ITSP: Automated Chromatographic SPE using the PAL Autosampler

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SPE: Why and How?

Solid Phase Extraction (SPE) is established as a preferred tool for isolating target analytes from complex matrices. This preference is the result of the availability of a diverse range of chromatographic sorbents allowing targeted approaches based on the specific chemistry of the analytes and matrices. In addition, SPE offers the ability to enrich or pre-concentrate analytes in samples. Enrichment is valuable because it allows one to match the analyte concentrations to the analytical approach used to measure them. Given these unique capabilities, it seems safe to say that SPE is the "gold standard" approach in analytical sample preparation.

Despite the seemingly "golden" rationale for using SPE, there are practical matters of execution that must be addressed if SPE is to be used for significant numbers of samples. Performing SPE using a cartridge on vacuum (or pneumatic pressure) manifold can be executed reliably in skilled hands. However, significant skill in achieving and maintaining proper liquid flow (varies with liquid level) is required and the manual labor involved limits the number of samples to 30 per day per technician. Furthermore, if enrichment is needed, a sample dry down step will be required, which will reduce throughput.

The number of samples can be increased by going parallel in tubes, pipette tips, cartridges, or 96 well plates. However, parallel liquid flow is highly variable tube to tube (or well to well). The increased variability in parallel flow results in increased variability in analyte recovery requiring the use of internal standards as well as acceptance of lower quality, specifically higher LLOQs and assay result variability. Furthermore, the parallel approach only addresses sample / solvent flow over the SPE sorbent and not pipetting sample / solvents to each well. So, going parallel only modestly increases sample throughput and adds the trade-off of increased probability of human error from the increased number of pipetting steps. Clearly, using SPE for large numbers of samples effectively, requires thoughtful consideration of a variety of trade-offs between quality and quantity.

Currently, the most common approach for achieving significantly increased SPE throughput is the parallel approach coupled with robots to perform the pipetting. This two workflow process (robotic SPE, then LC/MS/MS or GC/MS/MS measurement) achieves high throughput. However, this requires acceptance of lower quality results. Furthermore, there are significant additional costs for robots (200k-500k$), programming / integration, and technicians with robotic skills. From a results point of view, it seems to mirror the well known "speed, cost, quality triangle" where gains in any one of the three, takes away from the other two. It is important to realize that the speed, cost, quality triangle only exists if allowed. It is allowed by continuing to do SPE in the same way as before with nothing more than larger assembly lines (human or robotic). If one fundamentally changes or disrupts the SPE process, specifically the

single use SPE device, it is possible to change SPE and achieve benefit in speed, cost, and quality, all simultaneously.

ITSP – Instrument Top Sample Prep: The Art of the SPE Cartridge

Fundamental change of the SPE cartridge is the genesis of ITSP, a way to perform automated SPE rapidly, achieving the highest quality results, using the PAL autosampler that one probably already has in the laboratory. This genesis was born of the heart felt belief that SPE devices should change to become more automation capable ("the art") AND better from a fluidic point of view to maximize its potential chromatographic performance / sample volume efficiency ("the science"). This enthusiastic belief led to many spontaneous drawings of potential new SPE devices (>50 drawings in all) and the construction of more than a dozen prototypes. The dedicated enthusiasm continued for 6 years and was incredibly productive. The summation of the hand drawn art followed by building prototypes is shown in Figure 1.

The heart of the ITSP form of SPE is the patented[1-5] single use cartridge (Figure 1) containing customer-defined packed chromatographic media. The crimped-on septum and needle guide (upper 80% of cartridge) enable automation by facilitating accurate cartridge transport on a syringe needle. The automated SPE process starts using the syringe to perform cartridge conditioning, sample loading, and sample washing steps over a waste receptacle. Automation continues using the syringe to perform the elution step(s) over a clean vial(s) or well(s) and then discarding the used cartridge in a different waste receptacle. The automation finishes using the syringe to mix the freshly eluted sample and then inject it into the LC/MS/MS or GC/MS/MS that will be used to measure the sample. The locations of these events on a PAL autosampler are shown in Figure 2.

This automation approach is a simple single workflow process with the SPE integrated directly into the LC/MS/MS or GC/MS/MS software and workflow. It requires no additional skills beyond those needed to operate the LC/MS/MS or GC/MS/MS. Put simply, it is just a different method selection in the MS software already in use with any PAL autosampler equipped LC/MS/MS or GC/MS/MS.

In this online SPE by ITSP workflow, parallelism is achieved in a different way than it is with the two workflow process (robotic parallel SPE, followed by serial LC/MS/MS or GC/MS/MS). In the ITSP workflow, both SPE and LC/MS/MS (or GC/MS/MS) are performed serially, but in parallel with each other. This alternative approach to parallelism is depicted in the timeline shown in Figure 3. As shown, the cycle time is frequently a function of the LC/MS/MS (or GC/MS/MS) measurement and not the SPE. With this form of parallelism, the only cost in time for SPE is SPE of the first sample, and the process is considerably faster than the two workflow process.

This is achieved simply through good autosampler selection (CTC Analytics PAL System) and one must have an autosampler anyway.

ITSP – Instrument Top Sample Prep: The Science of the SPE Cartridge

The above covered the benefits of the upper 80% of the ITSP SPE cartridge (Figure 1), which enables the ability to accurately move the SPE cartridge, thereby enabling precise automation. Although smaller, the bottom 20% of the cartridge is every bit as important as the top. This is the part that does the chromatographic SPE, the part that expertly cleans and enriches the sample producing high quality results, and the part that one trusts to keep the LC/MS/MS or GC/MS/MS clean for many months of robust operation.

The ITSP SPE cartridge was designed to do this well. This can be seen in the syringe needle placement shown in Figure 1 where needle placement is directly at the frit containing the packed chromatographic sorbent, thereby allowing delivery of the sample, without significant dispersion, directly to the packed sorbent. Flow of the sample and solvents is positive liquid pressure, syringe pump driven (flow adjustable at a resolution of +/-10 nl/s) and is both precise and accurate. The extra-column volume of the cartridge (Figure 1, below sorbent) is just 16 μl. As a result, the ITSP SPE cartridge is truly a unique micro-SPE device with significant operational and performance advantages.

Operationally, this small volume SPE cartridge allows elution volumes of 50-100 μl, which facilitates significant control of the analyte concentrations delivered for measurement. As a result, the sample volume can be simply adjusted to match both the capability (sensitivity) of the measurement instrumentation and the specific needed cutoffs (LLOQs, S/N ≥20) for proper interpretation of the test results. This is achieved without the customary dry down step used with vacuum or pneumatically driven flow forms of SPE, which tend to require ≥5x larger elution volumes.

Perhaps this concept is best illustrated with common, in-use assay examples: For broad panel drug measurement in urine samples using a mid-range LC/MS/MS, 225 μl sample is loaded on the SPE cartridge, then eluted with 75 μl. This 3x enrichment of the analytes achieves the needed 1 ng/g cutoffs for low dose drugs and produces LC peaks for all drugs sufficiently intense for automatic integration across the concentration ranges observed in real samples. In the measurement of the same broad panel drug assay in oral fluid samples, 1 ml sample is loaded on the SPE cartridge, and then eluted with 75 μl. This 13x enrichment of the analytes achieves the same goals as urine samples, and in addition, the needed 0.2 ng/g cutoffs for low dose drugs. This concept can be amplified still further in the measurement of priority

pollutants in drinking water where 10 ml of water sample is loaded on the SPE cartridge followed by 50 µl elution in order to achieve 200x analyte enrichment and LLOQs at/near single digit part per trillion levels. In all of these example cases, the analytes are ready to measure immediately after SPE without performing dry down of the eluant.

The ITSP SPE cartridge is truly a unique micro-SPE device with significant chromatographic performance advantages. It has accurate syringe pump flow control over the packed sorbent bed allowing SPE separations to be performed at their van Deemter optimum velocity[6] as shown in Figure 4. This was an unexpected finding because the general expectation for single use SPE devices is that lower flow[7] always yields higher recovery. To the contrary, the data clearly shows that accurate flow control and optimization is just as important for SPE as it is for LC. With method optimization experiments performed in the same ways as done for LC, SPE recoveries can be systematically made both high and precise. The impact of performing SPE at this quality level is that the accuracy and variance in the final test results depends most on the LC/MS/MS (or GC/MS/MS) measurement (results CV = 3-5%) rather than the recovery of SPE sample preparation performed by the CTC PAL autosampler (Figure 2).

That's Not All

In skilled hands, method development / optimization experiments can be executed for multiple sorbents and solvents in an automated manner as a series of 5-6 run lists (DOE), where each list provides optima fed into the next list. The sum of these lists (including measurement / elimination of break-through of all condition, load, and wash steps, flow optimization, and a sample loading study) can be measured in as little as 3 lab days to yield a highly optimized SPE method. No other single use, disposable device SPE approach can achieve the precise chromatographic separations, pre-concentration of sample (without dry down), robust operation, and total automation achieved simultaneously by ITSP.

Furthermore, with PAL based SPE, one is not limited to a single dimension of SPE. Perhaps this concept also is best illustrated with in-use assay examples: For lipid profiling, uncharged oils can be isolated from fatty acids and phospholipids using anion exchange SPE. Then, the phospholipids can be isolated from the fatty acids using chelation in a second SPE step (easily achieved with a PAL). Finally, each of the three isolated samples (oils, fatty acids and phospholipids) can be directed to the LC/MS/MS or GC/MS/MS measurement approach best suited for that lipid class. Similarly, for proteomics, reverse phase SPE can be used for desalting, followed by a second chelation SPE to isolate peptides from phosphopeptides for separate LC/MS/MS measurement of the large numbers of peptides. In these examples of 2D SPE, the PAL often is operated as a stand alone sample preparation device, where it is equally

capable. This way, multiple instrument types and/or methods can process the different compound classes for the same samples in parallel. This capability also can be useful in any application where the analytical measurement time significantly exceeds the SPE time. For example, in the GC/MS/MS measurement of large pesticide panels in food, one stand alone PAL can use SPE to clean up enough QuEChERS extracts for continuous, around the clock measurement by five GC/MS/MSs.

ITSP truly is Smart SPE.[8]

Footnotes:

1. Gamble, K., US Patent 6969615, 11/1/2005.
2. Gamble, K., Martin, W., EU Patent 1174701, 09/11/2007.
3. Gamble, K., CDN Patent 2316648, 7/4/2004.
4. Gamble, K., Martin, W., US Patent 7001774, 2/21/2006.
5. Gamble, K., Fitzgerald, R., US Patent 7798021, 9/21/2010.
6. Neue, U.D., HPLC Columns: Theory, Technology, and Practice, Wiley VCH, 1997, p13.
7. Jordan, L., LC-GC, 1993, 11, 634-638.

8. Smart SPE is a trademark of ITSP Solutions Inc.

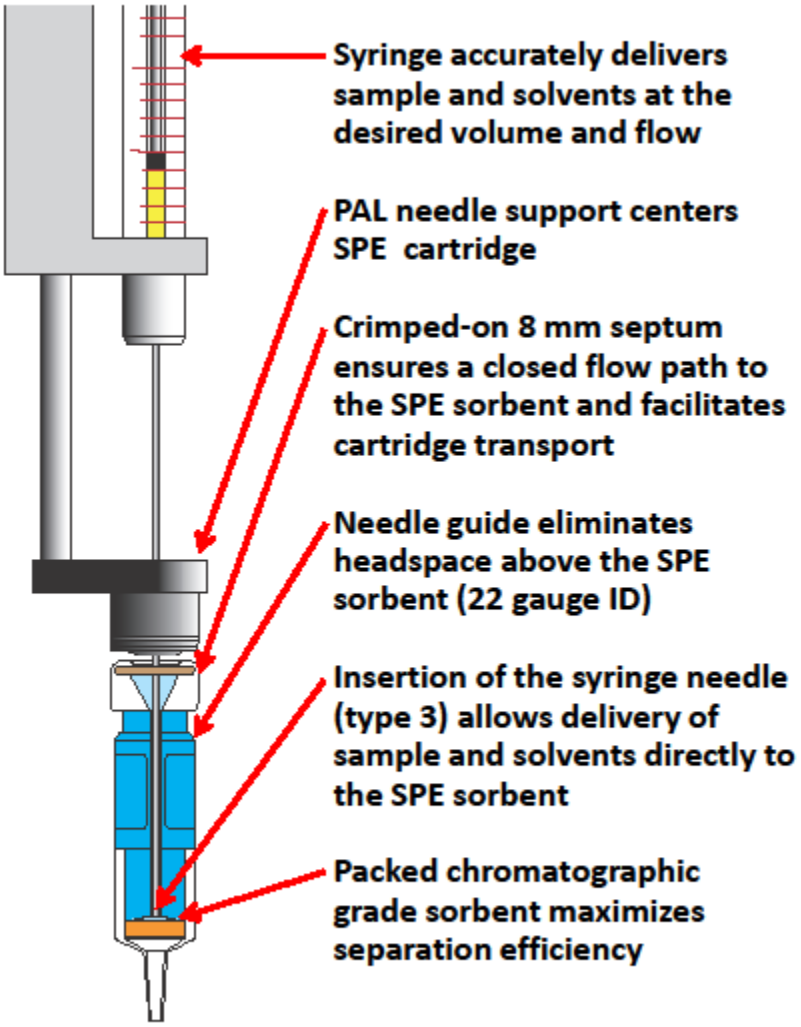

Figure 1. Internal view of an ITSP SPE cartridge and how it interfaces with the PAL autosampler syringe and needle support. This interface facilitates all SPE cartridge transport and delivery of sample and solvents.

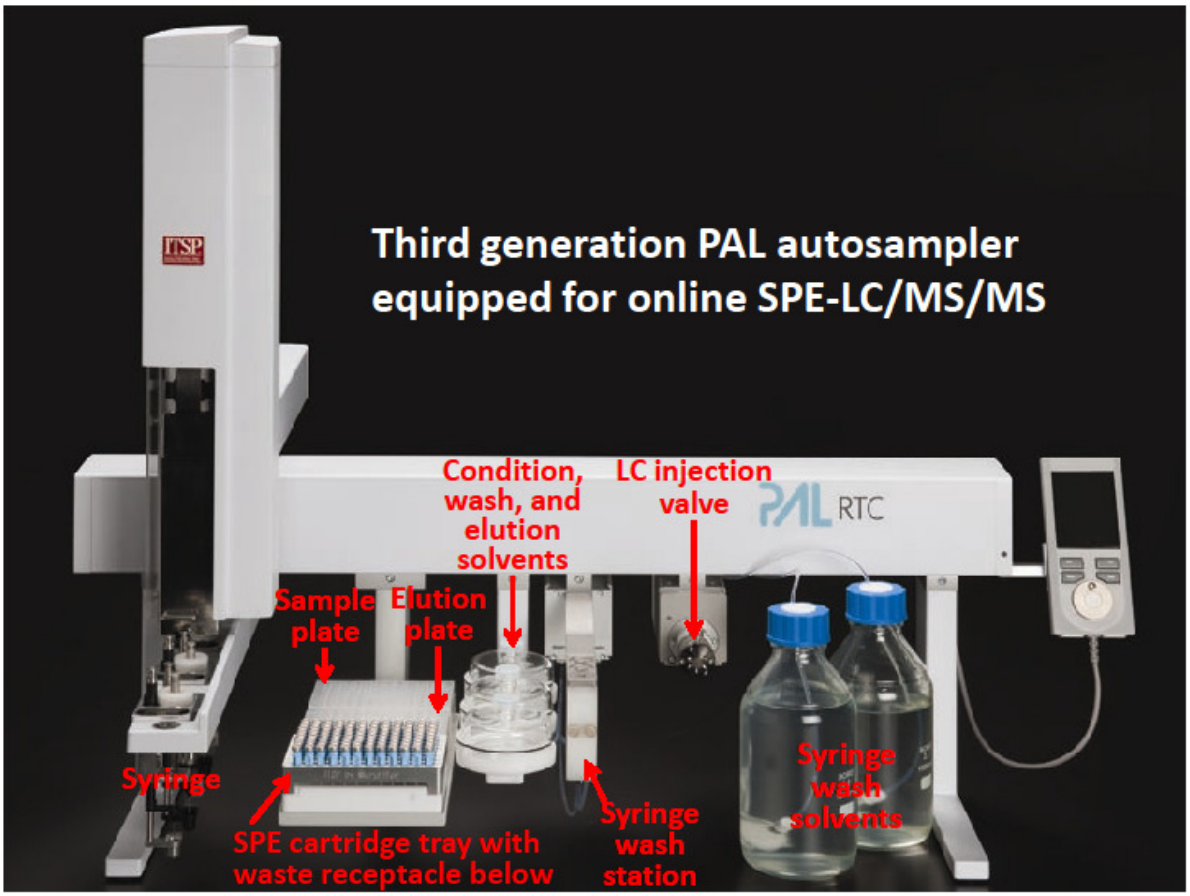

Figure 2. A PAL RTC autosampler equipped to perform online SPE-LC/MS/MS.
[Hoses connect the SPE cartridge tray and syringe wash station to an ordinary lab solvent waste container. Used cartridges are typically discarded by the PAL into a box under the wash station and LC valve.]

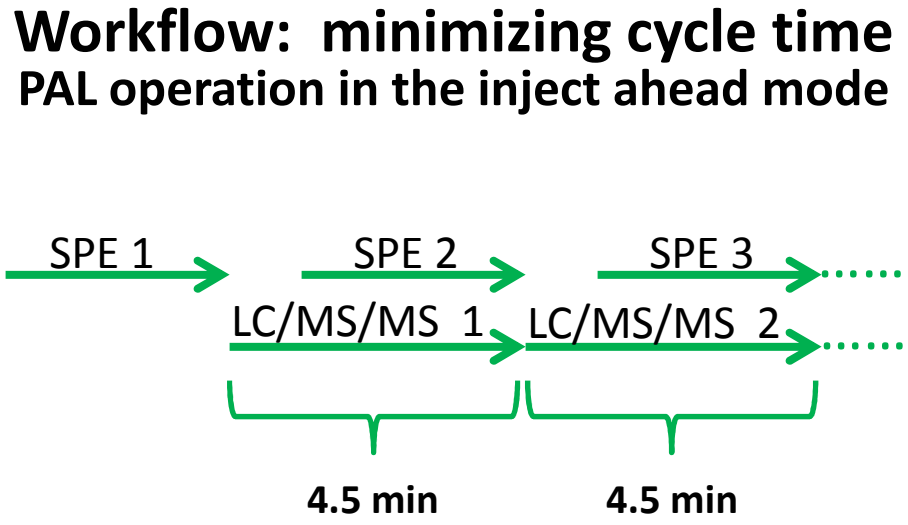

Figure 3. The execution timeline for SPE in parallel with LC/MS/MS for the analysis of drugs (71) in urine. In this process, overnight measurement of two 96 well plates of samples per LC/MS/MS is routine and the results are ready for review when the lab opens in the morning.

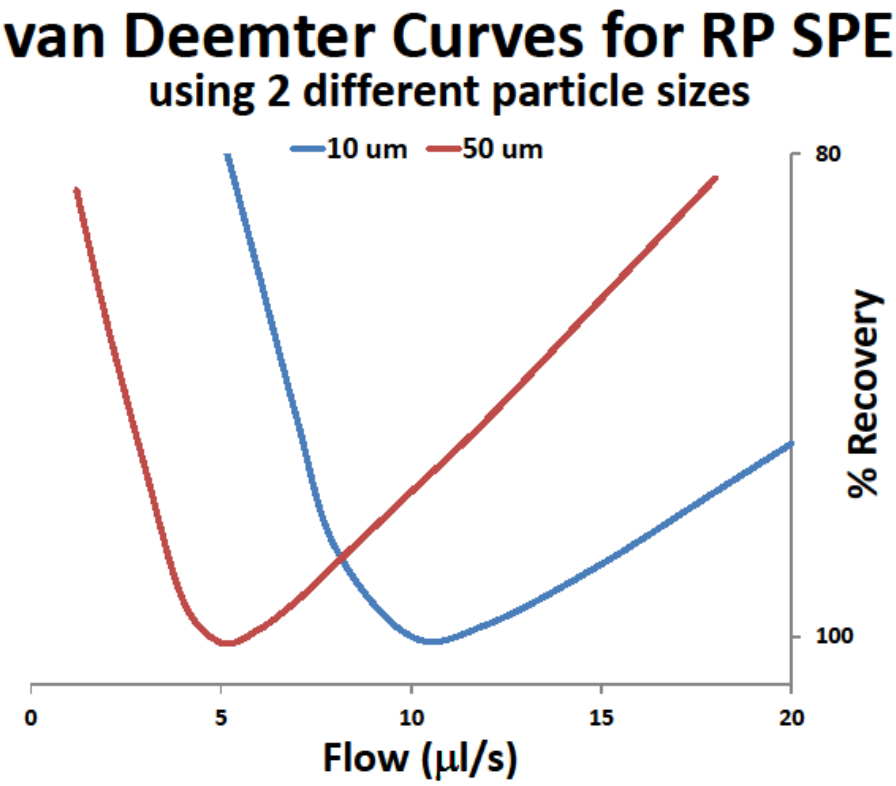

Figure 4. van Deemter curves for reverse phase (C_{18} endcapped) SPE using two different particle diameters (10 and 50 µm). This figure also shows the source of variable recovery with vacuum or pneumatically driven flow, because as flow drifts away from the optimum, there is always a price paid in recovery.

[%Recovery squared (relative variance, σ^2, in the amount of sample recovered) is the dimension plotted on the y-axis while the numbers indicated on the axis are %recovery as an aid to the reader. This approach is analogous to the %RSD approach to determining plate height described by Neue,[6] but appropriately, here it is used only for flow optimization.]