

Chromatography Sample Integrity with High-Density Sample Handling Solutions

Many chromatographers are investing in semi-automated and automated sample preparation systems to speed sample processing and make it more hands-free.

Introduction

Maintaining sample integrity to obtain ultimate sample separation and analysis is essential for chromatographers. It becomes even more critical – and more difficult – when using high-throughput, autosamplers and instruments that are compatible with the Society of Biomolecular Screening (SBS) standards. Although high-throughput systems allow for high-speed injections and sample processing while decreasing sample waste, they increase the risk of sample cross-contamination as well as raise issues regarding solvent resistance and compatibility.

Background

For the research analyst, ensuring high-performance sample analysis in a timely manner is job number one. At the same time, chromatography facilities face demands to analyze an increasing volume of samples without a concurrent increase in personnel, and a significant portion of a research analyst's time is spent on sample preparation. Therefore, novel solutions have been developed to allow the use of high-throughput protocols and automation techniques. The adoption of standard protocols for high-throughput batch preparation methods allows for more rapid analysis, but consideration must be given to the risk to sample integrity and accurate sample analysis. Automation-friendly, SBS-footprint, high-throughput plate systems are readily available from many manufacturers, with various configurations for sealing mats and closures. Many of these systems, however, contribute to sample cross-contamination or are incompatible with organic and/or volatile solvents, leading to questionable sample integrity and inaccurate sample analysis. In addition, many of these systems prevent efficient transfer of samples from one station to another, hindering the high-throughput process flow.

Solutions

Here we review the standard available formats for high-throughput plate systems, as well as the uniquely designed solutions to decrease the risk of cross contamination and allow for better process flow integration, resulting in the maintenance of sample integrity and accuracy of sample analysis.

Plates vs. Plates with Inserts

There are two popular available formats for use with high-throughput systems: sample plates and plates with inserts (Figure 1). Both plates and plates with inserts use standard SBS-footprints, in terms of base dimensions and layout. In 1996, the SBS established specific dimensions for microplates to standardize these plates and how they interact with various automation solutions. To maximize the fit and function of available chromatography sample plates with automation and semi-automation instruments and devices, most plates follow the standard dimensions set forth by the SBS. Not only is the standard footprint important, but the spacing between wells and/or inserts is equally important for use with multichannel pipettors or automated

and semi-automated dispensers and extractors. If the footprint of the plates are not standardized, multiple different instruments are required for the same task or modifications of instruments are needed between assays. These types of changes and modifications can lead to variances in data, and ultimately to a decrease in sample analysis accuracy. Thus, all sample plates are created in an 8 x 12 format (or 16 x 24 for 384 well plates), with equal distances between the center of each well and standard distances around the edge of the plate.



Figure 1 - (Left) Sample Plate and (Right) Sample Plate with Inserts

Sample plates are generally produced from polypropylene, a popular and standard resin that is relatively non-reactive and resistant to many reagents. Available in a range of sizes, based on recommended maximum sample volumes (correlated to height of the plate), wells can be square or circular with either round (U) or conical (V) bottoms. Important to note is that polypropylene, similar to many plastic resins, is not resistant to many organic solvents, thus glass is a preferred material for steps involving organic solvents.

Sample plates with inserts are, as the name implies, basically the available square or circular well sample plates with glass inserts that reside in each well. The use of inserts allows for multiple advantages over that of plates alone. First, inserts can significantly decrease the total assay volume required. Conical-bottom inserts allow for maximal recovery of sample. In addition, the use of inserts allows for the utilization of independently sealing closures, ensuring no cross contamination.

Sealing Mats

The most basic closure system for plates and plates with inserts are sealing mats and tapes. Sealing tape mats are easy to use and inexpensive, offering protection from evaporation and condensation. Sealing tapes are made from acetate, aluminum, or polyethylene, among other special derivatives. Select sealing tape mats are available without adhesive at the cavity areas, which can interfere with accurate analysis by contaminating samples during processing.

Sealing mats (Figure 2) are also easy to use and relatively inexpensive. Generally made of silicone or ethylene vinyl acetate (EVA), these mats

can also be coated with materials such as polytetrafluoroethylene (PTFE) to provide a level of resealability. Of note, silicone mats are resistant to alcohol, while EVA mats are resistant to many solvents. However, most sealing mats do not provide long-term viable seals and can contribute to sample cross contamination when removed. Neither sealing tapes nor mats are appropriate for volatile work flow processes.



Figure 2 - Sealing Mat with Sample Plate

Individual Closures

When using glass inserts with sample plates, individual closures can offer the best protection from cross contamination, thus protecting sample integrity. While a variety of snap caps are made from polyethylene with a limited silicone/PTFE septa, others are available made from polypropylene with various popular septa combinations (Figure 3). Importantly, select individual closures offer a secure snap seal that is equal to that observed with standard 12 x 32 autosampler vials, such as those offered as part of the patented MicroLiter Plate Sampling System (MPSS). The use of individual closures allows for single samples to be removed and used in external processes without risk of losing the sample. In addition, with the appropriate heating block and vice enclosures, these high-throughput solutions can be used for volatile reactions that normally have to be processed with standard gas chromatography screw-thread or crimp-top vials. Thus, individual closures prevent cross contamination, facilitate individual sample processing, and are a viable option for standard as well as highly volatile sample preparation protocols.



Figure 3 - Individual Closures with Sample Plate (with Inserts)

CapMat Closures

Unique closures are found with CapMat systems, which enable secure sealing of individual inserts while facilitating high-throughput workflow transfer of all 96 inserts as one (Figure 4). These polypropylene CapMats (Figure 5), with a selection of septa options, are a cross between individual closures and sealing mats. Importantly, those offered as part of the MPSS offer a secure seal equal to that observed with standard 12 x 32 autosampler vials with snap cap closures. Thus, these plates with inserts can be used to process volatile samples in high-throughput workflow processes.



Figure 4 - CapMat with Sample Plate (with Inserts)

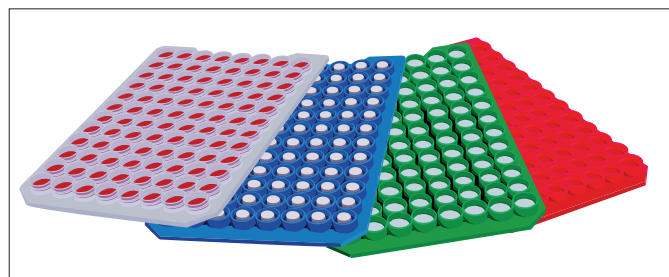


Figure 5 - CapMats with Selection of Septa Options

Conclusions

Research analysts are under pressure to perform a high volume of analyses, without a concurrent increase in work force. Thus, many chromatographers are investing in semi-automated and automated sample preparation systems to speed sample processing and make it more hands-free. The right high-throughput solution needs to be chosen, however, based on specific protocols to prevent cross contamination, ensure sample integrity, and to provide viable, accurate analysis. While various solutions are flooding the market, only specific purpose-designed solutions are appropriate for protocols involving volatile solvents and reactions. In addition, not all solutions provide the protection required to prevent cross contamination. To determine the most appropriate solution, a thorough review of workflow processes and identification of specific pain points are required.

Works Cited

Society for Laboratory Automation and Screening. (2004). ANSI/SLAS 2-2004 For MicroPlates.



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