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Review

Advances in sample preparation in electromigration, chromatographic and mass spectrometric separation methods

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Abstract

The quality of sample preparation is a key factor in determining the success of analysis. While analysis of pharmaceutically important compounds in biological matrixes has driven forward the development of sample clean-up procedures in last 20 years, today's chemists face an additional challenge: sample preparation and analysis of complex biochemical samples for characterization of genotypic or phenotypic information contained in DNA and proteins. This review focuses on various sample pretreatment methods designed to meet the requirements for the analysis of biopolymers and small drugs in complex matrices. We discuss the advances in development of solid-phase extraction (SPE) sorbents, on-line SPE, membrane-based sample preparation, and sample clean-up of biopolymers prior to their analysis by mass spectrometry.

Keywords: Reviews; Sample preparation; Solid-phase extraction; Membranes; Dialysis; Desalting methods; Mass spectrometry; Peptides; Proteins; DNA

Contents

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1. Introduction

Separation techniques such as chromatography, electrophoresis, and mass spectrometry are well suited for the analysis of complex multi-component samples. However, the analysis of analytes present in matrixes such as plasma, serum, whole blood, tissue homogenates, saliva or urine requires well designed sample preparation procedures. In the early years of reversed-phase chromatography, attempts were made to inject such samples directly onto a HPLC column [1–3]. It was quickly realized that this approach generally resulted in a rapid deterioration of the column's separation performance, an increase of background interferences, and a dramatic increase in column backpressure [1,4,5]. In addition, the chromatographic sorbent selectivity may be altered by irreversible adsorption of matrix compounds such as proteins.

It is clear that some useful means of sample preparation is required to reduce the sample complexity. Methods for sample preparation include, but are not limited to protein precipitation [4], liquid–liquid extraction (LLE), solid-phase extraction (SPE), and membrane methods, e.g., dialysis, and ultracentrifugation [6,7]. Of these techniques, SPE has gained the widest acceptance due to the ease of automation, high analyte recovery, extraction reproducibility, ability to selectively increase analyte concentration, and commercial availability of SPE devices and sorbents. While traditional sorbents have been based on silica material, many innovative types of sorbents have been introduced in the last 10 years [8,9]. The new sorbents posses high capacity, high hydrophobicity, better retention of hydrophilic compounds in a reversed-phase mode, and water wettability [10,11]. In addition, a variety of new formats have been introduced to speed up and simplify the SPE procedure, as well as increase the sample throughput, recovery and extraction reproducibility. Among these innovations are: 96-well SPE plates designed for rapid parallel batch sample processing [12–15], 47 mm SPE disk designed for rapid processing of large volumes of environmental samples [14,16–22], and miniaturized devices for desalting of biopolymers prior to MS analysis [23,24].

One active area in sorbent chemistry research is the development of molecularly imprinted polymers tailored to adsorb a specific analyte [25–28].

The rapid adoption of MS detectors to replace the traditional UV detectors has had arguably the most dramatic impact on sample preparation demands. Non-specific UV detectors require baseline separation of analyte from interferences; which necessitates long analysis times in order to maximize chromatographic efficiency. Selective MS detectors reduce the separation demands; this allows the analyst to increase the analysis speed at the expense of chromatographic resolution. In recent years, the typical chromatographic run time has decreased from ~15 min to less than 5 min. In some instances, analysts forego the chromatographic separation altogether to increase throughput. For example, analysis of peptides or DNA oligonucleotides by MALDI-TOF-MS requires only 5–20 s per sample [29–31]. This increase in sample throughput requires development of parallel methods for sample preparation.

Rational drug design focuses on the identification of disease mechanisms. Insight into the human genome and proteome helps to identify target molecules responsible for disease development and progress. Massively parallel analytical techniques used in human genome and proteome studies (e.g., 96-channel CE and MALDI-TOF-MS) are capable of tremendous sample throughput. While advances have been made in the automation of sample preparation for these analyses, it still remains the step which limits throughput.

This review is divided into two sections. In the first part we discuss the general trends in sample preparation techniques. We discuss SPE methods, on-line column switching, direct biological sample

injection, and membrane sample preparation methods. The second part focuses onto selected applications, namely approaches to the biopolymer sample clean-up. We pay special attention to the sample preparation of peptides and DNA oligonucleotides for mass spectrometry, particularly the methods for sample desalting and surfactant removal prior to MS. Sample clean-up for environmental analysis is beyond the scope of this paper; this topic was reviewed recently [8].

2. Objectives and historical perspectives of sample preparation

Sample preparation for HPLC, CE or MS is a complex operation that often causes the largest variability in analytical results. Ideally, sample manipulation prior to analysis should be as simple as possible, i.e., consists of homogenization and/or internal standard addition. The direct injection of biological samples into HPLC, CE, or MS systems was studied in the past with moderate success [32–37]. Injection of samples with large amounts of protein constituents result in short column longevity, poor separation performance, and a rapid increase in column backpressure. In CE, proteins precipitate and adsorb irreversibly to the silanol groups of the internal capillary wall [38]. This causes a local disturbance in electroosmotic flow, which often leads to a complete deterioration of separation performance. Once the capillary is coated with denatured proteins, it becomes a source of secondary interaction with analytes resulting in severe tailing of analyte peaks. Direct injection of biological samples into a mass spectrometer will rapidly contaminate the system and cause electrospray instability and sensitivity reduction.

Objectives of sample preparation for analysis of components in biological fluids are: (i) removal of macromolecular contaminants (mainly proteins), (ii) removal of other interfering components from the sample, (iii) concentration of the analyte of interest (if possible), and (iv) matching the sample solvent to the appropriate HPLC, CE or MS mobile phases.

A widely used method is to precipitate proteins with organic solvents, inorganic salts, or strong acids [4,6]. However, this fast and simple method removes proteins with limited efficiency, and still results in fast column deterioration. Precipitation with organic solvents makes it difficult to match the solvent strength with mobile phase, and the use of strong acids is incompatible with pH labile analytes. Subsequent removal of precipitated proteins by high-speed centrifugation or filtration is difficult to automate. In addition, precipitation gives relatively high background of impurities in the chromatogram. The interfering peaks present a problem for trace analysis.

LLE is another traditional method for sample clean-up. The choice of extraction solvent, its polarity, and different distribution coefficient, K_d, for different analytes offers better selectivity in sample preparation than simple protein precipitation, particularly for non-polar analytes. On the other hand, LLE is a laborious, relatively slow process, and organic non-polar solvents must be evaporated prior to injection of sample for RP-HPLC or CE. Evaporation at elevated temperature (typically several ml of extract) is time consuming, and cannot be easily used for volatile and semi-volatile analytes. Also, LLE is of limited use for highly polar analytes, due to unfavorable K_d. Although LLE can be automated, it is generally not as simple and straightforward as automation of SPE.

In the last 5 years, SPE has become the most widely used sample preparation techniques. After the introduction of SPE in 1972 for clinical analysis [39,40], it was slowly adapted over the next several years as various manufactures made SPE devices more readily available. The current popularity of SPE can be explained by the fact that it can be used almost generically for extraction of wide variety of analytes. It is a relatively simple method consisting of four basic steps: conditioning, sample load, washing steps, and elution. Careful selection of the stationary phase and washing conditions ensures removal of the sample matrix interferences and $>$90% recovery of target analytes at the same time. Reversed-phase sorbents are particularly compatible with water-soluble biological samples. Solvents such as methanol or acetonitrile that are typically used for elution are compatible with the most common RP-HPLC mobile phases. The time required for SPE sample processing (1–5 min) is in the range of the typical HPLC or CE separation run time. If faster

sample throughput is required, parallel SPE processing is easy to perform. Several SPE manufacturers have developed 96-well SPE plates for rapid sample clean-up [41]. Some researchers are exploring ways to achieve even faster throughput with 384-well plates [42,43].

Since 1990, reversed-phase SPE has become the sample clean-up method of choice in many analytical laboratories. However, several drawbacks were identified using alkylated silica-based sorbents. These are: (i) the incompatibility of these stationary phases to extreme pH. (ii) Relatively low capacity and retentivity for polar compounds. (iii) Relatively high elution volume necessary for quantitative recovery of analyte. (iv) Low recovery of basic analytes. (v) Sample breakthrough when SPE sorbent accidentally runs dry after the equilibration step.

These drawbacks motivated the development of a new generation of SPE polymeric sorbents with extended pH stability, minimal shrinking and swelling, and most importantly, the use of a hydrophilic–lipophilic balanced chemistry of polymer [10,11]. This class of sorbents does not require equilibration prior to the extraction of analytes. Both the high surface area and the high hydrophobicity ensure better extraction of polar analytes and higher capacity (retentivity) of sorbents. These sorbents are particularly suitable as a medium for parallel processing in 96- or 384-well plate format because they can run dry without the loss of extraction performance [10]. The success of this type of specifically engineered sorbent has led to the commercialization of other similar products (Abselut Nexus, Varian, Palo Alto, CA, USA; Isolute ENV+, Hengoed, UK; XTrelut NT, Merck Eurolab, Darmstadt, Germany).

On-line methods of sample clean-up were studied extensively in order to automate extraction and sample enrichment. The development is driven by the desire to make sample preparation and analysis faster, safer and less prone to human error. Membrane-based extraction or dialysis is a useful method both for HPLC [44–47] and CE [38]. On-line SPE (term overlaps with column switching [6,7,48,49]) requires relatively simple instrumentation set-up, but method development and validation may be more challenging than in case of off-line SPE [36,50]. The introduction of restricted access media, the stationary phases compatible with direct injection of biological samples, made the development of on-line SPE methods easier [48,51,52], although on-column protein precipitation still may take place when organic solvents are used as a mobile phase constituents.

On-line methods for sample clean-up for HPLC have been examined extensively in the last 20 years [5,36,53–55]. Sample is injected onto a small trapping column containing ~40 μm sorbent. Analyte is trapped on a sorbent with the effluent from the column diverted to waste. After sufficient washing, the trapping column is switched back in the stream of running mobile phase. Analyte is eluted onto the analytical column where the separation of the analyte from residual interferences is performed. An important consideration in the successful operation of these trapping columns is the appropriate choice of sorbent particle size and column frits. If the particle or frit size was too small, particles from the sample will rapidly plug them, leading to a rapid increase in backpressure.

Relatively affordable benchtop mass spectrometry systems have appeared on the market in last 5 years. The use of MS as a LC detector has improved sensitivity and selectivity of HPLC methods. The selected ion recording MS mode (SIR-MS) is more forgiving for quality of sample preparation. The monitoring of only one mass/charge ratio peak dramatically improves the selectivity of the analytical method. Therefore, sample preparation is often reduced to on-line SPE with direct injection of biological sample [56–58]. The protein fraction is diverted by switching valve to waste; the following fractions containing analyte are streamed to (ESI) MS [59].

One reocurring trend in analytical chemistry is miniaturization of instrumentation. Micro-SPE devices with ~0.5 μl bed volume were developed for desalting of biopolymers prior to mass spectrometry, especially for MALDI-TOF-MS [23,24]. Several applications of sample preparation on chip were described in literature, e.g., chip format desalting [60,61], or hollow fiber dialysis [62] as a sample clean-up for mass spectrometry.

Mass spectrometry, which has traditionally been used for structural characterization of individual molecules is now being routinely used for direct analysis of relatively complex mixtures. The extremely fast analysis cycle of MS creates a demand

for the development of high-throughput sample preparation techniques.

3. Solid phase extraction

3.1. Off-line solid-phase extraction – new stationary phases, strategies, mixed mode

Modern solid-phase extraction sample preparation and high-performance liquid chromatography share the same fundamental basis, but this underlying principle is often overlooked or ignored when analysts develop sample preparation procedures [63,64]. HPLC and SPE use similar principles of adsorption/distribution of analytes between mobile and stationary phases; sorbent chemistry and particle morphology is most often based on C_{18} alkyl surface modified silica, and instrumental design of SPE (syringe cartridges or barrels) resembles small chromatographic columns.

In some cases, such as on-line SPE, the borderline between solid-phase extraction and HPLC is blurred, which is reflected in the classification of this technique (on-line SPE is also known as a column-switching technique). On-line SPE will be discussed in detail in the next chapter.

Although similar in principle, off-line solid-phase extraction devices differ from chromatographic columns in several key characteristics: (i) off-line SPE devices are disposable, designed for a single use only. (ii) SPE devices are packed with larger particle size sorbents (typically 30–100 μm), whereas modern HPLC columns use 3–5 μm particles. The resulting lower backpressure of SPE is important when extracting viscous biological samples. (iii) SPE chromatographic sorbent bed length is significantly smaller than that of the HPLC columns. (iv) SPE devices are designed to easily load and elute samples into various collection devices, which means that there are significant amounts of dead volume. On the other hand, HPLC column design minimizes extra-column volumes. Due to the differences highlighted above, SPE devices offer dramatically lower chromatographic efficiency than HPLC column ($<$60 plates vs. $>$10 000 plates).

From the above mentioned differences it is clear that SPE can be successfully employed only when there is a significant difference in chromatographic selectivity between the analyte(s) of interest and the interfering matrix. One major use of SPE cartridges is purifying analytes from biological liquids for quantitative analysis; drugs of interest are retained on the sorbent, while most of the sample protein constituents are washed to the waste with aqueous buffer. A popular application for SPE is desalting of peptides and DNA oligonucleotides for subsequent MS analysis. While peptides and DNA oligonucleotides are retained on sorbent, non-volatile salts are washed out from sorbent with pure water [65–71].

SPE is frequently utilized for trace sample enrichment. Relatively large volumes of sample can be processed by SPE using larger cartridges or extraction disks. The most important considerations for preconcentration are: (i) high enough retentivity of analyte(s) in the volume of loading solvent (weakly retained analytes breakthrough easier than strongly retained ones) and (ii) capacity of the sorbent to fully adsorb the loaded mass of analyte (a large amount of analyte will breakthrough easier then a small mass load). Sample preconcentration is particularly important for environmental samples, where the requirement for sub-part per billion detection sensitivity challenges even the most sensitive detectors [16,72].

Some reasons for low sample recovery in SPE are: inappropriate cartridge conditioning, too-strong loading and wash solvent, too large volume (mass) of sample loaded, and too weak or too small volume of elution mobile phase. The strategies for SPE method development and troubleshooting were recently described by Bouvier [64]. Because sample preparation method development for every single analyte is tedious and time-consuming process, some manufacturers suggested generic procedures. For example, the extraction procedure for reversed-phase sorbents such as the Waters Oasis HLB SPE cartridge [73] consists of a few generic steps: sample load, wash of the interfering sample constituents with 5% methanol, and elution with 100% methanol mobile phase. The generic procedures are designed to provide high recoveries with minimal development time. However, such procedures may not be selective enough to remove matrix constituents that interfere with the analysis of the compound of interest. These conditions should be considered as a guideline for

further optimization. For example, cleaner extracts of ionizable analytes may be achieved by judicious choice of pH for the load, wash, and elution solutions.

Selective two-dimensional (2D) methods have been described which meet the selectivity and sensitivity goals for sample clean-up [73,74]. The choice of pH and mobile phase strength is critical for the best SPE selectivity. The published protocol highlights the SPE protocol optimization step by step: (1) identification of the maximum possible wash solvent strength to remove the interferences without loss of target analyte. The pH is chosen to suppress analyte ionization; non-ionized forms of the analytes are more strongly retained on reversed-phase sorbent. (2) Selective elution of the analyte without interferences. In this step, the mobile phase pH is chosen to ensure that the target analyte is fully ionized. Weakly retained ionized analyte is eluted with the weakest mobile phase (the lowest organic modifier content) still capable of its quantitative elution. The 2D method requires two series of experiments with two different pH values using set of different strengths of wash and elution solvents. Analysis and mass balance of SPE fractions is part of the optimization, which may be time consuming. It is, however, good practice to optimize the SPE procedure and perform mass balance calculations as part of any SPE method development. One should be aware that SPE method development performed with aqueous buffers of analytes (mimicking the sample matrix) may give different results than the extraction of analytes from matrices such as undiluted plasma. High protein content in plasma may affect the retention behavior of analytes or decrease their recovery [64]. In addition, the interferences from plasma may complicate the HPLC background and thus the quantitation of the target analyte.

Since the introduction of Sep-Pak cartridges in 1978, C_{18} reversed-phase sorbents have become the most widely used. Notwithstanding the success of silica-based reversed-phase sorbents, they do have drawbacks as mentioned in Section 2. Polymeric sorbents have also been employed for a long time for reversed-phase sample clean-up and concentration. In fact, the first commercially available SPE devices contained the styrene–divinylbenzene XAD-2 polymer [11]. However, polymer sorbents had significant drawbacks. The major problems were high levels of background interferences resulting from improper washing after the polymer synthesis and significant shrinkage/swelling in different solvents. More recently, manufacturers have overcome many of these early limitations.

One significant problem with silica-based reversed-phase sorbents is poor water wettability. C_{18} cartridges require an initial conditioning step with a water-miscible organic solvent. After the sorbent is wetted, water of aqueous buffer (sample) can displace the organic solvent in the pores. When the internal surface of sorbent fails to be wetted because of the omission of the conditioning step or if the sorbent runs dry, the accessibility of sorbent surface for adsorbing analytes is severely reduced. Low recovery of analytes was observed when C_{18} sorbent was accidentally dried down before sample application [10].

In 1996 Waters (Milford, MA, USA) introduced the Oasis HLB sorbent, which is a reversed-phase water wettable resin. Performance of this resin is unaffected by sorbent drying. This is especially important for parallel sample processing. Because flow-rates may vary in individual wells in 96-well extraction plates (due to particulates or different sample viscosity) drying will occur in some wells while others are still processed.

Varian recently introduced SPE cartridges Abselut Nexus for extraction of pharmaceuticals and other analytes. According to the manufacturer, samples of biological fluids can be directly applied on Abselut Nexus cartridges without a conditioning step, thereby simplifying analysis. This novel SPE medium is a blend of two different chromatographic sorbents: methacrylic ester-based polymeric resin and polystyrene resin mixed in particular ratio.

Alkyl-silica and polymer-based reversed-phase SPE sorbents are the most widely used SPE stationary phases. However, some other applications utilize different principles for SPE sample clean-up including normal phase, gel filtration, affinity SPE, molecular imprinted stationary phases, restricted access sorbents, ion-exchange sorbents, and mixed-mode sorbents.

Gel filtration spin cartridges are popular for clean-up and desalting of biopolymers. Spin columns are used for DNA sample preparation prior to CE

sequencing. The removal of non-volatile salts, dye terminators (mononucleotides) and PCR primers from DNA samples is essential for proper CE separation performance [75,76]. Electrokinetic injection from salt contaminated sample leads to signal suppression, and formation of highly conductive zones, which may cause capillary overheating and loss of the run. Gel filtration cartridges typically purify 50–100 μl of sample. Principles of gel filtration do not allow trace enrichment of analytes on a cartridge and sample concentration is usually performed by evaporation. Due to the limited efficiency of separation, repetitive gel filtration is often performed to achieve a sufficient desalting for MS applications [65]. Preparation of spin cartridges requires lengthy gel hydration. Some manufacturers now market cartridges in hydrated form. As apparent from the name, the spin columns are processed by centrifugation. Recently several manufacturers (Qiagen, Valencia, CA, USA; Edge BioSystems, Gaithersburg, MD, USA; Bio-Rad Labs., Hercules, CA, USA; Amersham Pharmacia Biotech, Uppsala, Sweden) have introduced a 96-well gel filtration plate configuration to increase the throughput of sample preparation. The necessity to centrifuge plates, however, complicates the automation.

Affinity interaction principles have been used for design of special immunosorbents for SPE. Strong and highly specific affinity interaction is useful for trace enrichment of specific analyte in very complex matrices. Selectivity and enrichment ratio of selected analytes is significantly enhanced when using affinity SPE. Preparation of affinity SPE sorbent (gel) may be complicated by the insufficient stability of antibodies. Binding of the selective antibody to the sorbent must be performed under gentle conditions to preserve its structure and activity. Specific immunosorbents were prepared towards several analytes, such as LSD [77], β-agonists [78], morphine [79], catecholamines [80], and costicosteroids [81].

The main drawbacks of immunosorbents are the difficulties related to the preparation, isolation, and storage of antibodies. Immunosorbents exhibit selectivity toward a single analyte or small class of analytes [82–84], affinity SPE is not viable for broad commercialization. In some cases a mixture of antibodies was used to increase utility of immunosorbent for structurally related class or analytes.

One new trend in molecular recognition sorbents is the synthesis of molecularly imprinted polymers (MIPs) [28,85]. Molecular imprinting is a technology in which recognition sites are created by copolymerization of a target molecule in a macromolecular matrix. The target molecule is subsequently chemically released from matrix, creating cavities at the molecular level. Complementary functional moieties correspond to the print molecule in similar fashion as antibody structurally corresponds to antigen. MIPs have been successfully used for the separation of amino acids, peptides and proteins [85], extraction of drugs [86–88], and for HPLC separation of chiral drugs [89,90]. MIPs exhibit high selectivity under normal-phase conditions, however, in polar solvents such are aqueous buffers hydrogen bonding and other polar interactions required for selective analyte binding are suppressed. Historically, the most difficult problem with MIPs has been their poor performance in reversed-phase mode.

One class of MIPs known as ''plastic'' antibodies is currently under development. MIPs mimicking molecular recognition of biological antibodies or enzymes are expected to play an important role in bio-catalysis, biomimetic sensors, as well as in sample preparation [25,28].

Selective sequence-specific hybridization of DNA strains has been used for construction of ''affinity''-like DNA clean-up and enrichment devices. Immobilized DNA probes [91–95], morpholino backbone modified oligonucleotides [96] and peptide nucleic acid oligonucleotides [97–100] were used for the capture of sequence specific RNA or DNA strands.

Some single-stranded oligonucleotides, possessing specific secondary structure, bind to proteins and other molecules with an affinity and selectivity resembling antibodies. Identification of these so-called DNA aptamers is a key step in their use as affinity probes [101]. DNA aptamers immobilized on solid supports may find broad application in future sample preparation and analysis [102].

RAM sorbents were developed for direct injection of biofluids containing a high content of macromolecular contaminants. The mechanism of restricted access depends on exclusion of bulky macromolecules from the internal surface of sorbent, and on modification of the outer surface of sorbent with

chemically bound hydrophilic coating. Small molecules in the sample are enriched by adsorption on the internal surface of sorbent particles; proteins are not retained. Different types of RAM sorbents have been described in a recent review [51]. RAM sorbents were originally used as packing for HPLC columns. Due to the incomplete wash of proteins from the column, resulting from their non-specific adsorption, their partial precipitation on-column occurs. The lifetime of RAM columns is, therefore, limited. Another disadvantage of RAM stationary phases is a secondary interaction of analytes with the hydrophilic surface coating that may cause peak tailing. RAM sorbents are well suited for on-line SPE (column switching), where lifetime and chromatographic performance is not as critical as in HPLC. SPE using RAM sorbents will be more thoroughly discussed in a later chapter.

Ion-exchange SPE has been used for the clean-up of polar analytes and for the isolation of proteins, peptides, oligosaccharides, and polynucleotides from biological samples [103–106]. The problem in processing biological samples is that there may be a high content of inorganic ions, which can overload the capacity of sorbent. Application of ion-exchange SPE may find broader applicability in the near future for the fractionation of complex protein mixtures for proteome analysis.

Mixed-mode sorbents were introduced in the mid-1980s to improve selectivity of sample clean-up as well as recovery of polar analytes that are poorly retained on reversed-phase SPE sorbents. Earlier approaches used the cation-exchange property of silanols to retain basic compounds [107]. Special C_{18}-OH (silanol rich) phases were developed by Varian and others [8]. Difficult and non-reproducible elution of bases from this type of sorbents initiated a development of alternative SPE cartridges, such as Bond Elut Certify, Bond Elut Certify II, and Isolute mixed mode. These SPE devices are packed with a blend of C_{18}-based and ion-exchange-based sorbents.

More recently, SPE products have been introduced containing both reversed-phase and ion-exchange functional moieties on one resin. Either cation-exchange or anion-exchange mixed-mode chemistries were developed. Cation-exchange mode improves clean-up of the sample when appropriate pH is chosen for wash step. The protocol suggested by the manufacturer [108,109] uses acidic loading buffer, in which charged basic analytes are retained by ionic interaction. The wash step consists of 0.1 *M* HCl (to wash out macromolecular interferences) followed by methanol. Methanol elutes non-charged interferences, while basic constituents remain adsorbed by ion-exchange mode. Ionic interaction is disrupted by changing pH, and elution is carried out by methanol–ammonium hydroxide mixture. Using this protocol, very clean drug extract is usually obtained. However, it should be mentioned here that basic interferences might be co-concentrated along with the analyte of interest.

Mixed-mode sorbents are useful for fractionation of analytes. Selective elution of neutral (acidic), and basic drugs can be achieved by controlling the pH of eluent [108]. An anion-exchange reversed-phase mixed-mode sorbent described recently [110] offers anion-exchange interaction for more selective SPE. Selectivity can be tuned up by the choice of load, wash and elution pH. While mixed-mode SPE does not bring the direct advantage for extraction of neutral analytes, it can give a cleaner extract using appropriate pH for load and wash of charged interferences (ion repulsion).

The most popular format for off-line SPE is a 1–6 ml syringe cartridge. In the early 1990s, extraction disks were developed for both large volume applications and miniaturized devices for small sample volume processing. Extraction disks are particularly useful for the processing of environmental water samples [16,72]. The disks are constructed from particle loaded PTFE membranes or glass fibers. The major benefit of the disk format is that it allows rapid processing of large sample volumes. The drawbacks are large elution volumes, blocking of membrane with particulates, and poor water wettability of RP sorbents typically used in disk SPE devices.

Miniaturization of SPE is driven by the limited availability of samples, the requirement of fast simple handling of small sample volumes (1–100 μl), and the need to minimize sample dilution in the elution step. Elution volume can be reduced by using cartridges packed with small amounts of sorbent. It should be noted, however, that loading capacity decreases as well. Fig. 1 shows how elution volume is affected by sorbent mass. Analytes from 10 and 5 mg plates can be eluted in as little as 100 μl of

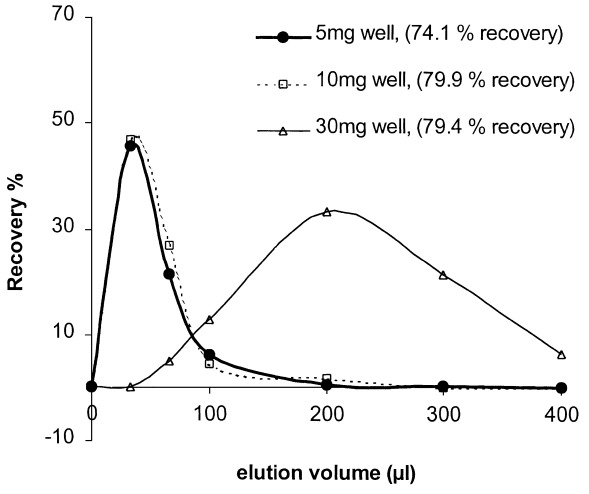


Fig. 1. Elution profiles of 25mer DNA oligonucleotide from 96-well extraction plate packed with different amounts of sorbent.

eluent. Besides chromatographic bed volume the elution volumes are affected by the volume of frits. Elution volume can be reduced by centrifuging the samples instead of using vacuum, or by drying of SPE cartridge prior to elution. Extraction disks show potential to minimize elution volume by elimination of frit induced sample channeling through the sorbent bed [18,19].

The miniaturized devices designed for sample processing in volumes of several microliters, such as pipette tips packed with ~0.1 mg of chromatographic sorbent, are discussed in subsequent chapters. Successful manufacturing of such small SPE devices requires methods for immobilizing the sorbent bead. Conventional approaches using packed beds are limited by large frit volumes. Fritless SPE cartridges may be constructed using monolithic chromatographic sorbent instead of a bed containing particles [111]. Monolithic stationary phases for chromatography are currently under investigation [112–115]. Mechanical stability, swell–shrinkage properties, and cleanliness of monolithic sorbents are the main considerations for design of monolithic SPE devices.

3.2. On-line solid-phase extraction (column switching)

The aim of SPE automation is to improve both throughput and reliability of analytical method. Two approaches have been taken to automate solid-phase extraction. The first is to employ robotic systems to automate extraction using traditional cartridges or extraction plates. The operator supplies the robotic system with a processing algorithm, appropriate buffer solutions, and cartridges. Sample preparation systems typically comprise an autosampler capable of injecting extracts onto the HPLC (CE, MS) system. Examples of such automated systems are Microlab SPE (Hamilton, Reno, NV, USA), Prospekt 2 (Spark Holland, Emmen, The Netherlands) and ASPEC XL (Gilson, Middleton, WI, USA). These systems belong to the class of at-line automation [38].

The second approach for sample clean-up automation uses the principles of column switching. This approach requires, in addition to standard a HPLC system, one additional mobile phase pump and one or more additional switching valves. The sample is loaded and concentrated onto a modified SPE cartridge (capable of high-pressure operation), which is located at the injection sample loop [36,116–118]. Contaminants are washed off the cartridge to waste using mobile phase from the second pump. Subsequently, a valve is switched and the remaining content of the SPE cartridge is eluted onto the HPLC column. The ASPEC XL typically performs off-line sample clean-up with disposable extraction cartridges (DECs). It can also be configured to work as an on-line SPE system using the high-pressure trace enrichment cartridge (TEC). This system is capable of automated sample dilution, mixing, internal standard addition and extraction. The selected volume of sample is loaded onto a trace enrichment cartridge via a six-port injection valve, and the sample contaminants are then washed from the micro-column with predetermined volume of mobile phase. Wash solvent is delivered by a piston pump via injection port. This approach greatly simplifies the instrument set-up. However, the length of microcolumn restricted by its backpressure, because sample load and wash is performed by low-pressure piston pump. When the main valve is switched to the inject position, the adsorbed analytes are eluted with mobile phase to the analytical HPLC column. During the HPLC analysis, the TEC cartridge is switched back to the load position, regenerated with appro-

priate buffers, and subsequent sample extraction is performed [119].

The column-switching approach brings several advantages over the at-line SPE approach. Because the system is basically a modification of a traditional HPLC autosampler, it could be relatively easily and inexpensively automated. Most instruments described in the literature are built or modified in the laboratory. Reusing the SPE pre-column for multiple injections decreases cost per analysis; pre-columns are often dry-packed devices that can be re-packed in the laboratory. Precise timing of column switching can be easily programmed since most of the modern instruments and chromatographic software can trigger or receive electronic signals from remote devices.

Most importantly, on-line SPE improves the sensitivity of the analytic method in comparison to at-line or off-line SPE, where typically only a small portion of the SPE eluent in subsequently analyzed by HPLC (CE) [120–124]. For example, if 10 μl of off-line SPE eluent is injected onto the HPLC column (after loading 1 ml of sample and eluting with 1 ml of mobile phase), only 1% of the recovered sample mass is actually analyzed. In on-line SPE, theoretical sample recovery is 100% [49,48]. When using MS detection, the detection limits are typically in the order of ng/ml or lower [125]. Off-line SPE can match the sample enrichment of the on-line method when time consuming eluent evaporation is used, and the sample reconstituted and injected in small volume of mobile phase.

The major drawback of the on-line SPE approach is the limited lifetime of the SPE cartridge/microcolumn. The Prospekt 2 system from Spark Holland uses small single use cartridges that are capable of high-pressure operation.

When a single cartridge (microcolumn) is used for repetitive sample extraction the instrumental set-up dramatically simplifies, but an additional problem occurs in the analysis of biological samples with high protein content. Contamination of the microcolumn with endogenous material is detrimental for its chromatographic performance and capacity. Typically, microcolumn lifetime is between 50 to 200 injections [126], depending on the volume and character of the sample injected. An increase in column backpressure, and decrease in the system chromatographic efficiency resulting in peak tailing and splitting was observed especially with plasma samples. Changes in column performance are mainly related to the precipitation of proteins in the microcolumn, which block sorbent pores and clog the microcolumn frits. To alleviate this problem, addition of SDS in a biological sample for protein solubilization is sometime used in conjunction with microcolumn backflush regeneration with a strong mobile phase after several injections [50,127,128]. Band broadening may be a problem in some on-line SPE systems due to an inappropriate combination of microcolumn and HPLC column stationary phase, and a mismatch of mobile phases used for elution. The broadening generally happens if the microcolumn retains analyte stronger than main HPLC column. Gradient elution helps to minimize this effect. Attention should also be paid to the compatibility of wash and elution mobile phases during the column switching.

More recently, the development of RAM sorbents offers an elegant solution for this problem, and improves the microcolumn lifetime [3,49,51,129,130]. As the name of the new class of chromatographic sorbents suggests, the accessibility of an internal surface of sorbent is limited to small molecules only. Macromolecules, such as proteins are excluded from pores and interact only with the outer surface of particle. In addition to the restricted access mechanism, the outer surface is coated/modified with hydrophilic functional groups assuring minimal adsorption of proteins. The classification and review of RAM chemistry was published recently [51].

RAM sorbents were used with moderate success as a packing for HPLC [131]. RAM are currently used in many on-line SPE applications. Although the compatibility of these stationary phases with direct biological sample injection is high, one still has to keep in mind that samples have to be centrifuged prior to injection to remove the samples' solid contaminants and protein precipitates. Even so, the remains of plasma may still precipitate on-column when eluting analytes onto the main HPLC column with mobile phases containing more than 10–20% of organic modifiers. The lifetime of RAM pre-columns is practically limited to ~500–1000 injections (50–100 ml of undiluted plasma) [49].

One new trend in on-line SPE its use as a fast sample clean-up method prior to MS. The method comprises direct injection onto a short column packed with large particle size sorbent (~30 μm), removal of the plasma proteins with low strength mobile phase (diverted to waste via a multi-channel valve), and elution of analytes of interest to MS using fast gradient of mobile phase [59,132]. The purpose of this ''ballistic gradient'' is to elute analytes to the MS as a concentrated narrow band without extensive chromatographic separation.

Ding and Neue [59] utilized on-line SPE column for off-line SPE method development. Retention behavior of analytes and plasma contaminants were studied in order to suppress the signal of sample background. The on-line developed method can be directly transferred to an off-line SPE, if required.

Because the time requirements for on-line SPE are similar to an HPLC duty cycle, this technique is well suited for serial sample preparation prior to HPLC. However, the development of fast chromatography will probably change the situation in future, when on-line SPE may become a rate-limiting step. In our opinion, a parallel off-line SPE will ultimately offer faster sample throughput.

3.3. Microextraction methods

Miniaturization of sample preparation has been a general trend in the last decade. This is driven by several factors: (i) limited amount of sample available for analysis (blood samples from small laboratory animals, proteins isolated from slab gels, single cell analysis). (ii) High sample cost (small scale of polymerase chain reaction reduces the cost of DNA analysis). (iii) Miniaturized techniques such as CE and capillary HPLC require less than a few microliters for analysis. (iv) Miniaturization of chemical processes increases apparent reaction rate due to reduction of macro-kinetics transport effects and enhanced sample mixing. This leads to the higher sensitivity of analysis (analysis and detection of chemical reaction on chip format). (v) Miniaturization of sample preparation devices allows for parallel processing of samples for high throughput (384-well or 1536-well SPE extraction plates).

In the last 5 years there has been a steady trend in sample preparation of biological samples towards smaller sample volumes, and higher sample enrichment efficiency [22]. The introduction of high capacity polymeric sorbents for SPE has allowed for a reduction in the amount of sorbent packing in a cartridge. 30 mg of polymer resin has a similar capacity as 100 mg of silica-based C_{18} sorbent [133]. The introduction of cartridges and extraction plates packed with 10 and 5 mg of sorbent and small size extraction disks follows this trend [9]. A device with a smaller amount of sorbent decreases the volume of mobile phase necessary for quantitative analyte elution, which results in lower sample dilution. This is demonstrated in Fig. 1. Elution profiles of 15mer oligodeoxythymidine DNA shows that cartridges packed with 5 and 10 mg of sorbent can be eluted with as little as 100 μl of mobile phase, whereas in case of 30 mg cartridge more then 400 μl of mobile phase is required. The excessively strong mobile phase used for elution (70% acetonitrile in water) was chosen in order to suppress any retention of analyte on stationary phase. The large elution volume obtained with 30 mg cartridge is in part caused by the dilution of mobile phase by the residual liquid trapped in internal pores of sorbent (~40 μl), and in the frits. Smaller elution volumes was achieved when using lower sorbent amount [18,19,65].

Several devices for sample preparation capable of processing samples in the volume range 1–100 μl have recently been reported. These are: (i) Microdialysis or electrodialysis devices. (ii) Micro HPLC columns for on-line desalting prior to MS analysis. (iii) Micropipette tips packed with small amount of chromatographic sorbent (Zip-Tips from Millipore, Supro Tips from Amica). (iv) Micro SPE cartridges (Monolith sorbent or powder packed tips). (v) Modified MALDI plates for on-target clean-up of biopolymers.

On-line interfaces for CE often use a sample clean-up principle of dialysis and electrodialysis. Two typical applications are described in the literature: salt removal, and removing the macromolecular interference prior to analysis of drugs in biological liquid [38,47,134,135]. Low-ionic-strength sample is required for an efficient electrokinetic injection in CE. Electrokinetic injection is the only option in special cases, when the capillary is filled with viscous molecular sieving matrix, and therefore pressure injection cannot be applied. Due to low

sample volume requirements, capillary electrophoresis fully benefits from the miniaturization of sample preparation. Membrane dialysis and electrodialysis sample preparation methods will be discussed in more detail in the next section.

On-line dialysis with SPE preconcentration of dialysate and on-line injection of the eluent on CE was described recently [136,137]. Literature on automated systems for sample preparation prior to CE described are rare. None of the systems are presently commercially available. Major technical problems involve proper electrical grounding of the system, concentrating of the sample to ~μl volume prior to injection, and maintaining the compatibility of the eluent/dialysate with CE in order to avoid peak broadening due to CE running voltage/current disturbances.

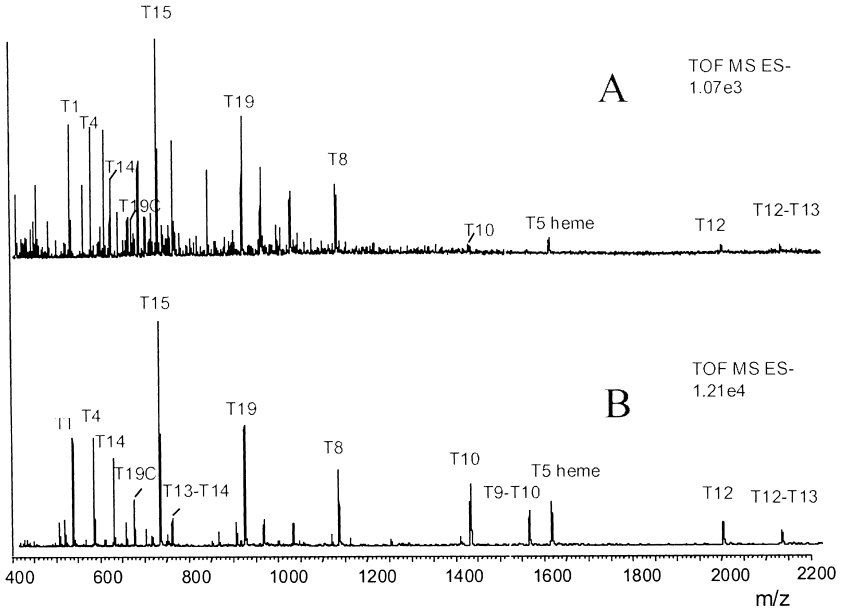
Both in-line and off-line sample preparation [38] typically dilute the samples prior to CE injection, which reduces sensitivity of the analysis. Sample injection volume of CE (few nanoliters) is not directly compatible with the large sample volumes. Therefore, a preconcentration technique is often used. Tandem isotachophoresis (ITP)–CE was used [138–141] for analysis of model samples or clean extracts. Transitional ITP in CE capillary is popular because no modification of a CE set-up is required. A large sample plug is introduced in to a CE capillary in low-ionic-strength buffer. Sample zone focusing occurs in the capillary due to the higher electric field strength (and faster mobility if ions) in the sample plug. After ion depletion, normal CE mechanism prevails, and focused zone is separated by CE [142–147]. Another technique using similar focusing principle is field-amplified injection [148–151]. Electroinjection is performed from sample solution with low ionic strength; the large preconcentration factor (~100) can be achieved without a modification of CE instrumentation. Both of these techniques, however, cannot be directly used directly for analysis of biological samples. For this simple reason off-line SPE is still the most popular method of sample clean-up for CE.

Analysis of biopolymers by mass spectrometry is a prime method for studying the proteins and DNA. Usually, biopolymer samples are enzymatically or chemically fragmented, and their structure is studied by MALDI-TOF-MS or ESI-MS. The presence of non-volatile ions in the sample (specific ionic strength, pH, and cations are required to support the proper catalytic activity of enzymes) causes the sample signal suppression (Fig. 2). Moreover, the ion adducts complicate the interpretation of the spectrum (Fig. 3). Several desalting methods were suggested for biopolymer MS analysis, including SPE.

Desalting of samples for ESI-MS can be in principal both on-line or off-line. On-line approach uses a microcolumn device placed into a stream of liquid prior to MS. It could be designed either as an electrospray tip packed with ion-exchange sorbent, or a microcolumn connected to the MS electrospray tip. The purpose of such a device is to scavenge cations from injected DNA samples and mobile phase.

Several groups described the on-line SPE–CE methods using reversed-phase sorbent [152–156] or sorbent loaded membranes [157–159]. Similar devices were developed for on-line preconcentration and analysis of peptides. SPE microcolumns were constructed in CE capillary by sandwiching a small amount of sorbent between two membranes. Such a micro SPE–CE tandem was directly coupled with MS. This micro SPE approach brings several advantages; most importantly high enrichment factor of 100–1000 was reported. Sample dissolved in a few μl can be easily concentrated in to a few nl volume in the CE capillary. The micro SPE cartridge and whole capillary is typically washed in several steps to minimize background contaminants, salts, and exchange buffer before the start of CE separation. The SPE microcolumn can be repeatedly used; durability of SPE device was high (up to several months) [152–155,157–159].

On-line preconcentrated sample is desorbed by the injection of a short plug of strong organic solvent (usually 6–15 s low-pressure injection of 60–90% ACN in desirable buffer) prior to electrophoretic separation. Either UV detection or (preferably) ESI-MS detection is used for monitoring of concentrated analytes. The flow-rate range (μl–ml) is directly compatible with mass spectrometry. SPE–CE–MS–MS technique is an excellent tool for identification of low abundant proteins isolated from cells by 2D gels. Although very powerful, this technique has also some drawbacks: (i) the construction of the SPE



peptide	sequence	MW
T1	N-Acetyl-GDVEK	588.6
T4	IFVQK	633.8
T14	YIPGTK	677.8
T19C	EDLIAY	722.8
T15	MIFAGIK	779.0
T13-T14	KYIPGTK	806.0
T19	EDLIAYLK	964.1
T8	TGPNLHGLFGR	1168.3
T10	TGQAPGFSYTDANK	1456.5
T5+heme	CAQCHTVEK-HEME	1634.5
T9-T10	KTGQAPGFSYTDANK	1584.7
T12	GITWGEETLMEYLENPK	2010.3
T12-T13	GITWGEETLMEYLENPKK	2138.4

Fig. 2. Effect of sample desalting on intensity and quality of ESI-MS peptide signal. A 10.9-pmol concentration of cytochrome *c* tryptic digest was infused into an electrospray mass spectrometer: (A) sample in 20 m*M* Tris–glycine buffer; (B) the same sample after SPE desalting. Figure adapted from Ref. [65].

device is performed manually in the laboratory. Equivalent CE–SPE capillaries are not commercially yet available. (ii) Mis-alignment of capillary–micro-column joins or tight sorbent packing is the source of increased backpressure. (iii) The low capacity of SPE, and high polarity of some peptides are the main reason of loss of weakly adsorbed analytes in preconcentration process. (iv) Relatively long sample preparation time in order of 10–20 min. (v) Embedded SPE bed often compromises separation per-

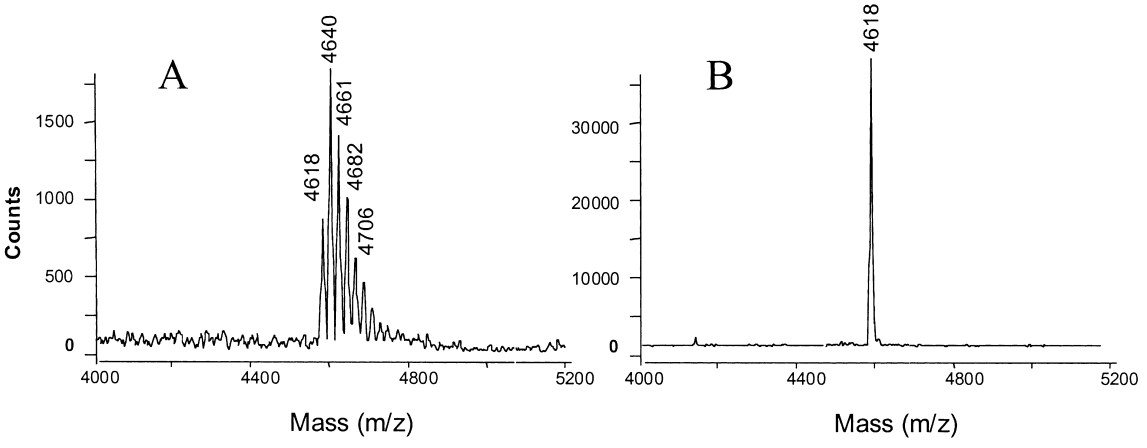


Fig. 3. Effect of DNA oligonucleotide sample clean-up on the quality of MALDI-TOF mass spectrum. A 1-μ*M* concentration solution of 15mer DNA oligonucleotide was prepared in 50 m*M* NaCl. (A) A 1-μl volume of this solution was applied onto the MALDI target, (B) 100 μl of 1 μ*M* 15mer DNA solution in 50 m*M* NaCl was desalted by SPE [65], and eluted in 100 μl of 70% acetonitrile solution in deionized water. A 1-μl volume of eluent was applied onto the MALDI target. Figure adapted from Ref. [65].

formance of CE. (vi) The laboratory-made SPE–CE–MS system is not rugged. It must be designed and run by a skillful operator.

The high enrichment ratio and direct MS coupling most certainly prevail over the disadvantages of this method.

The microcolumn construction for scavenging of non-volatile ions for μLC–MS is reportedly a less challenging task. Contrary to CE, the HPLC microcolumn does not contribute significantly to the band broadening; this is due to the fact the SPE device neither dramatically increases the void volume of system, nor changes the flow properties of HPLC [160,161]. Capacity of scavenging ion-exchange microcolumns is relatively high; they can be used for several hours without replacing or equilibration. Of course, the lifetime of microcolumns depends on the concentration of salts to which they are exposed.

Microdevices serving as a SPE–HPLC microcolumn for sensitive ESI-MS peptide analysis have been constructed in the microelectrospray needle itself [162,163].

Miniaturized solid-phase extraction devices were developed recently by Millipore [23]. The microtips are packed with small amount (~0.1 mg) of C_{18} reversed-phase sorbent of other sorbents. The volume of packed bed is about 0.5 μl, which allows to elute extracted analyte in small volume of mobile phase (1–5 μl). This volume range is compatible with MALDI-TOF-MS sample volume requirements. The Millipore ZipTip is designed to be compatible with laboratory pipettors and auto pipettors. C_{18} ZipTips are used primarily for biopolymer desalting for MALDI-TOF-MS; purified biopolymer can be eluted directly onto a MALDI target.

Similar approaches for construction of tip-like SPE devices has been used by other researchers [67]. Due to higher flow resistance of packed bed the vacuum or centrifugation is used for sample processing rather than pipetting. Packed tips were used in hydrophilic interaction chromatography (HILIC) mode [164]; affinity-like interaction was also employed for microextraction [165].

The high demand for biopolymer analysis in the last several years is a major reason why MALDI-TOF-MS is becoming a very popular technique in the last 2 years. The advantage of MALDI over ESI is that a simpler MS spectrum is obtained, consisting mostly of the molecular ion. The typical ESI spectrum contains multiple charged states of biopolymers, which complicates the spectra interpretation, and it is sensitive to ion suppression. Although MALDI-TOF-MS is relatively tolerant to the presence of non-volatile salts in sample, the sample desalting is desired to increase sensitivity and avoid ion adduct formation [61,67,68,166,167]. About 1–5 μl of sample is typically deposited onto a MALDI target plate, and evaporated to dryness prior to MS analysis. The on-target technique for sample preconcentration and desalting was developed recently [168–172]. The principle of this technique is an adsorption to the surface modified MALDI target. Either hydrophobic interaction or other modes of chromatography (similar principles to the SPE) may be used to extract and concentrate analytes from the sample on a target surface. This technique is known as a SELDI (surface enhanced laser desorption/ionization). Desalting of the biopolymers is one of the most frequent applications. Either hydrophobic [170] or ionic interaction [169] are used to adsorb molecules of interest. The plate is subsequently washed with deionized water, MALDI matrix is applied over the sample spot, evaporated to dryness, and plate is ready for analysis. Selective extraction of analytes of interest was performed with MALDI targets modified with antibodies [171,172], DNA oligonucleotides, and other surface ligand chemistries [173]. Some other reports describe an alternative approach of sample desalting using extraction of the analyte with the free beads of chromatographic sorbent in solution [174]. After centrifugation and wash steps the analyte is extracted with mobile phase and solution is spotted onto a plate. Alternatively, thoroughly washed beads are directly placed onto MALDI target and analyte is desorbed from the sorbent directly on the plate [175].

The desalting of biopolymers will be described in more detail in Section 5.

4. Membrane-based methods

4.1. Dialysis

Dialysis is a separation technique that utilizes the mass flux through a semi-permeable membrane

separating two chambers. The first chamber contains an acceptor liquid, the other (donor chamber) serves as a sample reservoir. Dialysis membrane of a particular molecular mass cut off (MWCO) provides a tool for molecular separation based on molecular hydrodynamic dimensions. Two primary applications of dialysis are for isolation of drugs (and other small molecules) from the macromolecular interferences in biological matrixes, and for removal of non-volatile ions from biopolymers such as DNA oligonucleotides and proteins.

The instrumentation used for dialysis can be as simple as a static drop dialysis on a floating membrane, or as complex as a fully automated system with continuous flowing liquids in donor and acceptor channels, on-line preconcentration by SPE, and on-line injection onto an HPLC or other separation technique. The most frequently used automated system for sample preparation by dialysis is the Gilson ASTED, consisting of an autosampler, two piston pumps, dialysis block, and trace enrichment (SPE) cartridge. This system can be coupled to an HPLC or CE system [38,136]. The drawback of dialysis is that this technique does not recover analytes quantitatively, and sample is usually diluted in acceptor liquid. It is therefore important to preconcentrate the sample using on-line SPE [38].

One popular approach, used mostly for buffer exchange and for biopolymer desalting, is on-line microdialysis in a hollow fiber submerged into a rinsing solvent [62,176–178]. This set-up was used for peptide desalting prior to ESI-MS. This technique of on-line microdialysis with direct MS infusion minimizes sample handling, which is particularly advantageous when dealing with small amounts of precious samples. However, it requires a few minutes to process a sample, and there may be a memory effect due to the tubing interface. A microdialysis junction was used as an interface for the separation of proteins, peptides, and other molecules by CE–MS. This junction was found to cause a ~20% loss of separation efficiency, but it also provided a significant sensitivity gain compared to the MS interface based on coaxial sheath flow [179]. Dialysis on a microfabricated chip device was also used for sample desalting prior to ESI-MS [61,180,181]. More complex set-up with two different MWCO membranes was designed for sample preparation of complex biological samples for mass spectrometry [60]. Practical considerations of dialysis (and other membrane-based sample preparation techniques) were discussed in an excellent review that was published recently [44].

Although dialysis offers an elegant solution for the separation of molecules that differ significantly in molecular mass (size), it has several inherent difficulties that limit its usefulness. Some of the major concerns are: (i) dialysis is driven by concentration gradient. In order to keep mass flux through membrane high, the acceptor chamber liquid must be continuously replenished. This leads to excessive dilution of dialysate, which requires subsequent concentration (usually by on-line SPE) [118,182]. (ii) Dialysis is a relatively slow process. Typical sample cycle time is on the order of minutes. This is not compatible with high throughput sample preparation. (iii) The analyte recovery is limited. The mass flux decreases over time due to the exhaustion of analyte in donor channel; it is impractical to recover more than 50% of analyte of interest [183]. (iv) The binding of analytes to proteins in the sample matrix has pronounced effect on a dialysis efficiency. Analytes have to be liberated from binding by addition of a release agent [184]. However, this is not effective in all cases. (v) Any change in the sample matrix will result in different dialysis efficiency for strongly protein-bound drugs. This presents a problem for calibration, choice of an internal standard, and for overall validation of the methodology.

The selection of the type of dialysis membrane may affect the dialysis performance. Binding of analytes to the membrane is a source of memory effects [45], and decreased dialysis efficiency. In some cases, the choice of releasing agents and membrane is crucial for successful sample clean-up [47].

One special mode of dialysis is in vivo microdialysis with microfibers implanted in the laboratory animal. This has been described by Hogan et al. [185]. Dialyzate was injected on-line to a CE system. In vitro monitoring of enzymatic processes using microdialysis sampling was also performed [47,186].

In principle, dialysis is not a selective technique. It is not possible to selectively recover the desirable analyte from a mixture of analytes of similar molecu-

lar mass. An alternative method to dialysis, membrane extraction, was developed recently. Donor and acceptor chambers are separated by a porous membrane, in which pores are filled with a water-immiscible organic solvent. This supported liquid membrane (SLM) works in a similar fashion as liquid–liquid extraction [46,150,187]. The analytes are dissolved in a membrane-entrapped non-polar organic solvent according to their distribution coefficient (K_d), and diffuse to the acceptor chamber. The choice of acceptor liquid pH assures appropriate ionization of analytes, which precludes their back extraction. Contrary to dialysis, the SLM has the potential to improve the selectivity of sample preparation with appropriate choice of membrane liquid, although choice of non-polar liquids is limited. SLM works best for ionizable analytes that are soluble in non-polar solvents when they are in the non-dissociated form. This condition is fulfilled for non-polar and moderately polar weak acids or bases. For highly polar compounds (that are usually weakly bond to sample matrix proteins), the dialysis sample clean-up is superior. For a more detailed description of SLM and other modes of membrane extraction, see a recent review by van de Merbel [44].

4.2. Electrodialysis

In both dialysis and SLM sample preparation techniques, diffusion of analyte through the interface is a rate-limiting step. In electrodialysis, two forces are employed to drive the analyte flux through membrane: a concentration gradient and an electric potential. The electric potential gradient causes charged analytes to electromigrate through the membrane towards the electrode with an opposite charge. Electro-driven flux overcomes back-diffusion, which leads to nearly quantitative transfer of analyte to the acceptor chamber. Because electromigration is only required to occur over a distance that corresponds to the thickness of the membrane (assume both chambers are stirred), a low voltage (5 V) is typically sufficient for fast ($<$1 min) electrodialysis. Efficiency of electrodialysis is low for samples with high ionic strength; at a fixed potential, the electric field strength decreases with the increase of sample/acceptor liquid conductivity since much of the IR drop such a system is through the solution rather than the membrane. In order to maximize recovery, pure water is used almost exclusively as an acceptor phase.

If weak acids or bases are electrodialyzed, the pH of both chambers needs to be controlled. Electrolysis of buffer can significantly affect the pH in both chambers, and negatively affect the sample recovery. Another complication is that the membrane can act as an electroosmotic pump, the pump rate of which varies with pH and acts as a non-selective means of solute transport.

One major benefit of elecrodialysis is the increased analyte recovery, and the enhanced rate of mass flux through membrane. Electrodialysis is limited only to charged analytes. Charge selective flux may increase the selectivity of sample clean-up [44].

A simple set-up was used for electrodialysis sample preparation prior to analysis of adenosine triphosphate and inositol phosphates by CE [134]. Capillary electrophoresis is compatible with electrodialysis due to the similar separation principles and instrumentation [44,185].

Electrodialysis/electroelution is a familiar technique used for isolation of biomolecules from agarose and polyacrylamide slab gels. Gel strip with the appropriate analyte band is placed into a compartment with semi-permeable membrane walls. After electroelution from the gel, the biomolecules are present in free buffer solution [188–191], and require further desalting.

4.3. Filtration

It is advisable to inject clean and homogenous sample into a HPLC and other separation systems. The failure to do so may result in a short column lifetime, poor separation, and low analysis reproducibility. In some cases dialysis can be used for sample preparation of colloidal solution or particulate laden samples, such as fruit juices, coffee, urine and plasma [44,192–194]. Unfiltered samples can also be applied to solid-phase extraction cartridges, as both the frits and packed beds provide some degree of filtration.

Besides these particular cases, sample filtration or centrifugation is frequently used to isolate liquid from solid particles. Centrifugation is traditionally

used in conjunction with protein precipitation. While centrifugation is simple, it is also time-consuming and difficult to automates, including the critical step of supernatant collection. Durability of an HPLC column with supernatant injection is about 100–500 samples [6,195], depending on sample injection size and composition. Part of the reason for shortened column lifetime is incomplete protein precipitation; however, increase of column back pressure is at least in part due to inefficient precipitate removal. The ideal sample preparation diluent is the actual eluent used for HPLC. If this is used then it is unlikely that a prepared sample will precipitate upon injection into a HPLC system.

Recently Biddlecombe and Pleasance [196] published a simplified method of sample preparation comprising automated precipitation of plasma protein, and filtration through a 96-well filter plate. Samples are evaporated to dryness using a 96-well dry down station, and then reconstituted and analyzed by LC–MS–MS.

5. Applications

5.1. Isolation of proteins from sodium dodecyl sulfate–polyacrylamide gel electrophoresis; surfactant removal from sample prior to mass spectrometry

Biological research has shifted from the study of single genes and proteins to comprehensive analyses of biological systems and pathways. This was a result of development of advanced separation techniques for protein analysis, such as 2D gels, which is capable of separating thousands of proteins in a single analysis. In the recent rendition of 2D gels, separation in the first dimension is performed on an immobilized pH gradient gel strip. Proteins are separated based on their isoelectric point (pI) using principles of isoelectric focusing. The gel strip with focused proteins is than attached to a slab gel presoaked with surfactant (SDS), and proteins are separated in the second dimension based on their effective size roughly corresponds to the proteins molecular mass (M_r). The result of the 2D separation is an array of separated protein spots that need to be visualized, isolated and characterized in further post analysis steps. Historically, 2D gels suffered from many problems, namely poor separation reproducibility, elaborate post separation protein visualization and excision, and incomplete protein recovery. Although separation reproducibility has improved with the introduction of pre-cast immobilized pH gradient gel strips and SDS gels, isolation of proteins from the gel and the subsequent sample preparation for further analysis remain difficult (Fig. 4).

Many protein isolation techniques have been developed using either manual staining techniques and spot excision from the gel, or gel blotting onto a membrane. Although the latter technique is more convenient and allows parallel recovery of all proteins separated by 2D gels, the drawback is incomplete protein recovery, and loss of proteins that are not well adsorbed to the membrane in the wash step. Further analysis utilizes protein digestion by appropriate proteolytic enzyme followed by mass spectrometry. Despite lower protein recovery from blots, membranes offers an advantage of easy sample clean-up, which reduces amount of salt and SDS

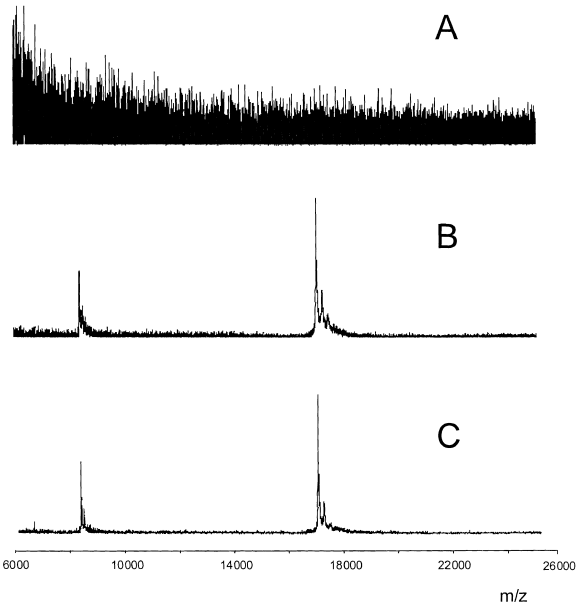


Fig. 4. MALDI-TOF-MS analysis of myoglobin extracted from polyacrylamide slab gel. (A) SDS–PAGE, (B) ALS–PAGE, (C) standard solution of myoglobin. Traces of SDS suppress MS signal. Acid labile surfactant (ALS) hydrolyzes during extraction and does not interfere with MS (adapted from Ref. [226]).

contamination prior to mass spectrometry analysis [197–200].

5.2. Sample preparation for protein/peptide analysis by mass spectrometry

The study of protein expression in is broadest sense is described as proteomics. This field faces the challenge of fractionating complex protein mixtures. Since the human proteome may be comprised of as many as 100 000 proteins, each of which can occur in at least dozens of variant forms (so called microheterogeneous forms), a single sample may be comprised of many thousands of proteins, many of which vary only slightly in property.

When cell lysate proteins (or other protein mixtures) are digested directly without prior protein separation, the mixture of resulting peptides is so complex that it is difficult to identify proteins present in the original sample. Therefore the primary route of analysis is to initially separate proteins by either 2D gels or by combinations of LC–LC or LC–CE techniques. Separated fractions are then digested by proteolytic enzymes, which results in complex mixture of peptides. Tens and hundreds of peptides originating from one or more proteins are routinely analyzed by MS or by combination of RP-HPLC–MS or CE–MS techniques [152,153,156,201–205].

Either 2D gels or a combination of other separation techniques is used to pre-fractionate protein mixtures prior to further digestion and peptide mixture analysis. In a sense, this fractionation is sample preparation for the terminal step of MS.

The number of samples generated by 2D gels after in gel digestion, or by tandem LC–CE is very high. Therefore, sample preparation for MS must be amenable to high throughput and automation. Thus, in this application sample preparation must meet three major requirements: (i) separate peptide mixtures from non-volatile salts, (ii) concentrate the analytes of interest, (iii) handle samples of only a few microliters in volume.

The methods described in the literature for microdesalting prior to MS were discussed in Section 3.3. Here we focus on selected applications.

MALDI-TOF-MS usually requires 0.5–1 μl of sample to be introduced onto a MALDI target. For the purpose of small volume desalting, small disposable pipette-like tips were developed, using small amounts of sorbent trapped in a tip. Millipore C_{18} ZipTips employ a C_{18} chromatographic sorbent embedded in a network of linear polymer. Large (100 μm) pores in the sorbent bed assure that liquid flow through this device is only moderately restricted. The sample can be aspirated and dispensed relatively easily using commercially available pipettors. The price paid for a low flow resistance in 100 μm pores is slow mass transfer of analyte to the stationary phase. Thus, several cycles of aspiration and dispension are recommended to improve the peptide adsorption on the sorbent. After a simple wash with deionized water, the peptides are eluted with 1–5 μl of mobile phase containing acetonitrile or methanol. In our hands ZipTips give us 50–75% recovery of selected peptides in 5 μl of eluent. However, this does not limit the usefulness of the ZipTip format for sample desalting for MS, since quantitative peptide recovery is not necessary for successful protein identification.

ZipTips are available which contain metal–chelating sorbents for selective recovery of phosphorylated peptides from complex peptide mixtures [206]. These tips are useful for the study of post-translational modifications of proteins. Other types of sorbents are also available.

Another extraction tip on the market is SuproTip from Amica. The internal walls of the tip are coated with a thin layer of sorbent. The sample flows freely through the open tip. As expected, stationary phase mass transfer of analytes in this system is severely limited; in our evaluation we observed only 1–10% recovery of peptides.

For complex peptide mixtures it is advisable to use at least one separation technique prior to MS. Recently, CE in combination with MS is gaining popularity. In order to concentrate sample for CE separations, micro SPE cartridges have been constructed by sandwiching PTFE membrane/frits that hold C_{18} sorbent particles in place inside plastic tubing sleeve. Sorbent was introduced by a methanol slurry using a gentle vacuum. CE capillaries are inserted into a sleeve and glued at both ends. The resulting C_{18} bed was approximately 1 mm long, and contained approximately 1 μg of material [152,153]. As mentioned in Section 3.3, SPE microcolumns negatively affect the quality of the CE separation.

The small dimension of the CE capillary allows construction of a sheath interface for CE–MS. This

approach increases the sensitivity of ESI-MS detection for low abundance peptides in complex digests. To reduce the dead volume in the separation system, a microcolumn for SPE–HPLC has been constructed from the microelectrospray needle itself [162,163]. After preconcentrating the protein digest on the sorbent packed needle, the non-volatile contaminants were washed off with buffers, followed by gradient elution of peptides. Microcolumns were constructed using either a membrane frit to hold chromatographic particles in place [163], or by using capillaries containing a smaller tip I.D. than the sorbent particles [162]. Prepacked electrospray columns are now commercially available from New Objective (Cambridge, MA, USA). Alternatively, ESI tips with sintered frits can be purchased and packed in the laboratory with the desired sorbent.

Special interest has been paid to DNA and peptide sample clean-up prior to MS directly on the surface of MALDI targets. Peptides were extracted using a target surface modified with C_{18} alkyl chains; after wash with the water the sample was desorbed by adding MALDI matrix in organic solvent [170]. A major limitation of hydrophobic SPE–MALDI-MS probes is the low wettability of the modified target surface. Ion pairing agents can be used to overcome this drawback [169]. Zhang and Orlando [168] improved adsorption capacity of monolayers by using a long polylysine chain chemically anchored to the MALDI target. Affinity-based protein extraction on MALDI target have also been described [171].

The quality of the MALDI-TOF-MS signal depends both on the quality of the sample and the homogeneity of crystallized MALDI matrix/sample [207]. Fast and reliable deposition of samples thus becomes a part of the sample preparation. Picoliter sample deposition for high-throughput MALDI analysis was developed recently using piezoelectric devices [31]. This flow-through device allows ''on-line'' MALDI coupling with HPLC [208,209]. Piezoceramic pulse-driven dispensers deliver 30–250 pl droplets of sample onto a target. This micromachined device has only 250 nl void volume between the capillary inlet and the dispenser nozzle. No significant difference in MALDI signal was found if the sample was applied to the plate before or after the matrix solution.

The complexity of samples for proteome research demands a combination of separation techniques to meet a separation goal. The techniques of SPE, CE, HPLC, and 2D slab gels were used for on-line or off-line fractionation prior to the final detection step, which is generally mass spectrometry. In this sense, all separation techniques can be considered as a sample preparation techniques for MS. Different schemes for 2D separations were suggested including HPLC–CE (HPLC fractions further separated by CE) [201], as well as a combination of SPE–HPLC–HPLC techniques utilizing affinity SPE for prefractionation of proteins [202]. General trends in the analysis of proteome samples were reviewed recently [205]. Mass spectrometry was used as a separation technique for differential display (comparison of two samples of the protein–peptide mixture obtained from the same organisms grown under different conditions). The labeling of one sample with one or two stable isotopes allows the direct comparison of the peptide peak intensity from both samples in one mass spectrum. This technique can identify upregulated and downregulated proteins in a very complex mixture [210].

5.3. Solid-phase extraction purification of DNA oligonucleotides

Four different applications of solid-phase extraction for DNA purification have been described: (i) isolation of genetic DNA from biological material [211–216], (ii) oligonucleotide desalting and primer removal prior to DNA sequencing [75], (iii) purification of crude synthetic oligonucleotides [217], and (iv) oligonucleotide desalting prior to genotyping by mass spectrometry [29,31,65,218–222]. Here we discuss the latter two applications.

DNA sequencing, quantitation, forensic analysis, as well as the modern methods of DNA genotyping are based on the biochemical technique known as polymerase chain reaction (PCR). PCR uses short single-stranded DNA oligonucleotides (primers) to amplify an interrogated region of double-stranded DNA. The amplification efficiency and selectivity depends on a sequence of selected primers and their purity.

With the introduction of DNA synthesizers, the synthesis of DNA primers has become an inexpensive and routine operation. Standard phosphoramidite chemistry allows for synthesis of short (typically 15–35mer) oligonucleotides in less than 30–60 min.

Even though every step of synthesis has high yield, the final purity of synthetic oligonucleotides is between 60 and 90%, depending on the primer length [223]. Prematurely halted fragments (so-called failure sequences) often need to be purified from full length DNA product.

While oligonucleotide synthesis is automated and relatively fast, the purification requires complex procedures, resulting in low sample throughput. The methods of choice are typically HPLC, slab gel electrophoresis or SPE. Although the last method does not provide high separation power, it can be successfully utilized for the ''trityl on'' method of oligonucleotide purification. ''Trityl on'' purification employs the hydrophobicity of the dimethoxytrityl (DMT) protecting group from the DNA synthesis. In the last step of DNA synthesis the DMT is not cleaved from the full-length oligonucleotide, which results in significantly greater retention of target product on reversed-phase sorbent. This mechanism is utilized for selective extraction of target oligonucleotide on the SPE cartridge, and selective wash of failure sequences from sorbent [66]. A recently published paper describes the high throughput purification of synthetic oligodeoxyribonucleotides on 96-well extraction plate [217]. The authors found that the loading flow-rate is a crucial parameter, which affects the loading capacity of cartridge. Slow mass transfer of large-molecular-mass analytes in the stationary phase causes a breakthrough at higher loading flow-rate. A 96-well extraction plate packed containing sorbent with smaller particle size improved extraction recovery. The purification protocol consisting of several steps can be fully automated using an autosampler: Sample load, selective wash of failure sequences from sorbent, on cartridge cleavage of DMT group by 2% trifluoroacetic acid, and final elution of purified product. Purity of target product is typically ~90% or higher.

With advances in the Human Genome Project, the main interest has shifted from de-novo DNA sequencing to the search for individual genetic variances. Many methods for genotyping have been proposed, mass spectrometry among them [29,31,65,218–222]. Both electrospray ionization and MALDI-MS sensitivity dramatically decreases when sample is contaminated with non-volatile ions. Presence of sodium salts (often required for PCR and other enzymatic reactions) also complicates the MS spectrum by formation of sodium adducts with DNA [224,225]. Fast and reliable methods for high throughput desalting were developed using 96-well extraction plates [65]. Typically 1–3 pmol of DNA PCR products is loaded onto a plate in triethylamine acetate (TEAAc) buffer, and non-volatile salts are washed off with few milliliters of TEAAc buffer followed by water. Desalted DNA is eluted in a small amount (20 μl) of 70% acetonitrile in water. Samples are evaporated at 60°C, reconstituted in MALDI matrix and a 1 μl volume placed onto the MALDI target.

The advantage of SPE for desalting is the high degree of purification, possible concentration and the ease of automation, particularly with 96-well (or 384-well) extraction plates. Other modes of SPE such as pipette tips packed with small amount of sorbent (ZipTip, Millipore), and on MALDI target sample clean-up were successfully used for DNA desalting. Automation of sample preparation prior to MS analysis is a major concern due to the fact that the number of samples is expected to be tens of thousands a day.

6. Conclusions and future prospects

Current trends in analysis are driven by both market and technological developments. High throughput sequencing of whole genomes and the proliferation of millions of new chemical entities by combinatorial means is ushering in a new age for chemical analysis. ''Big Science'', once only in the realm of nuclear and particle physics, has shifted to pharmaceutical and biochemical endeavors. Since most of the advances in scientific disciplines require and are often driven by the development of tools, it is clear the tools for increasing the throughput of chemical analysis are needed.

In the least decade, three key developments in analytical instrumentation have occurred: (1) mass spectrometric interfaces that allow generalized use of HPLC–MS, (2) MALDI-TOF-MS for the analysis of high-molecular-mass biopolymers, and (3) capillary electrophoresis for use in high throughput DNA sequencing. These instruments have revolutionized how analytical chemistry is and will in the future be done.

As mass spectrometry users shift from the physical chemist to the analytical chemist, mass spectrometer instrumentation manufacturers are designing smaller and more user friendly tools. Until recently, instruments required significant space and a high degree of user sophistication, while the latest instruments can easily fit on a benchtop. This means that a HPLC system is becoming more an interface or ''inlet'' to an MS system, and less a separation tool. Thus, the distinction between HPLC and SPE is blurring with an emphasis on high throughput analysis. Clearly, automation of SPE is, and will continue to be, an active area of sample preparation development. Commercialization of 384- and 1536-well SPE plates for high throughput parallel sample preparation is an active area of development; such devices with the ancillary robotics, will emerge in the near future.

Miniaturization of SPE devices for the analysis of biopolymers is an area of active development. The main factors driving this effort is a combination of the need for high throughput sample preparation, and the desire to both purify and concentrate samples. For instance, in the case of DNA analysis, the ability to concentrate a sample prior to MALDI-TOF-MS means that PCR, the step which often governs cost of analysis, can be conducted on both a smaller scale and with less cycles (i.e., faster).

The advent of a total analytical system (TAS) on a chip may mean that in the future sample preparation may become integrated on such devices. At this time the benefits of such integration are unclear. One exception is in handheld devices for field use [e.g., chemical and biological weapons (CBW) detection systems for first responders]. The rational for this statement is that in most research and development laboratories there is a desire for flexibility of operations, resulting in the use of workstations rather than integrated fully automated systems.

Methods of sample preparation will continue to evolve as the needs of the research community change. That will increase the fundamental need for sample preparation, that along with sampling often determine the success of an analysis.

7. Nomenclature

1D	One-dimensional
2D	Two-dimensional
ACN	Acetonitrile
ALS	Acid labile surfactant
CBW	Chemical and biological weapons
CE	Capillary electrophoresis
DEC	Disposable extraction cartridge
DMT	Dimethoxytrityl
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
HILIC	Hydrophilic interaction chromatography
HLB	Hydrophilic–lipophilic balanced
HPLC	High-performance liquid chromatography
I.D.	Internal diameter
ITP	Isotachophoresis
LIF	Laser-induced fluorescence
LLE	Liquid–liquid extraction
LSD	Lysergic acid diethylamide
MALDI	Matrix-assisted laser desorption/ionization
MIP	Molecularly imprinted polymer
M_r	Molecular mass
MS	Mass spectrometry
MWCO	Molecular mass cut off
μLC	Micro liquid chromatography
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
p*I*	Isoelectric point
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
RAM	Restricted access media
RP	Reversed-phase
SDS	Sodium dodecyl sulfate
SELDI	Surface enhanced laser desorption/ionization
SIR	Selected ion recording
SLM	Supported liquid membrane
SPE	Solid-phase extraction
TAS	Total analytical system
TEAAc	Triethylamine acetate
TEC	Trace enrichment cartridge
TOF	Time-of-flight

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References

[1] K.G. Wahlund, J. Chromatogr. 218 (1981) 671.
[2] B.R. Manno, J.E. Manno, C.A. Dempsey, M.A. Wood, J. Anal. Toxicol. 5 (1981) 24.
[3] D. Westerlund, Chromatographia 24 (1987) 155.
[4] J. Blanchard, J. Chromatogr. 226 (1981) 455.
[5] T. Arvidsson, K.G. Wahlund, N. Daoud, J. Chromatogr. 317 (1984) 213.
[6] R.D. McDowall, J. Chromatogr. 492 (1989) 3.
[7] D.C. Turnell, J.D. Cooper, J. Chromatogr. 492 (1989) 59.
[8] M.C. Hennion, J. Chromatogr. A 856 (1999) 3.
[9] R.E. Majors, LC–GC (Suppl.) May (1998) S8.
[10] E.S.P. Bouvier, D.M. Martin, P.C. Iraneta, M. Capparella, Y.F. Cheng, D. Phillips, LC·GC 15 (1997) 152.
[11] E.S.P. Bouvier, P.C. Iraneta, U.D. Neue, P.D. McDonald, D. Phillips, M. Capparella, Y.F. Cheng, LC·GC May (1998) S53.
[12] B. Kaye, W.J. Herron, P.V. Macrae, S. Robinson, D.A. Stopher, R.F. Venn, W. Wild, Anal. Chem. 68 (1996) 1658.
[13] J. Janiszewski, P. Schneider, K. Hoffmaster, M. Swyden, D. Wells, H. Fouda, Rapid. Commun. Mass Spectrom. 11 (1997) 1033.
[14] H. Simpson, A. Berthemy, D. Buhrman, R. Burton, J. Newton, M. Kealy, D. Wells, D. Wu, Rapid Commun. Mass Spectrom. 12 (1998) 75.
[15] S.L. Callejas, R.A. Biddlecombe, A.E. Jones, K.B. Joyce, A.I. Pereira, S. Pleasance, J. Chromatogr. B 718 (1998) 243.
[16] S. Chiron, D. Barcelo, J. Chromatogr. 645 (1993) 125.
[17] C.G. Markell, D.F. Hagen, V. Bunnelle, LC–GC 9 (1991) 332.
[18] G.L. Lensmeyer, D.A. Wiebe, B.A. Darcey, J. Chromatogr. Sci. 29 (1991) 444.
[19] G.L. Lensmeyer, D.A. Wiebe, T.C. Doran, Ther. Drug Monit. 14 (1992) 408.
[20] J. Hempenius, R.J. Steenvoorden, F.M. Lagerwerf, J. Wieling, J.H. Jonkman, J. Pharm. Biomed. Anal. 20 (1999) 889.
[21] D.D. Blevins, D.O. Hall, LC–GC (Suppl.) May (1998) S16.
[22] R.S. Plumb, R.D. Gray, C.M. Jones, J. Chromatogr. B 694 (1997) 123.
[23] M.G. Pluskal, Nat. Biotechnol. 18 (2000) 104.
[24] X. Jin, Y. Chen, D.M. Lubman, D. Misek, S.M. Hanash, Rapid Commun. Mass Spectrom. 13 (1999) 2327.
[25] K. Haupt, K. Mosbach, Trends Biotechnol. 16 (1998) 468.
[26] K. Mosbach, K. Haupt, J. Mol. Recognit. 11 (1998) 62.
[27] L. Ye, O. Ramstrom, M.O. Mansson, K. Mosbach, J. Mol. Recognit. 11 (1998) 75.
[28] O. Ramstrom, K. Mosbach, Curr. Opin. Chem. Biol. 3 (1999) 759.
[29] K. Tang, D.J. Fu, D. Julien, A. Braun, C.R. Cantor, H. Koster, Proc. Natl. Acad. Sci. USA 96 (1999) 10016.
[30] N.H. Chiu, K. Tang, P. Yip, A. Braun, H. Koster, C.R. Cantor, Nucleic Acids Res. 28 (2000) E31.
[31] D.P. Little, T.J. Cornish, M.J. O'Donnell, A. Braun, R.J. Cottler, H. Koster, Anal. Chem. 69 (1997) 4540.
[32] E. Riva, R. Merati, L. Cavenaghi, J. Chromatogr. 553 (1991) 35.
[33] A. Haque, J.T. Stewart, Biomed. Chromatogr. 13 (1999) 51.
[34] F. Jamali, A.S. Russell, B.W. Berry, C. Lehmann, J. Chromatogr. 310 (1984) 327.
[35] J. Ostrowski, J. Theumer, W. Gartner, K. Resag, H. Passing, J. Chromatogr. 309 (1984) 115.
[36] S.F. Chang, T.M. Welscher, R.E. Ober, J. Pharm. Sci. 72 (1983) 236.
[37] H. Breithaupt, M. Wilfling, J. Chromatogr. 230 (1982) 97.
[38] J.R. Veraart, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. A 856 (1999) 483.
[39] R.H. Kopp, Ind. Eng. Chem., Prod. Res. 14 (1975) 96.
[40] B.A. Quame, US Pat. 3567029, 2 March (1971).
[41] A.D. Wells, LC·GC 17 (1999) 600.
[42] G. Rule, M. Chapple, S. Lowes, J. Henion, presented at the ASMS 2000 Symposium, Long Beach, CA, 2000, poster WPH 299.
[43] S. Pleasance, R.A. Biddlecombe, C. Benevides, presented at the ASMS 2000 Symposium, Long Beach, CA, 2000, poster ThPD 155.
[44] N.C. van de Merbel, J. Chromatogr. A 856 (1999) 55.
[45] P. Chiap, B. Evrard, M.A. Bimazubute, P. de Tullio, P. Hubert, L. Delattre, J. Crommen, J. Chromatogr. A 870 (2000) 121.
[46] J.A. Jonsson, M. Andersson, C. Melander, J. Norberg, E. Thordarson, L. Mathiasson, J. Chromatogr. A 870 (2000) 151.
[47] T. Buttler, C. Nilsson, L. Gorton, G. Marko-Varga, T. Laurell, J. Chromatogr. A 725 (1996) 41.
[48] Z. Yu, D. Westerlund, K.S. Boos, J. Chromatogr. B 704 (1997) 53.
[49] Z. Yu, D. Westerlund, J. Chromatogr. A 725 (1996) 137.
[50] M. Dolezalova, J. Chromatogr. 579 (1992) 291.
[51] K.-S. Boos, A. Rudolphi, LC–GC 15 (1997) 602.
[52] W.R.G. Baeyens, G. Van der Weken, J. Haustraete, H.Y. Aboul-Enein, S. Corveleyn, J.P. Remon, A.M. Garcia-Campana, P. Deprez, J. Chromatogr. A 871 (2000) 153.
[53] M.W. Nielen, R.E. van Soest, H.E. van Ingen, A. Farjam, R.W. Frei, U.A.Th. Brinkman, J. Chromatogr. 417 (1987) 159.
[54] M.W. Nielen, A.J. Valk, R.W. Frei, U.A.Th. Brinkman, P. Mussche, R. De Nijs, B. Ooms, W. Smink, J. Chromatogr. 393 (1987) 69.
[55] J. De Jong, M.W. Nielen, R.W. Frei, U.A.Th. Brinkman, J. Chromatogr. 381 (1986) 431.
[56] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, J. Chromatogr. B 709 (1998) 243.
[57] R.S. Plumb, G.J. Dear, D. Mallett, I.J. Fraser, J. Ayrton, C. Ioannou, Rapid Commun. Mass Spectrom. 13 (1999) 865.
[58] J.S. Janiszewski, M.C. Swyden, H.G. Fouda, J. Chromatogr. Sci. 38 (2000) 255.
[59] J. Ding, U.D. Neue, Rapid Commun. Mass Spectrom. 13 (1999) 2151.
[60] F. Xiang, Y. Lin, J. Wen, D.W. Matson, R.D. Smith, Anal. Chem. 71 (1999) 1485.
[61] N. Xu, Y. Lin, S.A. Hofstadler, D. Matson, C.J. Call, R.D. Smith, Anal. Chem. 70 (1998) 3553.
[62] E.S.M. Lutz, M. Larsson, Chromatographia Suppl I. 49 (1999) S29.

[63] C.F. Poole, A.D. Ganatilleka, R. Sethuraman, J. Chromatogr. A 885 (2000) 17.
[64] E.S.P. Bouvier, LC–GC 13 (1995) 852.
[65] A. Belenky, B.H. Wang, E. Budman, C.R. Mallet, E.S.P. Bouvier, M. Gilar, presented at the DNA 2000 Symposium, Boston, MA, 2000, poster 9.
[66] K.M. Lo, S.S. Jones, N.R. Hackett, H.G. Khorana, Proc. Natl. Acad. Sci. USA 82 (1984) 2285.
[67] F. Kirpekar, E. Nordhoff, L.K. Larsen, K. Kristiansen, P. Roepstorff, F. Hillenkamp, Nucleic Acids Res. 26 (1998) 2554.
[68] G.B. Hurst, K. Weaver, M.J. Doktycz, M.V. Buchanan, A.M. Costello, M.E. Lidstrom, Anal. Chem. 70 (1998) 2693.
[69] Z. Yan, G.W. Caldwell, W.J. Jones, J.A. Masucci, Anal. Biochem. 277 (2000) 267.
[70] R.D. Bagshaw, J.W. Callahan, D.J. Mahuran, Anal. Biochem. 284 (2000) 432.
[71] L.K. Watkins, P.V. Bondarenko, D.C. Barbacci, S. Song, S.L. Cockrill, D.H. Russell, R.D. Macfarlane, J. Chromatogr. A 840 (1999) 183.
[72] S. Chiron, A.F. Alba, D. Barcelo, Environ. Sci. Technol. 27 (1993) 2352.
[73] Y.F. Cheng, U.D. Neue, L. Bean, J. Chromatogr. A 828 (1998) 273.
[74] Brochure WB025, Waters, Milford, MA, (1999).
[75] O. Salas-Solano, M.C. Ruiz-Martinez, E. Carrilho, L. Kotler, B.L. Karger, Anal. Chem. 70 (1998) 1528.
[76] M.C. Ruiz-Martinez, O. Salas-Solano, E. Carrilho, L. Kotler, B.L. Karger, Anal. Chem. 70 (1998) 1516.
[77] J. Cai, J. Henion, Anal. Chem. 68 (1996) 72.
[78] J. Cai, J. Henion, J. Chromatogr. B 691 (1997) 357.
[79] B.A. Rashid, G.W. Aherne, M.F. Katmeh, P. Kwasowski, D. Stevenson, J. Chromatogr. A 797 (1998) 245.
[80] E. Brandsteterova, K. Krajnak, I. Skacani, Pharmazie 50 (1995) 825.
[81] C.S. Creaser, S.J. Feely, E. Houghton, M. Seymour, J. Chromatogr. A 794 (1998) 37.
[82] C. Miege, M. Bouzige, S. Nicol, J. Dugay, V. Pichon, M.C. Hennion, J. Chromatogr. A 859 (1999) 29.
[83] P. Martin, I.D. Wilson, G.R. Jones, J. Chromatogr. A 889 (2000) 143.
[84] O. Bruggemann, K. Haupt, L. Ye, E. Yilmaz, K. Mosbach, J. Chromatogr. A 889 (2000) 15.
[85] M. Kempe, K. Mosbach, J. Chromatogr. A 691 (1995) 317.
[86] R.J. Ansell, K. Mosbach, Analyst 123 (1998) 1611.
[87] L.I. Andersson, R. Muller, G. Vlatakis, K. Mosbach, Proc. Natl. Acad. Sci. USA 92 (1995) 4788.
[88] G. Vlatakis, L.I. Andersson, R. Muller, K. Mosbach, Nature 361 (1993) 645.
[89] M. Kempe, L. Fischer, K. Mosbach, J. Mol. Recognit. 6 (1993) 25.
[90] M. Kempe, K. Mosbach, Int. J. Pept. Protein. Res. 44 (1994) 603.
[91] W.R. Hunsaker, H. Badri, M. Lombardo, M.L. Collins, Anal. Biochem. 181 (1989) 360.
[92] D.V. Morrissey, M. Lombardo, J.K. Eldredge, K.R. Kearney, E.P. Groody, M.L. Collins, Anal. Biochem. 181 (1989) 345.
[93] H. Tsurui, Y. Kumazawa, R. Sanokawa, K. Watanabe, A. Wada, T. Shirai, Nucleic Acids Symp. Ser. 25 (1991) 149.
[94] M. Mitsuhashi, C. Keller, T. Akitaya, Nature 357 (1992) 519.
[95] Y. Miura, Y. Ichikawa, T. Ishikawa, M. Ogura, R. de Fries, H. Shimada, M. Mitsuhashi, Clin. Chem. 42 (1996) 1758.
[96] J.M. Wages Jr., G.M. Wages, P. Matthews, D. Weller, J. Summerton, Biotechniques 23 (1997) 1116.
[97] P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt, Science 254 (1991) 1497.
[98] M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S.M. Freier, D.A. Driver, R.H. Berg, S.K. Kim, B. Norden, P.E. Nielsen, Nature 365 (1993) 566.
[99] D.P. Chandler, J.R. Stults, K.K. Anderson, S. Cebula, B.L. Schuck, F.J. Brockman, Anal. Biochem. 283 (2000) 241.
[100] H. Orum, P.E. Nielsen, M. Jorgensen, C. Larsson, C. Stanley, T. Koch, Biotechniques 19 (1995) 472.
[101] I. German, D.D. Buchanan, R.T. Kennedy, Anal. Chem. 70 (1998) 4540.
[102] R.A. Potyrailo, R.C. Conrad, A.D. Ellington, G.M. Hieftje, Anal. Chem. 70 (1998) 3419.
[103] W. Warren, M. Merion, BioChromatography 3 (1988) 225.
[104] K.A. Wreggett, R.F. Irvine, Biochem. J. 245 (1987) 655.
[105] K.A. Wreggett, R.F. Irvine, Biochem. J. 262 (1989) 997.
[106] Q.C. Meng, M.S. Cepeda, T. Kramer, H. Zou, D.J. Matoka, J. Farrar, J. Chromatogr. B 742 (2000) 115.
[107] R.C. Lewis, B.A. Phillips, J.R. Baldwin, D.T. Rossi, P.K. Narang, Pharm. Res. 9 (1992) 101.
[108] Brochure WB071, Waters, Milford, MA, (1998).
[109] Brochure WA00435, Waters, Milford, MA, (2000).
[110] J.J.P. Lee, J.E. O'Gara, D.P. Walsh, A.R. Pelissey, E.S.P. Bouvier, P.C. Iraneta, Y.F. Cheng, D.J. Phillips, presented at the 23rd International Symposium on High-Performance Liquid Phase Separations and Related Techniques, Granada, 1999, poster PB5/20.
[111] S.F. Xie, F. Svec, J.M.J. Frechet, Chem. Mater. 10 (1998) 4072.
[112] I. Gusev, X. Huang, C. Horvath, J. Chromatogr. A 855 (1999) 273.
[113] R.E. Moore, L. Licklider, D. Schumann, T.D. Lee, Anal. Chem. 70 (1998) 4879.
[114] E.C. Peters, M. Petro, F. Svec, J.M. Frechet, Anal. Chem. 70 (1998) 2288.
[115] E.C. Peters, M. Petro, F. Svec, J.M. Frechet, Anal. Chem. 69 (1997) 3646.
[116] W. Voelter, T. Kronbach, K. Zecg, R. Huber, J. Chromatogr. 239 (1982) 475.
[117] K. Zech, R. Huber, J. Chromatogr. 353 (1986) 351.
[118] A. van der Horst, P.N. de Goede, H.J. Willems, A.C. van Loenen, J. Chromatogr. 381 (1986) 185.
[119] M. Gilar, unpublished results.
[120] A. Sioufi, J. Richard, P. Mangoni, J. Godbillon, J. Chromatogr. 565 (1991) 401.
[121] X.H. Chen, J.P. Franke, K. Ensing, J. Wijsbeek, R.A. de Zeeuw, J. Anal. Toxicol. 17 (1993) 421.
[122] K.J. Swart, H.K. Hundt, A.M. Groenewald, J. Chromatogr. A 663 (1994) 65.

[123] M.C. Rouan, J. Campestrini, J.B. Lecaillon, J.P. Dubois, M. Lamontagne, B. Pichon, J. Chromatogr. 456 (1988) 45.
[124] R. Said, D. Robinet, C. Barbier, J. Sartre, C. Huguet, J. Chromatogr. 530 (1990) 11.
[125] M. Breda, G. Basileo, G. Fonte, J. Long, C.A. James, J. Chromatogr. A 854 (1999) 81.
[126] D.J. Anderson, Anal. Chem. 65 (1993) 434R.
[127] S. Schmitz, F.V. Warren, B.A. Bidlingmeyer, J. Pharm. Biomed. Anal. 9 (1991) 985.
[128] J.V. Posluszny, R. Weinberger, E. Woolf, J. Chromatogr. 507 (1990) 267.
[129] K.S. Boos, in: Proceedings from Solid-Phase Extraction Europe Symposium, Amsterdam, November 1994, 1994, p. 67.
[130] T.C. Pinkerton, J. Chromatogr. 544 (1991) 13.
[131] H. Tanaka, K. Takahashi, M. Ohira, J. Chromatogr. A 869 (2000) 151.
[132] D. Zimmer, V. Pickard, W. Czembor, C. Muller, J. Chromatogr. A 854 (1999) 23.
[133] E.S.P. Bouvier, unpublished results
[134] B.A.P. Buscher, U.R. Tjaden, J. van der Greef, J. Chromatogr. A 764 (1997) 135.
[135] P. Kuban, B. Karlberg, Anal. Chem. 69 (1997) 1169.
[136] J.R. Veraart, J. van Hekezen, M.C. Groot, C. Gooijer, H. Lingeman, N.H. Velthorst, U.A.Th. Brinkman, Electrophoresis 19 (1998) 2944.
[137] J.R. Veraart, M.C. Groot, C. Gooijer, H. Lingeman, N.H. Velthorst, U.A.Th. Brinkman, Analyst 124 (1999) 115.
[138] D.S. Stegehuis, H. Irth, U.R. Tjaden, J. Van der Greef, J. Chromatogr. 538 (1991) 393.
[139] D. Kaniansky, E. Krcmova, V. Madajova, M. Masar, Electrophoresis 18 (1997) 260.
[140] M.H. Lamoree, N.J. Reinhoud, U.R. Tjaden, W.M. Niessen, J. van der Greef, Biol. Mass Spectrom. 23 (1994) 339.
[141] F. Foret, E. Szoko, B.L. Karger, Electrophoresis 14 (1993) 417.
[142] A.J. Tomlinson, L.M. Benson, S. Jameson, S. Naylor, Electrophoresis 17 (1996) 1801.
[143] J.C. Waterval, C.J. la Porte, R. van 't Hof, J. Teeuwsen, A. Bult, H. Lingeman, W.J. Underberg, Electrophoresis 19 (1998) 3171.
[144] D.K. Lloyd, J. Chromatogr. A 735 (1996) 29.
[145] N.A. Guzman, S.S. Park, D. Schaufelberger, L. Hernandez, X. Paez, P. Rada, A.J. Tomlinson, S. Naylor, J. Chromatogr. B 697 (1997) 37.
[146] A.J. Tomlinson, N.A. Guzman, S. Naylor, J. Cap. Electrophoresis 2 (1995) 247.
[147] Y. Xiong, S.R. Park, H. Swerdlow, Anal. Chem. 70 (1998) 3605.
[148] Y. Zhao, C.E. Lunte, Anal. Chem. 71 (1999) 3985.
[149] Y. Zhao, K. McLaughlin, C.E. Lunte, Anal. Chem. 70 (1998) 4578.
[150] S. Palmarsdottir, E. Thordarson, L.E. Edholm, J.A. Jonsson, L. Mathiasson, Anal. Chem. 69 (1997) 1732.
[151] J.Z. Song, J. Chen, S.J. Tian, Z.P. Sun, J. Pharm. Biomed. Anal. 21 (1999) 569.
[152] D. Figeys, R. Aebersold, Electrophoresis 19 (1998) 885.
[153] D. Figeys, A. Ducret, R. Aebersold, J. Chromatogr. A 763 (1997) 295.
[154] M.A. Strausbauch, J.P. Landers, P.J. Wettstein, Anal. Chem. 68 (1996) 306.
[155] M.A. Strausbauch, S.J. Xu, J.E. Ferguson, M.E. Nunez, D. Machacek, G.M. Lawson, P.J. Wettstein, J.P. Landers, J. Chromatogr. A 717 (1995) 279.
[156] C.J. Herring, J. Qin, Rapid Commun. Mass Spectrom. 13 (1999) 1.
[157] A.J. Tomlinson, S. Naylor, J. Cap. Electrophoresis 2 (1995) 225.
[158] N.J. Clarke, F. Li, A.J. Tomlinson, S. Naylor, J. Am. Soc. Mass Spectrom. 9 (1998) 88.
[159] E. Rohde, A.J. Tomlinson, D.H. Johnson, S. Naylor, J. Chromatogr. B 713 (1998) 301.
[160] C.G. Huber, M.R. Buchmeiser, Anal. Chem. 70 (1998) 5288.
[161] B.H. Forngren, J. Samskog, S.A. Gustavsson, N. Tyrefors, K.E. Markides, B. Langstrom, J. Chromatogr. A 854 (1999) 155.
[162] C.L. Gatlin, G.R. Kleemann, L.G. Hays, A.J. Link, J.R. Yates III, Anal. Biochem. 263 (1998) 93.
[163] M.D. McGinley, M.T. Davis, J.H. Robinson, C.S. Spahr, E.J. Bures, J. Beierle, J. Mort, S.D. Patterson, Electrophoresis 21 (2000) 1678.
[164] P. Jeno, P.E. Scherer, U. Manning-Krieg, M. Horst, Anal. Biochem. 215 (1993) 292.
[165] K. Eguchi, Y. Hamaguchi, Y. Aso, T. Shioiri, M. Ogura, M. Mitsuhashi, Clin. Chem. 44 (1998) 2208.
[166] J.M. Schuette, U. Pieles, S.D. Maleknia, G.S. Srivatsa, D.L. Cole, H.E. Moser, N.B. Afeyan, J. Pharm. Biomed. Anal. 13 (1995) 1195.
[167] P.L. Ross, P.A. Davis, P. Belgrader, Anal. Chem. 70 (1998) 2067.
[168] L. Zhang, R. Orlando, Anal. Chem. 71 (1999) 4753.
[169] M.E. Warren, A.H. Brockman, R. Orlando, Anal. Chem. 70 (1998) 3757.
[170] A.H. Brockman, B.S. Dodd, R. Orlando, Anal. Chem. 69 (1997) 4716.
[171] A.H. Brockman, R. Orlando, Rapid. Commun. Mass Spectrom. 10 (1996) 1688.
[172] A.H. Brockman, R. Orlando, Anal. Chem. 67 (1995) 4581.
[173] M. Merchant, S.R. Weinberger, Electrophoresis 21 (2000) 1164.
[174] M. Fountoulakis, H. Langen, Anal. Biochem. 250 (1997) 153.
[175] G.J. Langley, J.M. Herniman, N.L. Davies, T. Brown, Rapid Commun. Mass Spectrom. 13 (1999) 1717.
[176] S. Kjellstrom, J. Emneus, T. Laurell, L. Heintz, G. Marko-Varga, J. Chromatogr. A 823 (1998) 489.
[177] T.R. Tsai, F.C. Cheng, L.C. Hung, C.F. Chen, T.H. Tsai, J. Chromatogr. B 736 (1999) 129.
[178] F.C. Cheng, L.L. Yang, D.Y. Yan, T.H. Tsai, C.W. Lee, S.H. Chen, J. Chromatogr. A 870 (2000) 389.
[179] J.C. Severs, R.D. Smith, Anal. Chem. 69 (1997) 2154.
[180] C. Liu, S.A. Hofstadler, J.A. Bresson, H.R. Udseth, T. Tsukuda, R.D. Smith, A.P. Synder, Anal. Chem. 70 (1998) 1797.

[181] C. Liu, Q. Wu, A.C. Harms, R.D. Smith, Anal. Chem. 68 (1996) 3295.
[182] N. Snippe, N.C. van de Merbel, F.P. Ruiter, O.M. Steijger, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. B 662 (1994) 61.
[183] N.C. Van de Merbel, J.M. Teule, H. Lingeman, U.A.Th. Brinkman, J. Pharm. Biomed. Anal. 10 (1992) 225.
[184] R. Herraez-Hernandez, N.C. van de Merbel, U.A.Th. Brinkman, J. Chromatogr. B 666 (1995) 127.
[185] B.L. Hogan, S.M. Lunte, J.F. Stobaugh, C.E. Lunte, Anal. Chem. 66 (1994) 596.
[186] F.C. Cheng, J.S. Kuo, H.M. Huang, D.Y. Yang, T.F. Wu, T.H. Tsai, J. Chromatogr. A 870 (2000) 405.
[187] S. Palmarsdottir, L. Mathiasson, J.A. Jonsson, L.E. Edholm, J. Cap. Electrophoresis 3 (1996) 255.
[188] H. Gu, D. Wilson, J. Inselburg, J. Biochem. Biophys. Methods 24 (1992) 45.
[189] D. Symons, C. Clarkson, Nucleic Acids Res. 16 (1988) 5208.
[190] W.G. Stroop, Anal. Biochem. 169 (1988) 194.
[191] U. Gobel, R. Maas, A. Clad, J. Biochem. Biophys. Methods 14 (1987) 245.
[192] P.F. Cancalon, J. AOAC Int. 82 (1999) 95.
[193] K.L. Tyczkowska, R.D. Voyksner, K.L. Anderson, M.G. Papich, J. Chromatogr. B 658 (1994) 341.
[194] K.J. Shingfield, N.W. Offer, J. Chromatogr. B 706 (1998) 342.
[195] R.D. McDowall, E. Doyle, G.S. Murkitt, V.S. Picot, J. Pharm. Biomed. Anal. 7 (1989) 1087.
[196] R.A. Biddlecombe, S. Pleasance, J. Chromatogr. B 734 (1999) 257.
[197] J. Bai, Y.H. Liu, T.C. Cain, D.M. Lubman, Anal. Chem. 66 (1994) 3423.
[198] X. Liang, J. Bai, Y.H. Liu, D.M. Lubman, Anal. Chem. 68 (1996) 1012.
[199] M.M. Vestling, C. Fenselau, Biochem. Soc. Trans. 22 (1994) 547.
[200] D. Fabris, M.M. Vestling, M.M. Cordero, V.M. Doroshenko, R.J. Cotter, C. Fenselau, Rapid Commun. Mass Spectrom. 9 (1995) 1051.
[201] H.J. Issaq, K.C. Chan, G.M. Janini, G.M. Muschik, Electrophoresis 20 (1999) 1533.
[202] M. Geng, J. Ji, F.E. Regnier, J. Chromatogr. A 870 (2000) 295.
[203] P. Cao, M. Moini, Rapid Commun. Mass Spectrom. 12 (1998) 864.
[204] M. Mann, Nat. Biotechnol. 17 (1999) 954.
[205] A. Pandey, M. Mann, Nature 405 (2000) 837.
[206] C.L. Brucato, A. Schmitzer, W. Kapaciewicz, presented at the 19th International Symposium on the Separation and Analysis of Proteins, Peptides and Polynucleotides, Delray Beach, FL, 1999, poster 115.
[207] P. Onnerfjord, S. Ekstrom, J. Bergquist, J. Nilsson, T. Laurell, G. Marko-Varga, Rapid Commun. Mass Spectrom. 13 (1999) 315.
[208] P. Onnerfjord, J. Nilsson, L. Wallman, T. Laurell, G. Marko-Varga, Anal. Chem. 70 (1998) 4755.
[209] T. Miliotis, S. Kjellstrom, J. Nilsson, T. Laurell, L.E. Edholm, G. Marko-Varga, J. Mass Spectrom. 35 (2000) 369.
[210] S.P. Gygi, B. Rist, S.A. Gerber, F. Turecek, M.H. Gelb, R. Aebersold, Nat. Biotechnol. 17 (1999) 994.
[211] G. Konat, G. Gantt, I. Laszkiewicz, E.L. Hogan, Exp. Cell Res. 190 (1990) 294.
[212] J.D. Harding, G. Gebeyehu, R. Bebee, D. Simms, L. Klevan, Nucleic Acids Res. 17 (1989) 6947.
[213] S. Parzer, C. Mannhalter, Biochem. J. 273 (1991) 229.
[214] P. Chomczynski, K. Mackey, R. Drews, W. Wilfinger, Biotechniques 22 (1997) 550.
[215] W.R. Boom, H.M.A. Adriaanse, T. Kievits, P.F. Lens, US patent 5234809 (1993).
[216] M. Colpan, J. Schorr, P. Moritz, US patent 5990301 (1999).
[217] M. Gilar, E.S.P. Bouvier, J. Chromatogr. A 890 (2000) 167.
[218] D.P. Little, A. Braun, M.J. O'Donnell, H. Koster, Nat. Med. 3 (1997) 1413.
[219] A. Braun, D.P. Little, H. Koster, Clin. Chem. 43 (1997) 1151.
[220] S.J. Laken, P.E. Jackson, K.W. Kinzler, B. Vogelstein, P.T. Strickland, J.D. Groopman, M.D. Friesen, Nat. Biotechnol. 16 (1998) 1352.
[221] L.A. Haff, I.P. Smirnov, Nucleic Acids Res. 25 (1997) 3749.
[222] L.A. Haff, I.P. Smirnov, Genome Res. 7 (1997) 378.
[223] W.J. Warren, G. Vella, Mol. Biotechnol. 4 (1995) 179.
[224] C.G. Huber, A. Krajete, J. Chromatogr. A 870 (2000) 413.
[225] C.G. Huber, A. Krajete, Anal. Chem. 71 (1999) 3730.
[226] E.K. Brown, E.S.P. Bouvier, J. Ding, R.G. Herbert, J.J. Lee, presented at the 19th International Symposium on the Separation and Analysis of Proteins, Peptides and Polynucleotides, Delray Beach, FL, 1999, poster 213.