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Review

Automating solid-phase extraction: current aspects and future prospects

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Abstract

This paper reviews current trends and techniques in automated solid-phase extraction. The area has shown a dramatic growth the number of manuscripts published over the last 10 years, including applications in environmental science, food science, clinical chemistry, pharmaceutical bioanalysis, forensics, analytical biochemistry and organic synthesis. This dramatic increase of more that 100% per year can be attributed to the commercial availability of higher throughput 96-well workstations and extraction plates that allow numerous samples to be processed simultaneously. These so-called parallel-processing workstations represent the highest throughput systems currently available. The advantages and limitations of other types of systems, including discrete column systems and on-line solid-phase extraction are also discussed. Discussions of how automated solid-phase extractions can be developed, generic approaches to automated solid-phase extraction, and three noteworthy examples of automated extractions are given. The last part of the review suggests possible near- and long-term directions of automated solid-phase extraction.

Keywords: Reviews; Solid-phase extraction; Automation; Sample preparation

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1. The automation trend

The use of automation in solid-phase extraction sample preparation is now increasing dramatically. In 1989, after years of struggle, only a few published papers had appeared. A decade later, it is becoming easy to find good, relevant applications of automated solid-phase extraction in most branches of analytical chemistry literature. Since 1995, these applications have been in the areas of environmental science [1–8], food science [9–16], clinical chemistry [17–36], pharmaceutical bioanalysis [37–98], forensics [99–105], analytical biochemistry [106–111] and organic synthesis [112], but examples of automated solid-phase extraction can be found in other areas of analytical chemistry where high throughput is required. The chart in Fig. 1 shows the number of published examples of automated solid-phase extraction, demonstrating a dramatic growth rate of more than 100% per year between 1989 and 1999.

This paper reviews the current trends and approaches for automated solid-phase extraction. It explores the capabilities and limitations of these varied approaches and attempts to answer the obvious question of why automated solid-phase extraction is making big leaps forward after a debut that, while widely heralded, was slow and unimpressive. In the most ambitious part of this undertaking, we attempt to predict future directions and growth areas for the automated solid-phase extraction experiment, technologies that are established but, by no means, mature.

2. Advantages and limitations of automated solid-phase extraction

In order to effectively apply automated solid-phase extraction it is necessary that the advantages and limitations of the approach be understood. Some of the major potential advantages and limitations are summarized in Table 1. One long-standing advantage of automated solid-phase extraction systems was that unattended operation and minimal operator interven-

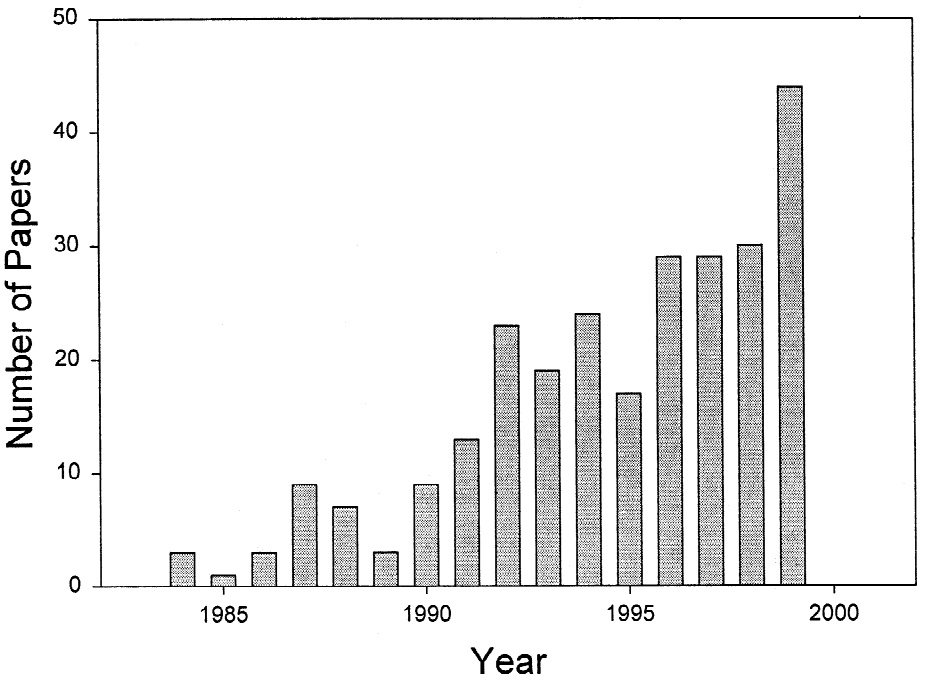

Fig. 1. Bar chart showing the number of automated solid-phase extraction papers published in the past 20 years. The number for the year 1999 is an estimate, based on the number of papers (22) appearing between January and June of this year.

Table 1
Some significant potential advantages and limitations of automated solid-phase extraction

Advantages	Disadvantages
Time savings	Carryover can limit performance
Higher throughput through the use of a parallel processing algorithm	Systematic errors can occur undetected and error recovery is sometimes a problem
Improved precision and accuracy	Precision is worse with systematic errors
Safety. Automation lessons exposure to pathogenic or otherwise hazardous samples	Sample stability (physical or chemical) is occasionally a problem when sequential processing is used
Reduced assay tedium	
Automated method development is possible	

tion allowed for timesaving. Analysts could redirect their time to other tasks during the course of the automated solid-phase extraction.

A more recently appearing advantage has been that automated systems could provide higher sample throughput that could be obtained from manual systems. This advantage has been made possible by utilizing of the concept of **automated parallel–sample processing**. With early, automated systems, individual samples were processed in series [113]. The next sample in the series was not started until the preceding sample had been completed or was well on its way. With serial sample processing, automated solid-phase extraction systems were slower than manual systems, but because they could operate continuously during the day, night or weekend, timesavings were still achieved. Although not efficient in terms of process time, this approach did prove to be effective and is still in use today. The fastest serial processing equipment currently extracts 25 to 50 samples per hour [114].

About 10 years ago, automated parallel processing solid-phase extraction was introduced and demonstrated to be practical [115]. Under this algorithm, numerous samples are extracted simultaneously. Although the equipment requirements for parallel processing can be more specialized, or at least more cleverly designed, the great pay back occurred in terms of dramatically improved throughput. After this parallel processing became commercially available in the form of the Zymark Rapid Trace [100], the speed of automated systems began to overtake that of manual approaches. As will be described below, the fastest automation systems in existence today, including most 96-well microtiter plate systems, are parallel processing systems. The fastest parallel processing systems can achieve speeds of up to 400 samples per hour [114].

The ability of an automated solid-phase extraction system to improve assay precision and accuracy is variable and depends on factors such as the consistency of analyte retention and the frequency of systematic errors, such as clogged cartridges and poor volume transfers. All things being equal, the best assay precision and accuracy is obtained by an expert, highly motivated analytical chemist who is not influenced by extraneous factors such as tedium. In terms of performance, automated systems come next. Analysts with average skills and a normal propensity to boredom can be outperformed by automated systems when faced with moderate to high sample load [116]. Humanistic factors, such as safer handling of hazardous materials are also important advantages for automation. The ability to perform automated solid-phase extraction method development is an underutilized advantage of many modern workstations [117]. This capability is described later in this review.

The limitations of automated systems, although overshadowed by the advantages, are real and should be kept in mind. One practical limitation is analyte carryover. Carryover is dependent on many variables, including the particular apparatus being employed, the range of analyte levels, the adsorption properties of the analytes, the matrix, sensitivity requirements of the assay, the extent of flushing, tip changing and similar operations. At best, carry over can limit dynamic range of an assay by giving erroneously elevated analyte response at low levels. At worse, carryover can severely affect the precision and accuracy of an assay method and give falsely positive results. For a given apparatus, carryover that

is acceptable for one application can be completely unacceptable for another application. For this reason, carryover should always be evaluated over a wide, realistic range when automated solid-phase extractions are planned. Extent of carryover ranging from 0.01 to 0.5% is typical, and the smaller the carryover, the better the performance of the assay will be.

An important feature now available on many liquid-handling workstations is liquid level sensing. By using the presence or absence of electrical conductance between different areas on the liquid transfer tips, liquid level sensing can detect whether or not liquid, in a suitable form for transfer, is present. If liquid is not present, the transfer tip can be repositioned to reattempt the transfer. This feature has practical significance when clots, flocculent or other inhomogenieties are present in dirty samples. In many cases, the workstation can be programmed to make several attempts at a suitable sampling of liquid. Typically, if a suitable sampling of liquid cannot be obtained, the workstation will skip the sample. This approach is helpful for decreasing the number of systematic errors made by sample inhomogenieties such as clots or protein globules in mammalian plasma, but not as reliable as avoiding and working around the clot in a manual transfer.

Another disadvantage for some automated solid-phase extraction systems is the physical or chemical stability of the samples. This issue is most problematic when the serial-processing algorithm is being used with unstable samples. For example, if a drug substance is determined in plasma, the overall processing time is 3 min per sample and 60 samples are to be processed serially, it is important to know if the drug is stable for 3 h. In addition, the physical stability of plasma comes into play. If protein flocculent begins to occur in the plasma due to denaturization, the incidents of clogged solid-phase cartridges or wells will increase and the extraction failure rate can skyrocket at the end of a run. Similar problems can occur with foods or other biological samples. Parallel processing algorithms are less susceptible to sample stability requirements because the time required to process all samples is usually brief (10 to 20 min) and because removal of the analyte from matrix can physically or chemically stabilize the sample. For this reason and because of higher throughput, parallel processing approaches to automated solid-phase extraction have advantages over serial processing methods.

3. A brief review of equipment for automated solid-phase extraction

An excellent hardware review for automated solid-phase extraction has been recently published by Smith and Lloyd [114]. In this review, Smith and Lloyd present an overview of at least 18 different commercially available systems for automated solid-phase extraction and discuss 13 important attributes, such as the degree of automation, type and quantity of work, cost, functionality, etc. Their discussion is intended as a guide for selecting a suitable system and it is highly recommended for this purpose. We will not attempt to redo this excellent review. Instead, we have classified the available systems into three categories: (1) on-line techniques, (2) discrete column workstations, and (3) 96-well workstations. Their descriptions and a discussion of their advantages and limitations follow.

3.1. On-line solid-phase extraction approaches

Three recently introduced on-line extraction techniques have been applied to biological samples. These techniques involve direct injection with restricted access media (RAM) [118,119], turbulent flow chromatography [120] and on-line solid-phase extraction.

The concept of restricted access media combines a hydrophilic external surface and a hydrophobic internal surface in silica particles with controlled pore sizes (Fig. 2). Large biopolymers, such as proteins, are prohibited from entering the pores of the packing and are not well retained by the column. Therapeutic drugs and other small molecules permeate the pores of the column packing material, where they partition and retain. This approach is, in principle, a combination of size-exclusion and partition chromatography. In practice, to obtain a reasonable amount of chromatographic efficiency, it is often necessary to perform a column- or solvent-switch with this approach, sometimes using a back flush setup. It occasionally takes a few days to

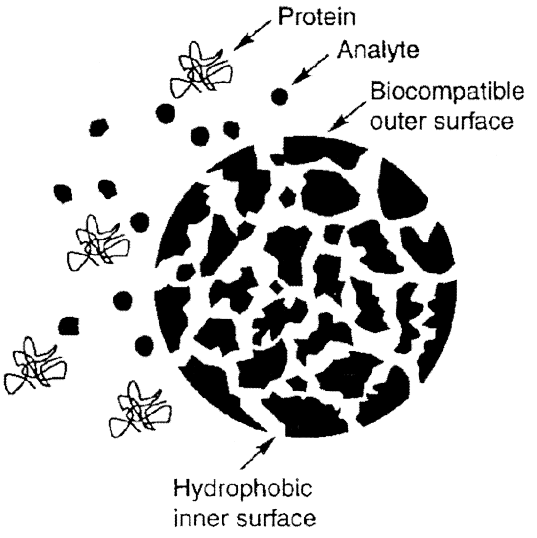

Fig. 2. Schematic representation of a sorbent particle for restricted-access media chromatography. This media allows proteins and macromolecules to be excluded and elute in the solvent front, while small analyte molecules enter the pores and are retained.

develop suitable conditions, and sample volumes are limited to 10–50 μl. The restricted access medium column typically requires 1.1 to 2 ml of solvent for washing after each injection and this can usually be done while analytes are eluting from a downstream analytical column. A limitation associated with this arrangement is that injection-to-injection cycle time can be long (8 to 15 min) and sample instability (both matrix and analyte) can be problematic when a large number of samples is involved. Injection-to-injection analyte carryover, although not well characterized, is also a potential problem.

Turbulent flow chromatography is a direct-inject sample-preparation technique that is accomplished on a special chromatography column. The technique has some applicability toward plasma and serum. The special column combines large particle (50 μm) and frit size (20 μm) with high flow-rate (5 to 10 ml/min) to achieve eddies and non-laminar flow. Under this arrangement, improved mass transfer and flow equilibration increases the analyte diffusion within the pores of the packing material. The net result is a separation of large biological matrix components from small analyte molecules. Sample pretreatment can be reduced to only a simple centrifugation step, and the column can be back-flushed and re-equilibrated as necessary to rid the system of insoluble components. Method sensitivity can be nearly equal to off-line preparation (low ng per ml range for 50-μl sample size), and there is a significant loss of chromatographic efficiency in the form of peak fronting or tailing (Fig. 3). Variable analyte recovery is prevalent. For many compounds, inter-injection carryover (0.15 to 0.5%) seems to limit the dynamic range and utility of this technique.

The most widely applied on-line solid-phase extraction apparatus continues to be the Prospekt [36,37,39,44,69,72,94,97,121]. This device incorporates custom solid-phase extraction cartridges into an analytical-scale high-performance liquid chromatography (HPLC) separation using three electrically actuated switching valves. Several hundred cartridges can be loaded into the instrument prior to initiation of a run. Under program control, samples in a 96-well format can be directly injected onto the head of the cartridge, undesired components washed to waste, then analytes eluted to an in-line HPLC column, followed by detection. As with all column switching arrangements, elution solvents are limited to those that are compatible with the downstream components, and must be carefully selected to maintain acceptable chromatography. To circumvent this limitation, some investigators have gone so far as to propose eliminating the analytical column entirely [122]. Again, as with other serial processing techniques, sample stability must be considered.

There are advantages to on-line serial processing approaches. Although their throughput is lower than that for pure, parallel processing systems, they can gain back some lost efficiency by a direct link between the sample preparation and the downstream separation/detection. Fewer liquid transfers are made. The trade-off to be made is that the downstream separation and detection need to be adapted to the on-line extraction in terms of timing and solvent selection, etc., and could be sub-optimal.

3.2. Discrete column workstations

One current arrangement for solid-phase extraction workstations is to use discrete solid-phase extraction columns in commercially available syringe barrel sizes. Two systems that have successfully used this approach are the Rapid Trace (Zymark) and the

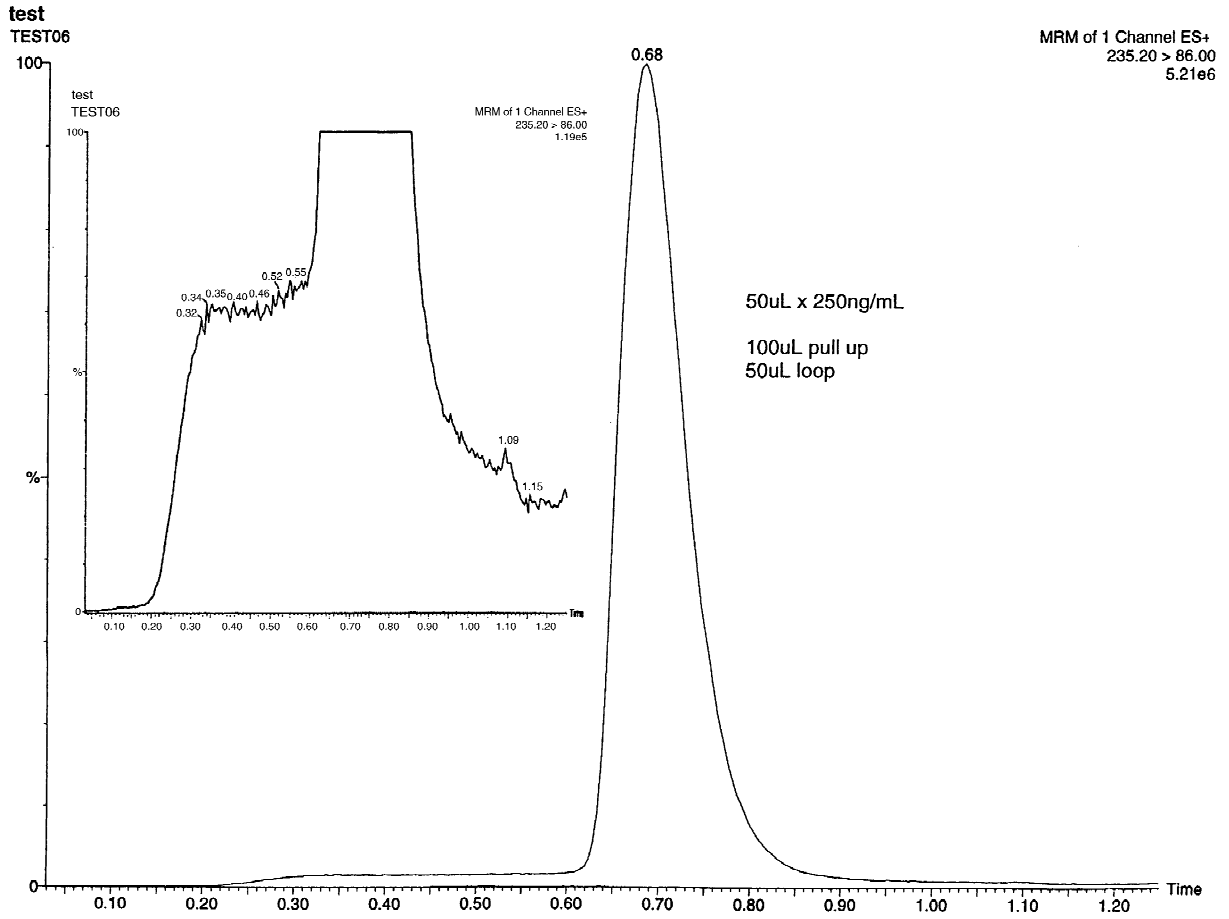

Fig. 3. Representative chromatogram for a typical turbulent-flow chromatography experiment (50 μl injection), showing both fronting and tailing behavior. The number of theoretical plates (N) for this compound is ~350. Time scale in min.

Spe-ed Wiz (Applied Separations). The biggest advantage of these systems is the wide selection of phase availability in the syringe barrel format. This advantage could diminish in time, as additional sorbent types become available for 96-well workstations. Another advantage is that each of these systems is, to some extent, a parallel processing system; so at least some throughput advantages are enjoyed, relative to serial processing. In this regard, the Rapid Trace is a hybrid serial/parallel processing system; consisting of up to 10 modules operating in parallel, each module can process up to 10 samples serially.

A limitation of the currently available discrete column workstations is that ancillary tasks, such as building standards and reagents, extract dry-down, and direct autosampler compatibility are not available. Discrete column workstations were introduced years before 96-well workstations and seem to be losing ground to them, primarily due to throughput and speed limitations. The ability of a discrete column work station, such as the Rapid Trace, to finely tune conditions for a solid-phase extraction is, however, unmatched [80,123].

3.3. 96-Well workstations

The recent explosion of automated solid-phase extraction is directly related to the commercial availability of 96-well format workstations and solid-

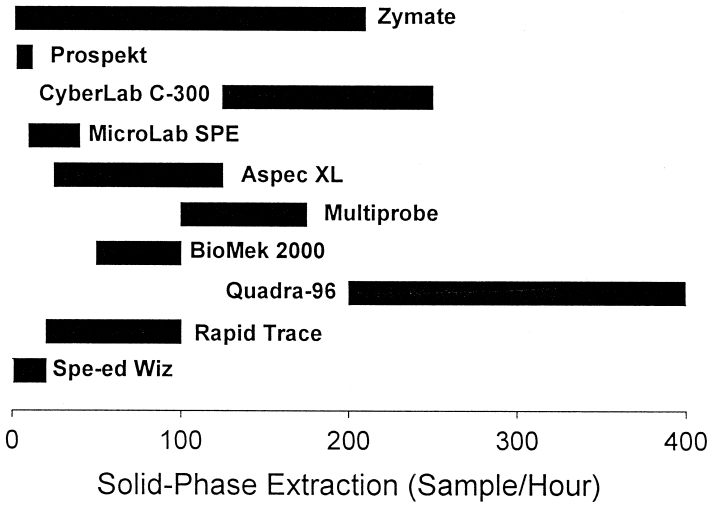

Fig. 4. Comparative sample throughput for several automated solid-phase extraction work stations, adapted from Ref. [114].

phase extraction materials in 96-well format. Of the 10 automated workstations ranked for throughput in Fig. 4 [114], those six with the highest sample throughput are 96-well (read: parallel processing) systems. Unless there is a radical, unforeseen breakthrough in the serial processing approach, serial sample processing will not be able to compete for throughput with parallel sample processing.

Not all 96-well workstations were created equal. There is a definite relationship between price, functionality and complexity. The greater the functionality, the greater the complexity and price. Many 96-well workstations such as the Packard Multiprobe II and the Beckman Biomek 2000 have been adapted from general-purpose use. These systems were designed as liquid handlers long before they ever touched a solid-phase extraction column. As such, these automated workstations have more flexibility and capabilities at the expense of efficiency. They allow for almost completely automated approaches, while displaying greater facility and lower throughput efficiency. The 96-well manifolds, similar to that shown in Fig. 5, have been widely adapted to these workstations.

The 96-well systems are not the best available technology in terms of producing precisely tuned solid-phase extractions. Yet, because of the insatiable demand among some analytical groups for increased speed and throughput, 96-well workstations seem destined to gain popularity at the expense of other approaches until they are eventually supplanted them or are supplanted by something better.

4. How to automate solid-phase extraction

For an automated solid-phase extraction to be worth while, a minimum number of samples are required. In the past, this break-even number was a few hundred samples. The work required to automate an extraction demanded the assay of this many samples before a return was obtained on the automation investment. As automated solid-phase extraction has become more commonplace and as better off-the shelf solutions have become available, this break-even number has decreased. It now can be as low as 5 or 10 samples, depending on the system available and the analytical chemist's comfort level with automation.

There are few absolute rules in solid-phase extraction. One rule that stands out is that if a procedure is to ultimately be automated, it should be automated from the onset of method development, using the workstation on which it will be run. It is counter-productive for an extraction protocol to be developed manually then automated, as there are enough differences in pressures, flow-rates and solvent composition that transferring from manual to

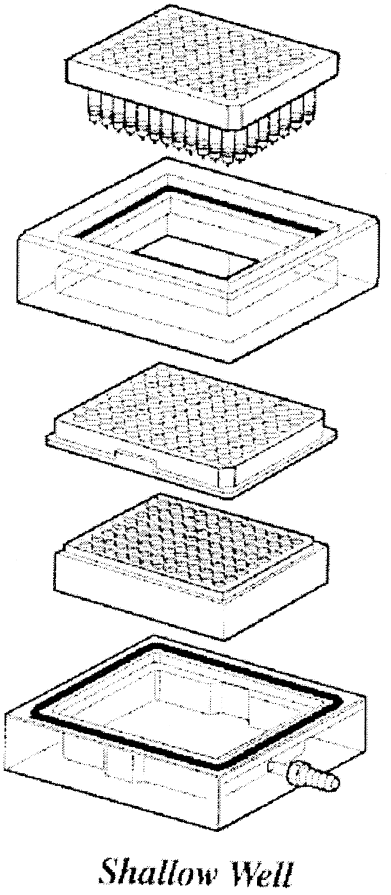

Fig. 5. Typical shallow-well 96-well format solid-phase extraction vacuum manifold adapted for use with many liquid handling workstations.

automation is like starting from scratch [116]. In addition, automated workstations have developed to the point where a number of efficiency advantages can be gained by doing the solid-phase extraction method development on the workstation [124]. Initial steps in automated solid-phase extraction, therefore, include selection of a workstation, based on assay requirements, on which to begin method development [114].

As the initial experiment, a solid-phase extraction method development paradigm might involve recovery evaluation at one concentration, in duplicate, on several different reversed-phase sorbents (eight samples plus four blank extracts for spiking as recovery standards). To achieve a first approximation of best case recovery, wash and elution solvents are chosen so that the analytes have the best chance of being retained and eluted. For a wash solvent in a reversed-phase extraction, aqueous–organic (95:5) could be chosen, and the converse (aqueous–organic, 5:95) could be chosen as an elution solvent. Because the extraction selectivity is sub-optimal at this juncture, the use of a highly selective detection method is desirable. The evaluation of recovery using non-matrix samples is not recommended because interaction of analyte molecules with matrix components will affect recovery. On the basis of recovery, one or two sorbents could then be selected for further evaluation. An example of these experimental results is shown in Fig. 6.

Several sorbent manufacturers can supply 96-well solid-phase extraction method development kits. These kits are designed so that a different sorbent is located in each row of a single 96-well microtiter-plate. Using one of these plates, a number of different sorbents can be evaluated in a single set of automated experiments.

Next, five or six wash solvents containing various amounts of organic (possibly 5, 10, 20, 30, 40 and 50%) are tested. This also requires duplicate determinations and recovery standards (18 samples). An example of these experimental results is shown in Fig. 7. From this plot, it is easy to select an appropriate wash solvent composition to maximize recovery.

This experiment could be followed by an evaluation of elution solvents at typical organic compositions of 70, 80, 90 and 100% (12 samples), with seventy percent being a practical limit for evaporating extracts in a reasonable time. If drydown is to be omitted from the procedure, then this restriction need not apply.

A fourth experiment would be an evaluation of precision and recovery at one to four concentration levels ($n=6$ to 24, plus a recovery standard), plus recovery of an internal standard ($n=3$, plus recovery standard). As a minimal method development exercise, therefore, 60 to 70 spiked samples would be prepared and extracted within one day. The experiments need to be performed sequentially because the results from each will impact how subsequent experiments are designed.

Selectivity is assessed through the course of the

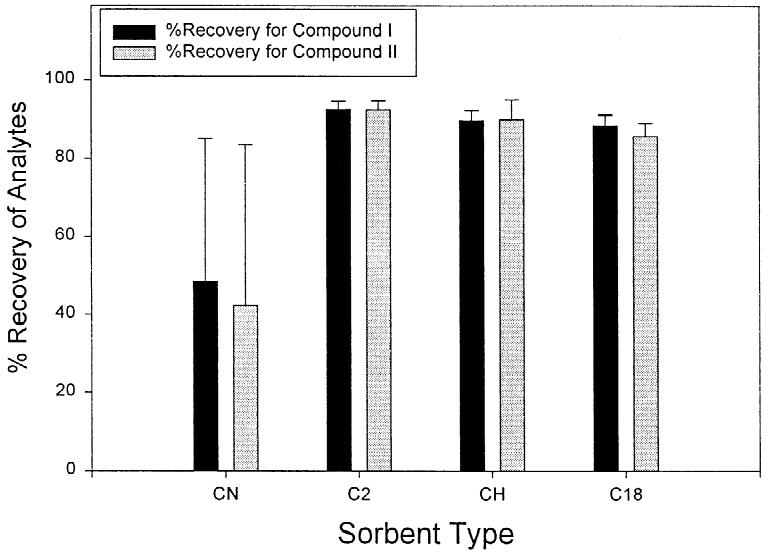

Fig. 6. Solid-phase extraction recovery of two acidic compounds from four different solid-phase sorbents using a wash solvent of 5% acetonitrile and an elution solvent of 100% acetonitrile. The solid lines indicate the relative standard deviation associated with each determination [123].

method development. Overall method selectivity, of course, results from the combined selectivity of the solid-phase extraction, the down-stream separation (liquid chromatography, gas chromatography, capillary electrophoresis, capillary electrochromatography, etc.) and the detection technique (photometric absorbance, fluorescence, flame ionization, tandem mass spectrometry, etc.). The lower the selectivity of the down-stream processes, the greater emphasis is placed on solid-phase extraction selectivity. Therefore, if the analytical chemist has access to more selective separation and detection techniques, less time is spent on solid-phase extraction selectivity development.

With many types of solid-phase extraction workstations, especially those with computer control of flow and pressure, some initial experience is required to select these parameters. After this experience has been gained, the same or similar settings can be used for a variety of applications, without adjustment.

As mentioned earlier, carryover is a problem for automated solid-phase extraction workstations and carryover performance should be investigated. This set of experiments can be as simple as running a few matrix blanks in the same workstation positions after a few high-level samples or standards have been processed. If carryover is observed, then additional wash steps or transfer tip exchanges need to be added to the procedure and the carryover assessment is repeated. If carryover cannot be eliminated in this way, then it could be possible to decrease carryover by selecting a more appropriate wash solvent for increased analyte solubility and decreased surface adsorption. For example, if the analytes are amine-containing compounds and demonstrate stickiness to surfaces, a mixture of methanol, water and trace formic acid would be a better wash solvent choice than pure acetonitrile.

Carryover limits the performance and usefulness of some solid-phase extraction systems, automated or manual. Some degree of carryover is always present and can be observed if the detection technique is sensitive enough or the analyst looks hard enough. It is, therefore, important that realistic levels of carryover are assessed. If a method will be used to quantify an analyte over 1 to 1000 parts-per-billion, carryover from a 10 parts-per-million sample may not be reasonable if this level will not be encountered in sample assay work. Abnormally high analyte levels do occur sporadically in most analytical work and these occurrences must be recognized. The final options for eliminating carryover from automated

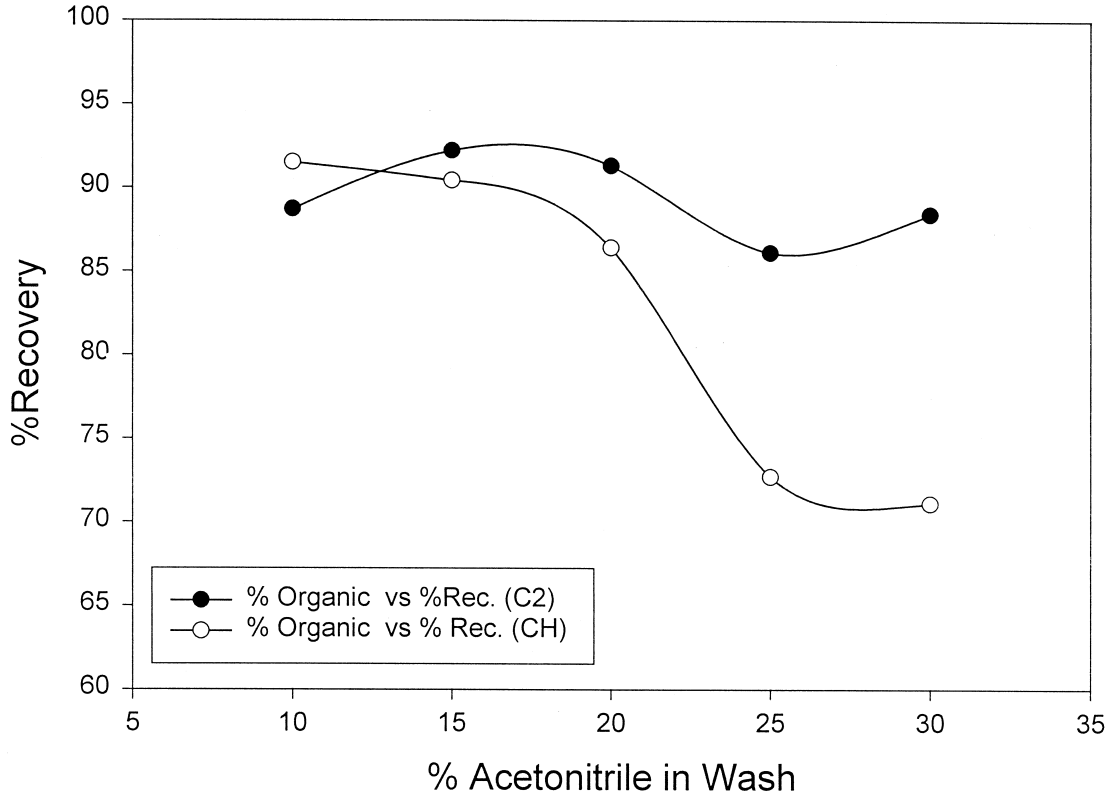

Fig. 7. Solid-phase extraction recovery of a compound as functions of wash solvent concentration and sorbent. Solid dots (●) indicate recovery for C_2 sorbents, and hollow dots (○) indicate recovery for CH sorbent using 100% acetonitrile elution solvent [123].

methods are: (1) to deliberately limit the low-end dynamic range of the method so that false positives are not encountered and (2) avoid using an automated system for the extraction. If it is not feasible to increase the limit of quantitation, a different option must be found.

5. Generic approaches to automated solid-phase extraction

Because of the widespread use of liquid chromatography–tandem mass spectrometry (LC–MS–MS) for drug bioanalysis, there is currently less of a necessity for finely tuned solid-phase extractions that there once was in this area. Instead, generic solid-phase extraction conditions that can accommodate many different analyte structures using the same extraction conditions have become more interesting. To make a solid-phase sample preparation useful for LC–MS–MS it must remove as much of the sample salts as possible in order to reduce the effects of ion suppression [125–127] and it must remove as many nonvolatile matrix components as possible so that the instrument ion source is not quickly fouled. Because the LC–MS–MS instrument is inherently so selective, added assay selectivity, per se, is no longer an objective of solid-phase extraction.

Examples of this approach have been reported by Janiszewski et al. [128] and others [129] by utilizing extraction disks in a 96-well format to perform quick, automated solid-phase extractions under very simple wash and elution conditions. In one approach [128], a Tomtec Quadra 96-well workstation was used to perform the semi-automated solid-phase extraction with Empore C_2 extraction disks. The

advantage of this piece of equipment is that it allows liquid to be transferred to or from all 96 wells simultaneously, thus giving the greatest throughput advantage.

The generic protocol for this type of extraction requires the sorbent to be conditioned with pure organic or organic water, then water prior to sample loading. After the wells are processed with aliquots of wash solvent (typically water or buffer–organic mixture), a 96-well shallow plate is manually inserted into the collection position and the elution solvent (pure organic or organic with a small amount of acid or base) is added to each well. It is also possible to use multiple solvent elution steps to elute different classes of compounds separately. With extraction disks or small mass (10 mg) packed-bed sorbents, the elution volume can be kept in the 30- to 150-μl range. As a simplification to the procedure, the eluate is not dried down. Rather, the contents of each well is diluted with a small volume of water or buffer to give an injection solvent with a composition (20 to 40% aqueous) that is compatible with the liquid chromatography mobile phase.

This approach provides minimal sample cleanup with little or no method development effort. The solid-phase extraction desalts and deproteinizes the samples: method selectivity is furnished by the LC–MS–MS system. An example of the best-case method selectivity is shown in Fig. 8. The approach is capable of high throughput (up to 400 samples/h) and appears to work a large percentage of the time for discovery-phase drug-compounds. In this way, it is well suited for drug discovery support.

6. Recent examples of automated solid-phase extractions

6.1. An environmental example with a discrete column workstation

Synthetic organic pesticides have become major pollutants because large amounts have been used during last several decades. To satisfy detection limit demands, it is critical to preconcentrate the trace amount of pesticides in water and food supplies to protect public health. Solid-phase extraction is the most commonly used technology for the task because of good analyte selectivity and recovery, and especially because it is capable of isolating and enriching the trace materials from aqueous samples. Categories of sorbents used for isolation and advantages of solid-phase extraction have been discussed in detail [130]. Because the monitoring of pesticide concentrations is becoming a routine task, more attention is being paid to the automation of the extraction.

A representative application in this area is the use of the Zymark Autotrace, a workstation that utilizes the discrete column approach. The operator has discretion in choosing cartridges with different dimensions and phases. The apparatus removes manual intervention from cartridge conditioning, sample loading, cartridge washing and analyte elution. Up to 100 samples can be loaded onto the system and solvent volumes for other steps such as conditioning, rinsing and eluting can be relatively large. In an example reported by Quayle et al., 16 organochlorine pesticides in deionized water have been detected and quantified simultaneously [6]. The detectable concentrations of the pesticides were between 1–5 ng/l. The analyte recoveries were impressive (92–105%) and relative standard deviations were less than 12%. An additional manual evaporation step is usually performed after elution because the elution volume can reach 10 ml. This report investigated direct high-volume injection (~100 μl) of the eluent to onto a capillary gas chromatography column or, alternatively, used a relatively small volume of elution solvent (2 ml) to eliminate the dry down step. The precision and accuracy of the method were acceptable using either approach. Discrete column workstations such as the Autotrace provide relatively simple automation for limited step extractions and shows advantages in automation when large sample volume is necessary to get enriched trace analytes to be detectable.

6.2. A biological fluid example using a 96-well workstation

Sample preparation methods based on 96-well format have been widely used with bio-fluids to achieve high throughput. In most cases, single plate processing cannot meet the high sample-throughput demand. Some efforts have focused on the integration of several robotic systems to achieve full

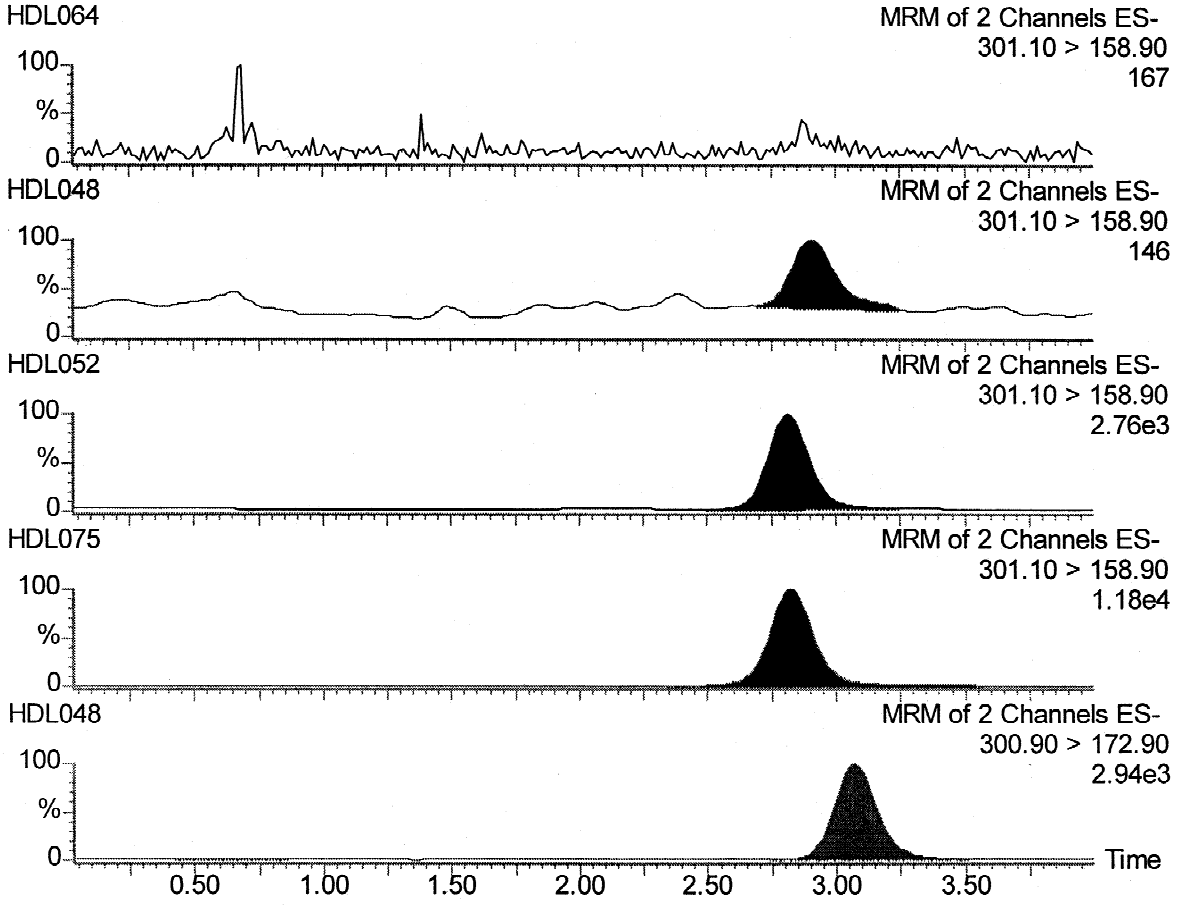

Fig. 8. Representative multiple reaction monitoring (MRM) chromatograms for extracted dog plasma samples (from top to bottom) at concentrations of (a) blank, (b) 5 ng/ml (spiked), (c) 100 ng/ml (spiked), (d) 425 ng/ml (0.5 h after receiving an oral dose of drug, and (e) internal standard at an effective concentration of 100 ng/ml [123]. Time scale in min.

automation of the entire sample preparation and one example is discussed below.

Glucocorticoid fluticasone propionate (FP) is being considered for the treatment of asthma. A sensitive, robust and high throughput solid-phase extraction method has been developed for LC–MS–MS assay of FP in human plasma [95]. The extraction system (Fig. 9) consists of a custom-built solid-phase extraction workstation based on Packard Multiprobe technology. The samples were extracted on 96-well MicroLute II plates packed with 50 mg of Varian C_{18}. The station incorporated a vacuum manifold, a reagent addition strip (dispensing eight samples each time) and a solvent switching valve for selecting a maximum of nine solvents. The 96-well workstation was integrated with a robotic sample processor (RSP) Multiprobe 104 DT. A refrigerated carousel served as a warehouse for all extraction labware and storage of final collecting plates. A Zymate XP robot loads and transfers extraction blocks and collection plates among the workstation, RSP and carousel. The unique design of this system is that not only are extraction steps such as cartridge conditioning, washing and eluting automated, but multiple plate management and connection among several units are also automated and integrated. User-friendly software can control both a Zymate XP robot (plate management) and RSP. Incorporating the MultiProbe 104 DT into the system provides more capable liquid handling, such as converting indi-

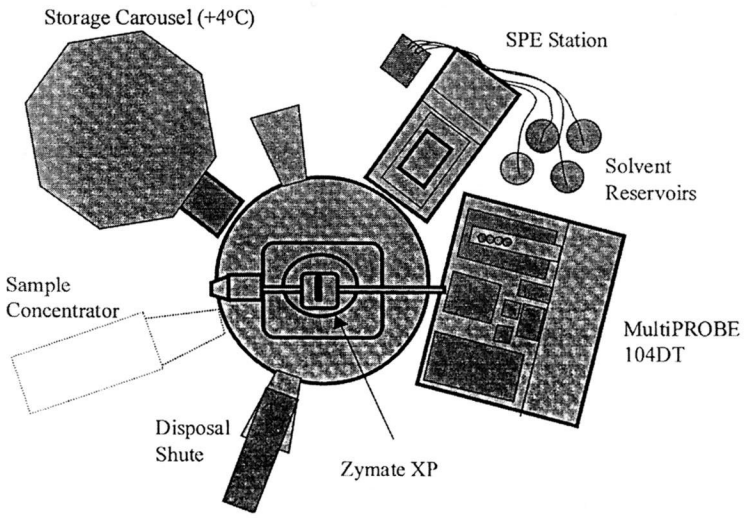

Fig. 9. Schematic of a Zymark 96-well solid-phase extraction robotic system for use with biological fluids [95].

vidual sample to 96-well format, sample dilution and standards preparation. Individual tip (four tips total) liquid sensing and other functions in the MultiProbe effectively manipulates biological fluids.

The extraction is fully automated from beginning to end and this is very powerful when large numbers of samples are being prepared for LC–MS–MS. With the reported solid-phase extraction–LC–MS–MS method, a quantitation limit of 20 pg/ml (0.5 ml plasma) was achieved. Excellent precision and accuracy (<6%) for inter- and intra-run was obtained. The automated extraction proved to be a very successful replacement for manual or semi-automated extractions. This system also has the potential to integrate more functionality such as an evaporator, a necessary step in many solid-phase extractions.

6.3. On-line solid-phase extraction for pharmacokinetics of a drug

The most attractive feature of on-line solid-phase extraction is that it almost entirely automates the sample handling process. One approach to this, the Prospekt, uses individual disposable cartridges so that cross-contamination will be minimized. The extraction time is synchronized with chromatographic separation time. Sometimes, however, this becomes a drawback because when fast (1 to 2 min) chromatography is used, the sample preparation is rate limiting.

McLoughlin et al. carried out on-line solid-phase extraction to simultaneously assay 10 drug candidates in dog plasma using LC–MS–MS with simultaneous reaction monitoring [72]. The cycle time of the assay was 8 min per injection, and was limited by the chromatographic separation. After preconditioning an IST Isolute CN cartridge, 50 μl of each sample (internal standard added before loading) was loaded to the cartridge. The cartridge was washed to waste with water and acetonitrile–water (10:90, v/v) at an optimized flow-rate. The compounds were eluted directly onto the analytical column using chromatographic mobile phase (organic–aqueous, 50:50), then separated and detected by MS (Sciex, Thornhill, Canada). Validation results for the extraction efficiency, and precision and accuracy are shown in Table 2. A quantitation limit of 2.5 ng/ml was achieved for most compounds.

7. Future directions in automated solid-phase extractions

If the trend depicted in Fig. 1 is real, then it appears likely that automated solid-phase extraction will continue growing and gaining popularity. Instrument manufacturers have adequately addressed some

Table 2
Extraction efficiency, accuracy and precision (mean % recovery±% RSD) of ten drug substances assayed simultaneously

Substance No.	Extraction efficiency (%)	Plasma concentration (ng/ml) of quality control samples (n=6)		
		5	50	500
1	86	102±27	80±13	77±16
2	46	100±19	101±16	99±14
3	97	121±14	119±18	108±12
4	102	107±10	118±8	110±9
5	106	119±18	108±12	108±12
6	119	93±18	108±8	106±12
7	88	93±8	108±7	109±6
8	95	78±19	112±11	108±9
9	96	90±11	106±6	101±9
10	104	112±21	99±13	104±9

of the issues that have slowed the growth of automated solid-phase extraction. Other issues, such as operator interventions, are still rate limiting.

Until recently, low throughput had been a problem. In the early days of automated solid-phase extraction, an average chemical analyst could outproduce an automated system and multiple operator interventions were commonplace. Only in the last seven or eight years have commercial parallel-processing systems been available. Higher throughput 96-well systems have been available for about half that time. With these systems, the throughput has exploded and the number of operator interventions, while not disappearing across the board, has decreased convincingly. With many semi-automated systems such as the Tomtec Quadra-96, the Biomek 2000, the Multiprobe and any system that relies on a vacuum manifold such as shown in Fig. 5, operator intervention between sorbent washing and analyte elution is still required.

As was true for many years in the personal computer industry, software capabilities have lagged behind hardware in automated solid-phase extraction workstations. Early systems required detailed specialized knowledge of spatial coordinates, operating systems, electronics and computer programming [131]. Today, although a few of the low-end systems still rely on primitive software, all of the best 96-well workstations have modern graphical user interfaces and are macro-programmable, They can be operated with minimal training. Some popular on-line systems have seen the light and are introducing graphical user interface versions as well.

Because throughput continues to drive analytical chemistry, future challenges in the automated solid-phase extraction workstation realm could involve a combination of parallel 96-well liquid handling (as demonstrated by the Tomtec Quadra-96) with fully automated extractions. This total automation could include the method development steps such as standard preparation and sorbent/solvent selection, removal of operator interventions such as positioning of collection tubes, and injection of samples into the chromatographic or electrophoretic system. Along this line, it seems inevitable that a dedicated solid-phase extraction workstation with all of these features will appear in the next one to two years.

Beyond this practical and inevitable short term picture looms the dual-headed phantom of microfluidics and assay miniaturization [132–134]. Although not practical at this time, microfluidics and miniaturization hold great promise in terms of throughput advantages. Chip-to-mass spectrometry interfaces are being developed in this way to meet the needs of emerging drug discovery paradigms (for example, see http://www.abs-lcms.com). This technology could provide high-throughput sample assays for evaluating and characterizing drug targets and drug candidates by combining microchip-based separation devices with electrospray mass spectrometry. Although some success has been achieved in these areas of micro-channel plate liquid handling and separation, enormous technical battles in the area of separation and detection sensitivity need to be fought and won before this type of approach can be possible, let alone practical. These battles will be

won eventually and the resulting technology will allow automated solid-phase extraction on the nanoliter scale, with thousands of channels being processed in parallel and total assay time shrinking from hours to minutes or seconds.

8. Conclusion

The current trends indicate that automated solid-phase extraction is now more widely used than in the past. Much of this recent growth stems from increased capabilities of commercially available workstations. In past years, automated systems allowed analytical chemists to redirect time to other tasks while maintaining approximately equal throughput with manual methods. Newer workstations utilize a parallel processing approach to achieve greater efficiency and dramatically improved sample throughput. Because they can offer greater sample throughput than either on-line or discrete column approaches, it appears likely that 96-well systems will supplant these other approaches.

Generic approaches to automated solid-phase extraction have become feasible. One generic strategy exploits the ultra-high selectivity of LC–MS–MS to minimize solid-phase extraction method development. The solid-phase extraction contributes desalting and protein removal. It can be applied to diverse drug molecules in biological fluids, with little refinement of wash and elution solvent compositions. By using disks or small bed-volume packings, elution solvent volumes are minimized and dry-down is replaced by a simple dilution to weaken the injection solvent strength. The driving force is, again, throughput.

Near term trends for automated solid-phase extractions suggest that a more integrated, dedicated system with decreasing operator intervention is on the horizon. This system would utilize parallel processing to maintain the high sample throughput of today's most efficient systems. Longer term, microfluidics and miniaturization could allow parallel processing to take on a whole new meaning, as 96-well processing escalates to hundreds or thousands, as needed. Small sample volume requirements will decrease and place great demands on detection sensitivity. The future of automated solid-phase extraction, as an analytical growth area seems secure.

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