multiplexing [14] increases sample throughput. Recent reports indicate that MALDI-TOF-MS has the potential to meet the above mentioned requirements [12,14,15,33–35]. In addition to SNP detection, MALDI-TOF-MS (and ESI-MS) is also capable of de novo SNP discovery. However, the MS analysis of samples such as protein/DNA enzymatic digests, sequencing reactions, and PCR reactions is compromised in the presence of salts and other reaction mixture components. Sample clean-up is currently a bottleneck of high throughput MS biopolymer analysis.

Several methods have been reported for biopolymer sample purification prior to MS analysis. These include dialysis [36,37], ultrafiltration, size-exclusion [38,39], affinity purification [12,34,35,40–43], and solid-phase extraction (SPE) [15,44–46]. Affinity-based DNA purification employing a hybridization of target oligonucleotide to the complementary streptavidin–biotin anchored oligonucleotide was described [40,47]. Although it is an elegant and efficient method, it increases the cost of genotyping assay. Ultrafiltration and size-exclusion spin columns usually do not provide a sufficient degree of desalting for MS [38]. Dialysis allows for a high degree of desalting, but it is difficult and costly to automate.

Reversed-phase (RP) SPE is an efficient technique for both peptide and DNA oligonucleotide sample clean-up. With a few exceptions [46,48], the SPE devices offered on the market are available in relatively large format. The dilution associated with this method requires subsequent sample lyophilization, which is time-consuming and may present an additional problem (see below).

In this paper we describe the development of a rapid and effective RP-SPE method for DNA oligonucleotide and peptide desalting. The selection of the type of stationary phase was driven by three main requirements:

(i) The support must have minimal silanol activity to reduce cation-exchange retention mechanism, and therefore eliminate the secondary retention of Na^+ and K^+ cations and their possible coelution with the sample.

(ii) Samples should be eluted in a small elution volume. Sample volume should be compatible with MALDI-TOF-MS and ESI-MS requirements.

(iii) SPE performance should not be affected by sorbent drying during the extraction. The 96- or 384-well extraction plate will be used for high throughput sample clean-up; some wells may run dry during the extraction process.

96-Well Oasis® HLB extraction plates (Waters, Milford, MA, USA) were chosen for sample preparation. Oasis HLB is a reversed-phase resin based on a copolymer of divinylbenzene and *N*-vinylpyrrolidone. Due to its higher sorbent capacity compared to classical C_{18} silica sorbents, the extraction plates are available in low bed volume configurations. This reduces considerably the re-

quired elution volume, especially when using extraction devices packed with 10 and 5 mg of sorbent per well [49–51].

Due to its chemical composition the Oasis HLB sorbent exhibits interesting chromatographic properties. Highly hydrophobic divinylbenzene moiety provides the reversed-phase interaction necessary to retain analytes, while more hydrophilic *N*-vinylpyrrolidone increases the sorbent water wettability, and interacts with analytes by H-bonding mechanism. The water wettability preserves analyte retention even when the sorbent runs dry; high-performance is maintained even after the sorbent bed has been processed hours after SPE cartridge conditioning or sample loading [51].

In this work we utilized 1-ml Oasis HLB cartridges for method development. The oligonucleotide desalting method was directly transferred to a 96-well extraction plate.

2. Experimental

2.1. Materials

Triethylamine, 99.5%; glacial acetic acid, 99.99%; acetonitrile, HPLC grade; 3-hydroxypicolinic acid (HPA), ammonium citrate (AC), and ethylenediaminetetraacetic acid, 99+% (EDTA) were purchased from Aldrich (Milwaukee, WI, USA). Tris(hydroxymethyl)aminomethane (Tris), boric acid, trypsin, cytochrome *c*, and sodium chloride were purchased from Sigma (St. Louis, MO, USA). Water (18 MΩ cm) was deionized in the laboratory using the Milli-Q system (Millipore, Bedford, MA, USA). DNA oligonucleotides were obtained from Hybridon (Hybridon, Milford, MA, USA). Peptide mixture was prepared by digestion of cytochrome *c* with trypsin. 96-Well extraction plates and cartridges packed with Oasis HLB sorbent were obtained from Waters.

2.2. MALDI-TOF-MS analysis

The experiments were carried out on a PE-PerSeptive Biosystems Voyager DE Linear mass spectrometer (PE-PerSeptive Biosystems, Framingham, MA, USA) equipped with a 337 nm N_2 laser. All mass spectra were acquired in the positive ion mode with delayed extraction. The accelerating voltage was 20 kV with the grid voltage set at 92.5%. The delay time was 400 ns. The guide wire voltage was set at 0.1%. Typically 20 individual spectra were added to generate a spectrum.

Oligonucleotide samples dried in Eppendorf tubes were reconstituted in 5 μl of matrix solution; an aliquot of 0.6 μl of the solution was then deposited on a stainless steel target. The solvent was evaporated at room temperature. The matrix solution consisted of HPA and AC in acetonitrile–water (1:1). The concentrations of HPA and AC were 70 g/l and 0.04 *M*, respectively. Both HPA and AC were used without further purification. Prior to use matrix solution was treated with cation-exchange resin (400 mesh; Bio-Rad Labs., Hercules, CA, USA) in ammonium form.

2.3. ESI-MS analysis

Mass spectra were acquired using an LCT system from Micromass (Manchester, UK). For analysis of peptides the ESI-MS capillary voltage was set at 2.8 kV, sample cone, and extraction cone at 35 V. The spectra of oligonucleotides were acquired using capillary voltage 2.8 kV, sample cone 35 V, and extraction cone 5 V. Desolvation gas flow-rate was set at 520 and 310 l/h, respectively. Typically 25–30 individual spectra were added to generate a summed spectrum. Sample was desolved in 50–70% aqueous acetonitrile solution and infused at a flow-rate of 5 μl/min using the Pump 11 (Harvard Apparatus, Holliston, MA, USA).

2.4. HPLC analysis

Ion-pairing reversed-phase chromatography was carried out using the following Waters instruments: Model 515 pumps, a Model 2700 sample manager, a Model 996 photodiode array detector. Separation was carried out using a C_{18} HPLC MICRA NPS ODS-IIIE column, 53×4.6 mm, 1.5 μm non-porous particles (EIChrom, Darien, IL, USA). The column was operated at 35°C. The flow-rate was 0.7 ml/min with mobile phase A: 0.1 *M* triethylammomium acetate (TEAA), 0.5 m*M* EDTA, pH 7.0. Mobile phase B: 25% acetonitrile in 0.1 *M* TEAA, 0.5 m*M* EDTA, pH 7.0. The gradient consisted of three linear

Table 1
SPE procedure for biopolymer sample clean-up prior to MS analysis

	DNA oligonucleotides	Peptides
Equilibration	1 ml of acetonitrile	1 ml of acetonitrile
	1 ml of 0.1 *M* TEAA, pH 7	1 ml of 0.1% TFA
Sample load	0.1–1 ml of sample in 0.1*M* TEAA, pH 7	0.1–1 ml of sample in 0.1% TFA
Wash 1	2 ml of 0.1 *M* TEAA, pH 7	2 ml of 0.1% TFA
Wash 2	1 ml of deionized water	1 ml of deionized water
Elution	0.1 ml of 70% acetonitrile	0.1 ml of 70% acetonitrile

TFA=Trifluoroacetic acid.

steps: 0–30% B in 4 min, 30–40% B between 4 and 8 min, then fast ramp up to 100% B in 1 min.

2.5. SPE biopolymer sample clean-up prior to MS

SPE was performed using the vacuum manifold UniVac 3 from Whatman Polyfiltronics (Rockland, MA, USA) or the extraction plate manifold kit (Waters). Vacuum was regulated to maintain the flow-rate at 2–4 ml/min. We used extraction cartridges and 96-well extraction plates packed with 30, 10, and 5 mg of Oasis HLB resin (Waters). The extraction conditions for biopolymer purification are summarized in Table 1. Sample lyophilization was performed prior to MALDI-TOF-MS using the Savant Speedvac Plus SC210A (Forma Scientific, Marietta, OH, USA). Centrifugation was also used for sample elution from SPE using the Forma Scientific centrifuge, Model 5681 (Forma Scientific). Centrifugation force was set at 600 *g* for 30 s.

The oligonucleotide elution profiles were obtained by subsequent elution of analytes from SPE devices with small increments of mobile phase (70% acetonitrile in water). Eluted fractions were diluted to 1 ml with 0.1 *M* triethylammonium acetate buffer, pH 7 and the concentration of analyte was measured using a UV spectrophotometer. Other SPE conditions and buffer compositions are specified in Table 1.

3. Results and discussion

MS of oligonucleotides is sensitive to the presence of non-volatile sample contaminants, as demonstrated by Fig. 1. A 1-μ*M* solution of a 15-mer oligonucleotide was prepared in 5 μ*M*–50 m*M* sodium chloride solutions. A 1-μl volume of sample was mixed with 1 μl of MALDI matrix and 0.8 μl was spotted on the stainless steel target and analyzed by MALDI-TOF-MS. It is evident that higher Na^+ concentration suppresses the ionization efficiency of the oligonucleotides (Fig. 1). Moreover, sodium ions form sodium adducts with the polyanionic backbone of the oligonucleotides, which results in reduction of the signal/noise ratio (S/N) due to the splitting of the MH^+ peak into multiple signals, and causes difficulties in determining of the oligonucleotide molecular mass. The quality of the spectrum becomes acceptable only when sodium concentration drops below the 0.5 m*M* level (Fig. 1C).

A typical sample volume required for ESI-MS analysis is about 10–20 μl, and only ~1 μl is typically used for MALDI analysis. In order to improve sensitivity of MS, the sample is usually concentrated prior to analysis. The desalting methods such as dialysis, ultrafiltration, and gel filtration chromatography are principally not capable of sample concentration during the clean-up; the sample is usually concentrated by drying. Unfortunately, the evaporation concentrates the sample as well as trace levels of non-volatile contaminants. Fig. 2 demonstrates how the trace enrichment of sodium and potassium cations affects the MALDI-TOF-MS quality. A 10-pmol concentration of the oligonucleotide sample was dissolved in 200–800 μl of HPLC-grade water, lyophilized, and reconstituted in the MALDI matrix. As seen from Fig. 2B and C, the K^+ and Na^+ concentrations exceeded the tolerable level. According to conductivity measurements, HPLC-grade water contains a relatively high concentration of alkali metal salts (~100 μ*M*). For good performance of desalting methods the water quality is

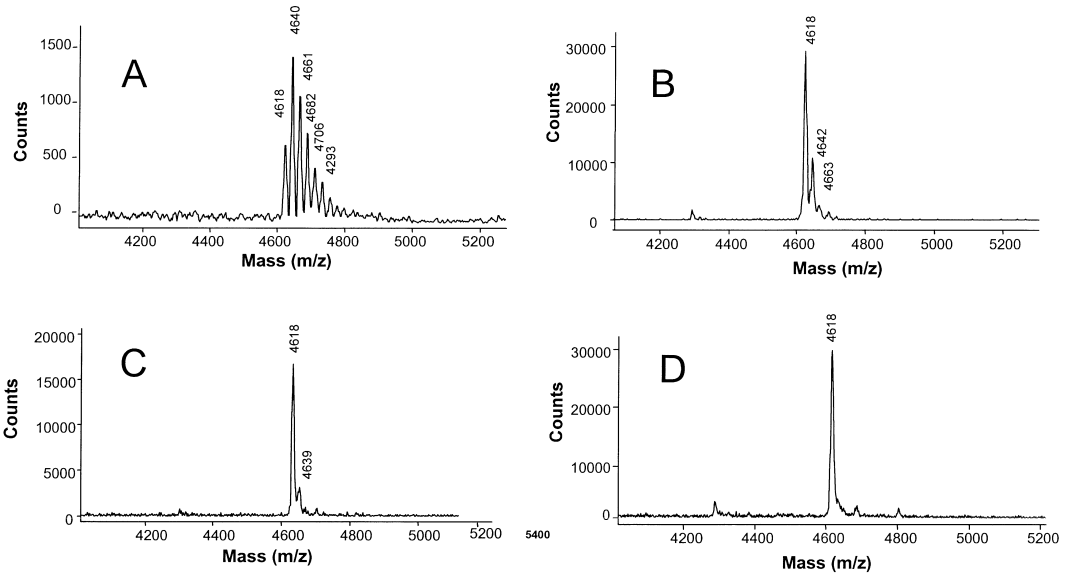

Fig. 1. Effect of sodium chloride concentration on the quality of 15-mer DNA oligonucleotide MALDI-TOF-MS signal. A 1-μ*M* solution of oligonucleotide was prepared in (A) 50 m*M* NaCl, (B) 5 m*M* NaCl, (C) 0.5 m*M* NaCl, (D) 5 μ*M* NaCl.

critical. When using deionized water, no significant increase in Na^+, K^+ adduct formation was observed upon sample lyophilization (data not shown).

Our goal was to eliminate the lyophilization step in order to improve the degree of desalting, simplify sample handling, and increase the sample throughput. RP-SPE is well suited for both sample desalting and concentration; elution volume can be significantly smaller than the volume of loaded sample. We evaluated the elution profiles of DNA using 96-well extraction plates packed with different amounts of Oasis HLB sorbent. After loading we eluted DNA sample with small increments of 70% acetonitrile in water (Fig. 3). Quantitation of DNA in eluted fractions was performed using a UV spectrophotometer; fractions were diluted to 1 ml and absorbance measured at 260 nm.

Similar to chromatography, the SPE sample elution volume decreases with the decrease of the sorbent bed volume (mass). A comparison of elution profiles of the 15-mer oligonucleotide from three different SPE devices is shown in Fig. 3. As expected, the broader elution profile was obtained for 30 mg extraction plate than for 5 and 10 mg plates (Fig. 3A). Elution profiles for wells packed with 5 and 10 mg appear to be similar; in this particular case the void volume of frits exceeds the volume of sorbent bed, which distorts the elution profile of the 5 mg plate. Approximately 400 μl of eluent is required for the elution of oligonucleotide from the 30 mg extraction plate. The elution volume is reduced to 100–150 μl in the case of 5 and 10 mg extraction plates (Fig. 3A).

In order to further minimize elution volumes we employed centrifugation for 30 s at 600 *g* for the removal of liquid remains trapped in the sorbent bed, and the frits. We used a centrifugation also for sample elution, which allowed for a convenient collection of small liquid volumes. In this experiment we used finer elution increments (20 μl) than it was possible for vacuum processed SPE. As a result, sharper elution profiles were obtained. The difference in elution profiles between 5 and 10 mg plates is clearly visible (Fig. 3B). Surprisingly, the absolute recovery of the oligonucleotide was significantly higher than with vacuum-driven SPE.

Cartridges and plates packed with a smaller amount of sorbent have lower mass capacity for sample load. We studied the capacity of the 5 mg cartridges for the extraction of 3–36-mer DNA oligonucleotides. The recovery was linear for the sample mass load in the range from 0 to 50 pmol of

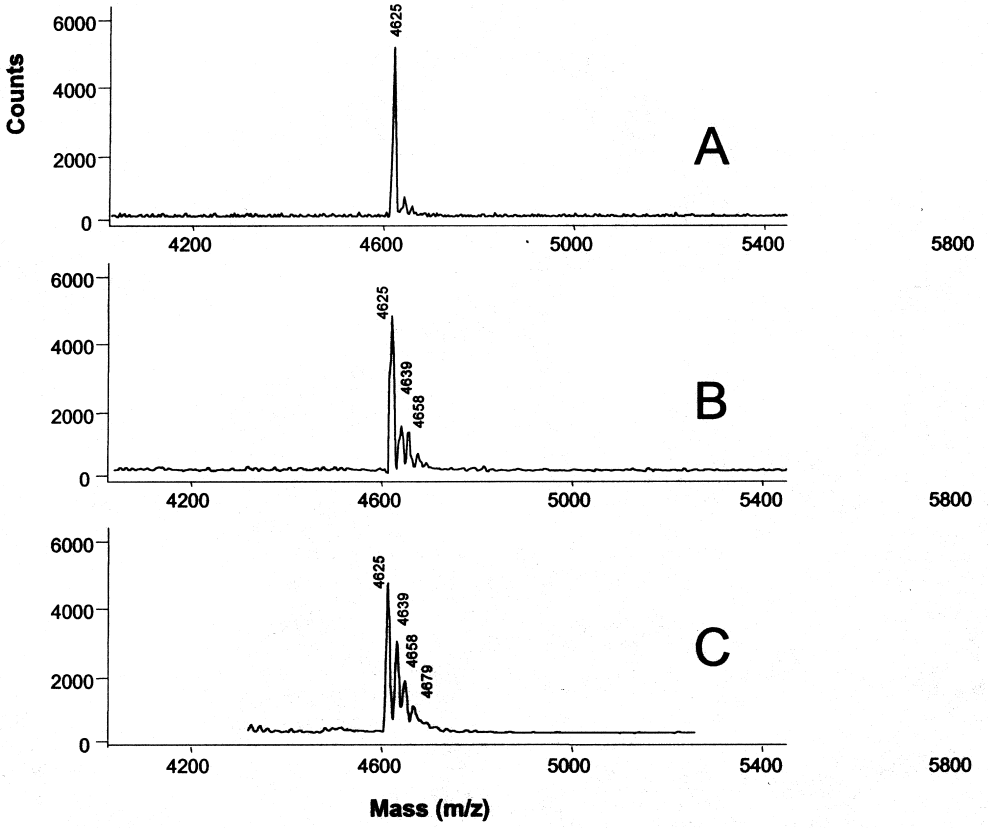

Fig. 2. Contamination of DNA sample by trace amounts of K^+ and Na^+ ions. A 3-pmol concentration of 15-mer DNA oligonucleotide was dissolved in corresponding volumes of HPLC-grade water (A) 200 μl, (B) 400 μl, (C) 800 μl. Samples were lyophilized to dryness and analyzed by MALDI-TOF-MS. Traces of alkali cations were concentrated by evaporation.

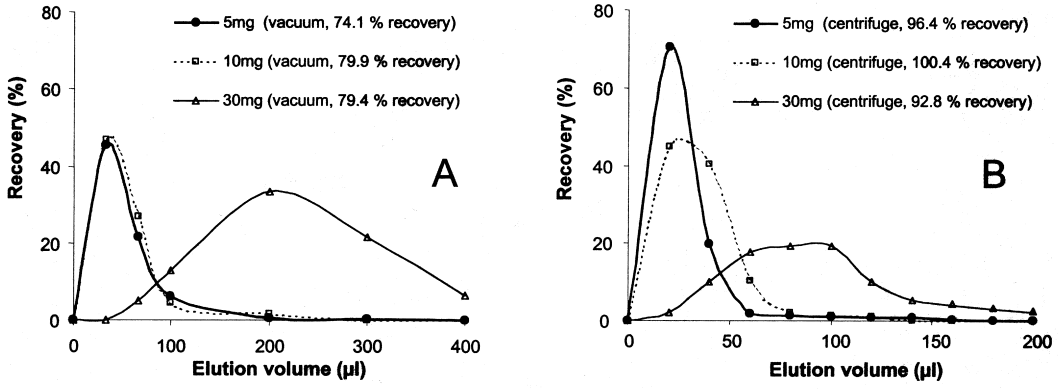

Fig. 3. Elution profile of 15-mer DNA oligonucleotide from extraction plates packed with 5, 10, and 30 mg of Oasis HLB sorbent. (A) Vacuum elution, 33.3 μl elution step; (B) elution of sample was performed by centrifugation at 600 *g* for 30 s, 20 μl elution step.

DNA (the data are not shown). We conclude that 5 mg of sorbent has sufficient capacity for purification of genotyping samples taking into account the typical mass load of oligonucleotide samples generated by PCR. A 1–20-pmol concentration of 3–40-mer DNA is typically used for MS analysis.

We evaluated the SPE desalting of DNA oligonucleotides under the conditions shown in Table 1. No size discrimination was observed for different fragment lengths. Recovery was rather uniform from 3-mer up to 36-mer oligonucleotides. Good quality of the MALDI-TOF-MS spectrum was obtained with SPE sample clean-up (Fig. 4); the direct analysis of identical sample prior to the SPE purification does not show any DNA signal. Fig. 4 shows no significant sodium adduct formation. The intensity of MS signal decreases with the length of oligonucleotide. In our current set-up we routinely detect ~1 pmol of the 35-mer oligonucleotide (after SPE desalting).

Due to the poor ionization efficiency of MALDI-TOF-MS for long DNA oligonucleotides (>60-mer), some genotyping methods were designed to generate short (3–10) DNA fragments. If dialysis (currently the most powerful desalting technique) is used for desalting of such samples, it may result in a significant loss of small oligonucleotides (Fig. 5). Dialysis for several hours (using 0.025 μm membrane, molecular mass cut-off 3000) is necessary for high quality desalting performance. Unlike dialysis, RP-SPE permits desalting of short DNA fragments without a loss of recovery (Fig. 5B).

ESI-MS is known to be less tolerant to the presence of non-volatile salts in the sample than MALDI-TOF-MS. Therefore we also evaluated the utility of SPE for biopolymer sample clean-up prior to ESI-MS. 0.1 mg of 25-mer phosphorothioate DNA oligonucleotide (M_r 7776) was dissolved in 1 ml of 20 m*M* NaCl, mixed 3:7 (v/v) with acetonitrile, and infused directly into the ESI-MS system (LCT, Micromass). No signal was observed (Fig. 6A). A 0.1-ml volume of the sample was desalted by RP-SPE using the following steps: 0.1 ml of sample was lyophilized prior to SPE sample clean-up, dissolved in 0.1 ml of 100 m*M* TEAA, pH 7, loaded onto an SPE cartridge, and processed according the method described in Table 1. The quality of MS spectra after RP-SPE sample clean-up was good (Fig. 6B).

Analysis of a peptide mixture prepared by digestion of cytochrome *c* by trypsin in 20 m*M* Tris–glycine buffer is shown in Fig. 7. Salt contaminants in the sample significantly reduce the *S*/*N* ratio; signal of some peptides cannot be distinguished from background noise (Fig. 7A). When RP-SPE desalting is used prior to ESI-MS analysis, all peptides can be clearly identified in the mass spectrum (Fig. 7B).

The 5 mg Oasis HLB cartridge was used to study how the resin performance is affected by drying. We found that sample recovery was practically un-

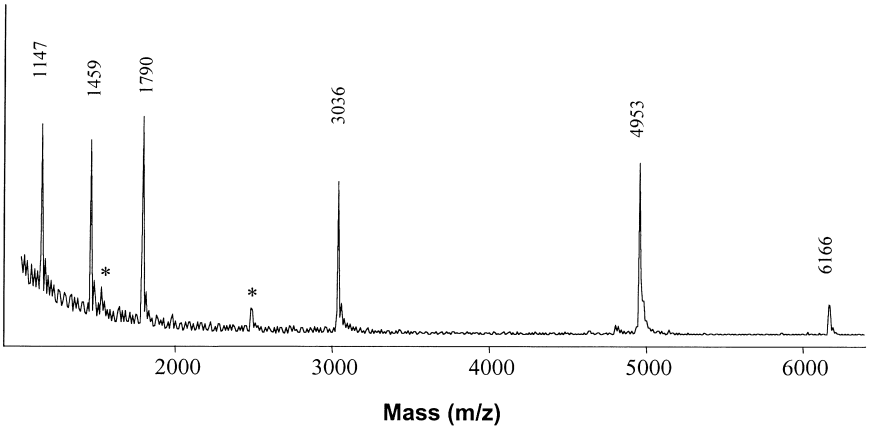

Fig. 4. 4-, 5-, 6-, 10-, 16-, and 20-mer DNA oligonucleotides (10 pmol of each) were desalted by SPE using the 5 mg Oasis HLB cartridge. Sample was eluted by vacuum in 150 μl of 70% acetonitrile, lyophilized and reconstituted in 10 μl of MALDI matrix. A 0.8-μl volume of sample was spotted onto the MALDI target for analysis. No sodium adducts were detected. Peaks labeled by an asterisk are signals of doubly charged 10- and 16-mer oligonucleotides.

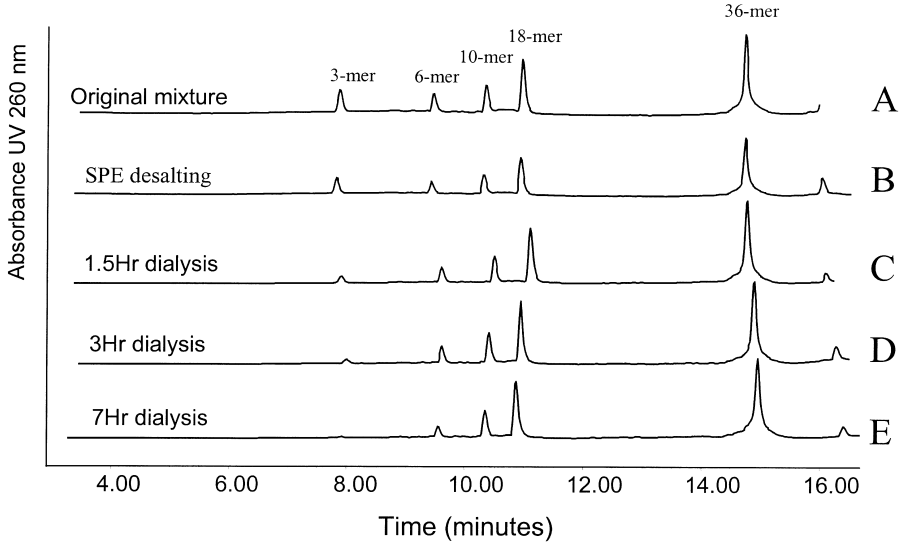

Fig. 5. Desalting of DNA oligonucleotides (3–36-mer). Compare original sample (A) with sample processed by RP-SPE (B), and dialysis (C–E). Losses of short oligonucleotides increase with the dialysis time.

changed by the sorbent drying under vacuum before the sample load (Fig. 8A). Similarly, the recovery of DNA was not affected by sorbent drying before the sample elution (Fig. 8B). DNA sample was eluted with 50 μl of mobile phase using a vacuum manifold. This experiment shows that the extraction procedure is rugged and the elution with small amount of mobile phase provides reproducible results.

We utilized centrifugation to significantly reduce

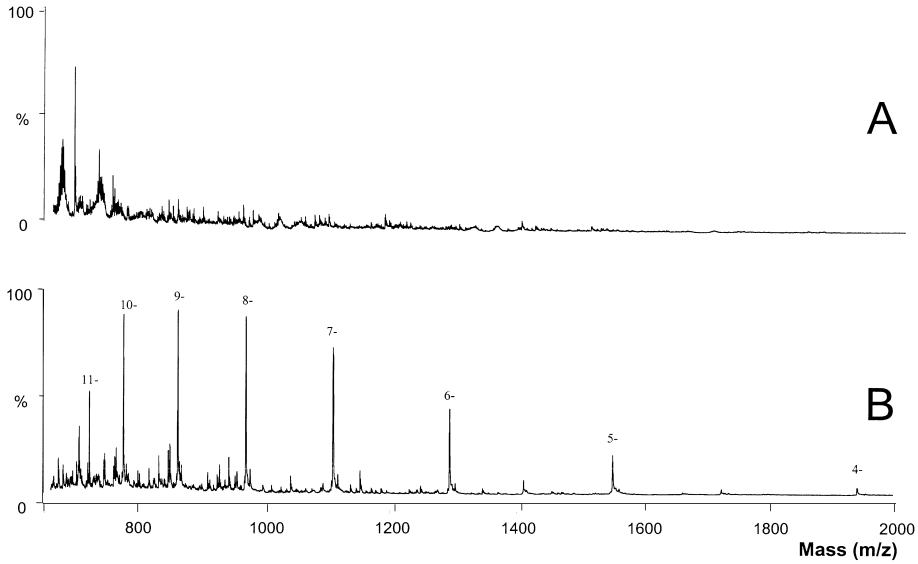

Fig. 6. ESI-MS analysis of 25-mer phosphorothioate DNA. Oligonucleotide solution was prepared in 20 m*M* NaCl and diluted with acetonitrile (3:7, v/v). (A) Direct infusion without desalting, (B) after SPE desalting. Sample was infused at the flow-rate 5 μl/min for 30 s. MS spectrogram was generated by adding 30 spectra. For other conditions see Experimental.

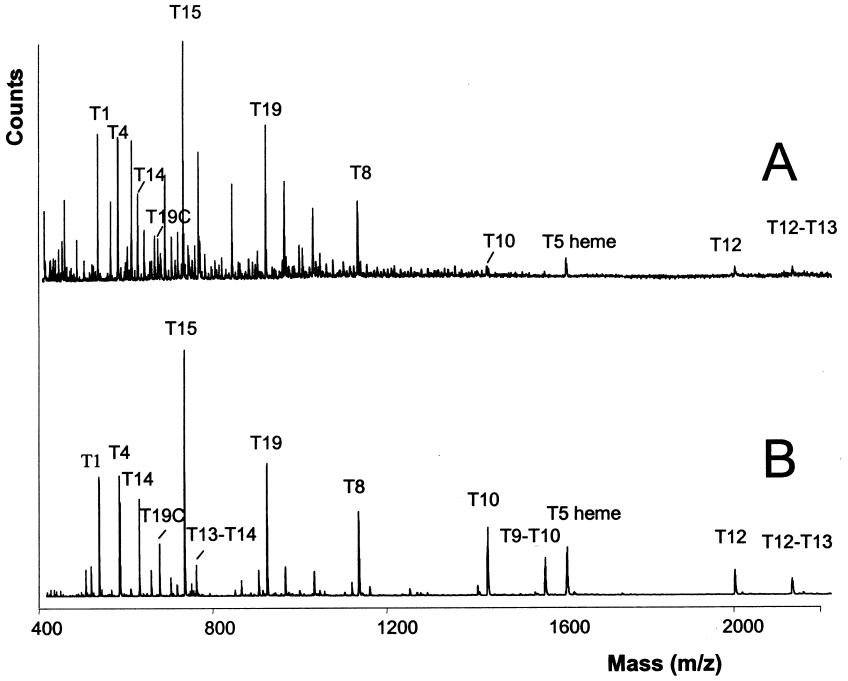

Fig. 7. Cytochrome *c* was digested with trypsin in 20 m*M* Tris–glycine buffer. (A) Peptide solution was diluted with acetonitrile (3:7, v/v), and directly infused into the ESI-MS system. (B) The same sample after RP-SPE desalting. Samples were infused at 5 μl/min for 30 s. MS spectrogram was generated by adding 30 spectra. Total mass of sample infused was equivalent to 10.9 pmol of digested cytochrome *c*. For other conditions see Experimental.

sample elution volume. Approximately 70% of adsorbed sample can be recovered from the 5 mg plate in as little as 20 μl of eluent (see Fig. 3). We also studied the possibility to use MALDI matrix instead of buffer for elution of oligonucleotides. 96-Well extraction plate packed with 5 mg of Oasis HLB sorbent was dried down with vacuum prior to elution. Centrifugation with 20 μl of MALDI matrix (comprising 50% acetonitrile) recovered approximately 10–15 μl of eluent. Eluent was mixed thoroughly and 0.8 μl was directly applied onto the MALDI target for MS analysis. This process completely eliminates the need for lyophilization step. MALDI signal intensity of oligonucleotides is com-

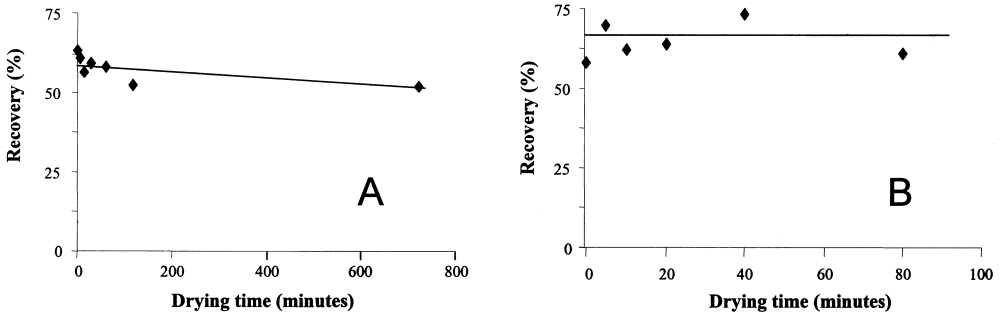

Fig. 8. 5 mg Oasis HLB cartridge extraction performance was not affected by sorbent drying under vacuum (A) before DNA oligonucleotide loading or (B) before DNA elution. Oligonucleotide was eluted with 50 μl of mobile phase; for other conditions see Table 1.

parable with the sample preparation comprising from vacuum elution with 20 μl of 70% acetonitrile, eluent lyophilization, and reconstitution in 10 μl of MALDI matrix.

Finally, we evaluated the performance of the 96-well Oasis HLB plate packed with 5 mg of resin for clean-up of 3 pmol of a 20-mer oligonucleotide sample. The number of samples processed was 960 (10 96-well plates); the averaged results are summarized in Table 2. Every plate (96 samples) gives us on average 92 good MALDI-TOF-MS signals. 1–3 samples per plate required extensive manual search through the MALDI spot to acquire an acceptable spectrum. A few samples (0–4 samples per plate) failed due to a prominent cation adduct formation. Surprisingly, we observed predominantly potassium cations. We believe that appearance of potassium–DNA adducts in MS spectra is due to the post-clean-up sample contamination. It is advisable to work in a dust-free environment, and to wash all polyethylene pipette tips with deionized water prior to manipulation with desalted samples.

The processing of 960 samples using single vacuum manifold takes approximately 2 h, followed by 1 h of lyophilization. When eluting samples by centrifugation (sample load and wash are still performed by vacuum) one can eliminate the lyophylization time, however, overall time saving is not significant. Assuming that the MALDI-TOF-MS data acquisition rate is 10–15 s per sample, the total MS data acquisition time for 960 samples is nearly equal to the sample preparation time. The results clearly demonstrate that the Oasis HLB 96-well extraction plates are useful for high-throughput sample desalting prior to MS analysis. Advances in sample preparation (384-well extraction plates, automation) will increase sample throughput in future.

Table 2
Desalting of DNA oligonucleotide using 96-well extraction plates packed with 5 mg of Oasis HLB

	No. of samples	% of samples
SPE processed samples	960	100
Good MS signal (automated acquisition)[a]	919	95.7
Low MS signal (manual search)[b]	20	2.1
Prominent K^+ ion adduct formation	21	2.2

Ten SPE plates were processed and results were averaged. Samples were analyzed by MALDI-TOF-MS. MALDI matrix was spiked with internal standards; mass accuracy with internal molecular mass calibration was within ±0.07%.

[a] Automated MALDI-TOF-MS acquisition comprises from 10 laser shots in two series. Spectra are collected when $S/N>15$ for target peak. Average MS signal was 1100±550 ion counts for target oligonucleotide.

[b] Automated MALDI-TOF-MS acquisition failed to acquire spectrum with $S/N>15$. Data with $S/N>15$ were recovered manually.

4. Conclusion

Single nucleotide polymorphism is the most frequent genetic variation. Therefore, the number of genotyping samples is expected to be very high. Current MS instrumentation is capable of performing several thousand analyses per day. The RP-SPE method was developed to comply with demand for high sample throughput. The desalting procedure utilizes the 96-well SPE format. Successful automation of purification procedure is made possible by the choice of Oasis HLB sorbent; a multi-parallel sample extraction using a vacuum can be performed without danger of retentivity and capacity loss even if sorbent bed runs dry. The quality of desalting meets the requirements for both MALDI-TOF-MS and ESI-MS. Extraction plates packed with 5 or 10 mg of sorbent per well permits the sample elution in a small volume, which eliminates excessive sample lyophilization. Elution from SPE can be performed with as little as 20 μl of eluent.

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