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BY LOY SHICK, TECHNICAL SERVICES MANAGER, THERMO FISHER SCIENTIFIC

THE IMPORTANCE OF PRE-CLEANED CHROMATOGRAPHY VIALS

MASS SPECTROMETRY is a powerful analytical technique commonly coupled with differing chromatography methods for sample separation. Since it is increasingly being adapted for more diverse applications, contamination issues affecting sensitivity and reproducibility must always be considered. Advances in GC practice, such as employing on-column injection, and newer techniques employing finely packed HPLC columns, require the elimination of as much particulate matter as possible from the sample stream. The quality of sample vials used can potentially compromise final analysis and this provides a compelling case for the use of chromatography vials which are cleaned to remove trace level contaminants and tested as standard. This article investigates the effectiveness of the industry's first pre-cleaned chromatography vials, the new National Scientific Mass Spec Certified Vials from Thermo Fisher Scientific for highly sensitive applications where chromatography is coupled with mass spectrometry.

Introduction

Detection of compounds can be accomplished with very minute quantities in mass spectrometry. This means that compounds can be identified at very low concentrations, down to the femtogram level, in chemically complex mixtures. Advances in sample separation techniques and materials have greatly improved the reliability, sensitivity, precision and accuracy of mass spectrometry, expanding its versatility and application base. Mass spectrometry, therefore, now provides valuable information in biotechnology, for example, on the analysis of proteins, peptides and oligonucleotides; and in the pharmaceutical industry in areas of drug discov-

Figure 1 LC/MS background scan, positive electrospray ionization.

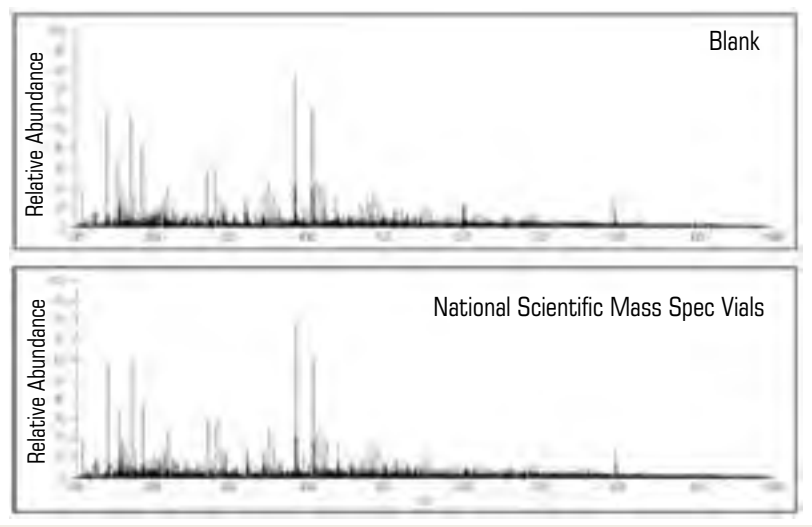
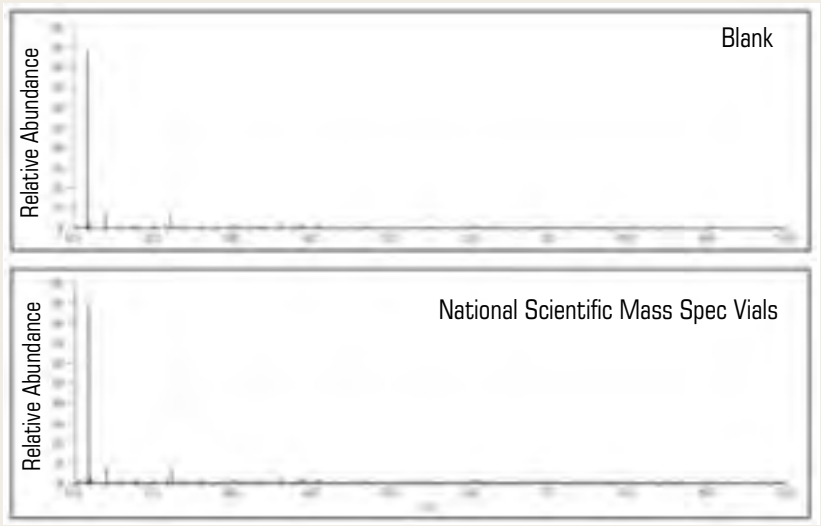


Table 1 Typical Cumulative Particle Counts.

VIAL	≥0.1µm	≥0.15µm	≥0.2µm	≥0.3µm	≥0.5µm	≥2.0µm	≥5µm	≥10µm	≥15µm
Competitor Vials	5,677	3,809	2,755	1,709	1,051	307	76	4	0
Nat Scientific Vials	356	264	218	192	176	160	45	8	3

Figure 2 LC/MS background scan, negative electrospray ionization.



ery, combinatorial chemistry, pharmacokinetics and drug metabolism. It is also used in the environmental industry for monitoring polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), water quality, and food contamination; and in the geological industry for investigating oil composition. In addition, mass spectrometry is proving ever more versatile in the clinical setting for neonatal screening, hemoglobin analysis, drug and other analyte testing, proteomics, and biomarker analysis.

Chromatographers have always depended on high temperature glass forming methods to burn off organic contaminants in a vial that might be detected by instrumentation during analysis. Residual compounds that might survive the glass forming process were either not detected by traditional chromatography techniques, or were present in concentrations too low to affect separation and analysis. Recent advances in instrument construction and separation techniques, however, have resulted in cases where high temperature glass forming procedures cannot guarantee the production of a vial with no detectable background.

The presence of inorganic sub micron particles in all glass vials as a byproduct of the manufacturing process is a phenomenon that has not been extensively studied. Gas chromatographers depend on injection port liners to act as traps for particulates while the HPLC chromatographer takes extensive steps to eliminate them during sample preparation or trap them with guard columns. This has been an effective strategy for routine analytical methods, but the need to work with ever lower concentrations of analytes creates the possibility of interactions with compounds of interest. As chromatographic instruments and techniques continue to advance, it can be seen that an unprocessed sample vial can potentially limit the ability to produce reliable results.

Low Particulate Background

It has therefore become apparent that pre-cleaned vials are now required for today's highly sensitive hyphenated techniques. As a result, the industry's first pre-cleaned chromatography vials, National Scientific Mass Spec Vials, undergo a proprietary cleaning process, in a GMP compliant, fully validated clean room environment, which greatly reduces the background contaminants and their potential effect on high sensitivity chromatography. These certified mass spec vials undergo additional processing to remove trace artifacts introduced by manufacturing methods. Each production lot is then tested for particle counts, LC/MS and GC/MS background, according to a sampling plan designed to assure consistently superior performance from every vial. After processing, the vials are immediately packed into pre-cleaned, inert vial trays. Ultra high purity bonded PTFE/Silicone closures are packaged in an airtight, resealable container to further reduce the possibility of contamination. A series of studies have been undertaken to evaluate and verify the effectiveness of these pre-cleaned vials.

In the first study, particulate analysis of a typical unprocessed vial compared to the National Scientific Certified Mass Spec vials was carried out. All National Scientific Mass Spec Vials were processed and tested for background particulates. Table 1 shows the processed vial to have a significant reduction in total particle counts. A

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Figure 3 GC/MS background scan, positive electrospray ionization.

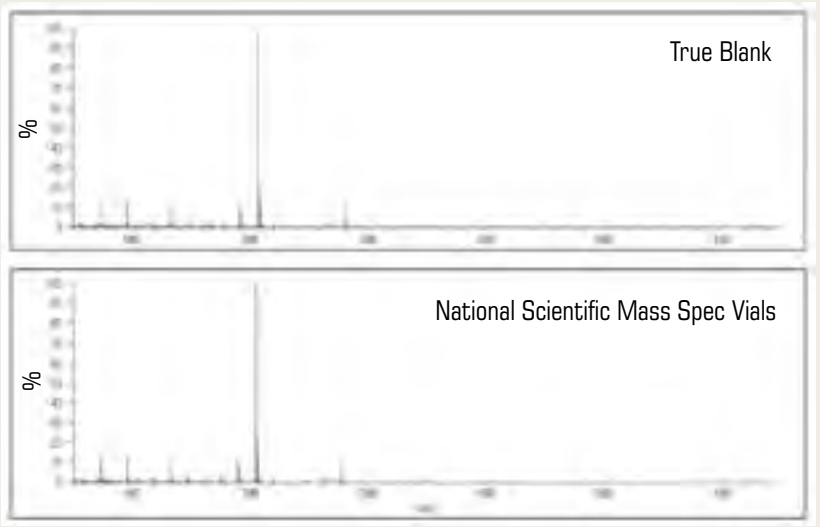
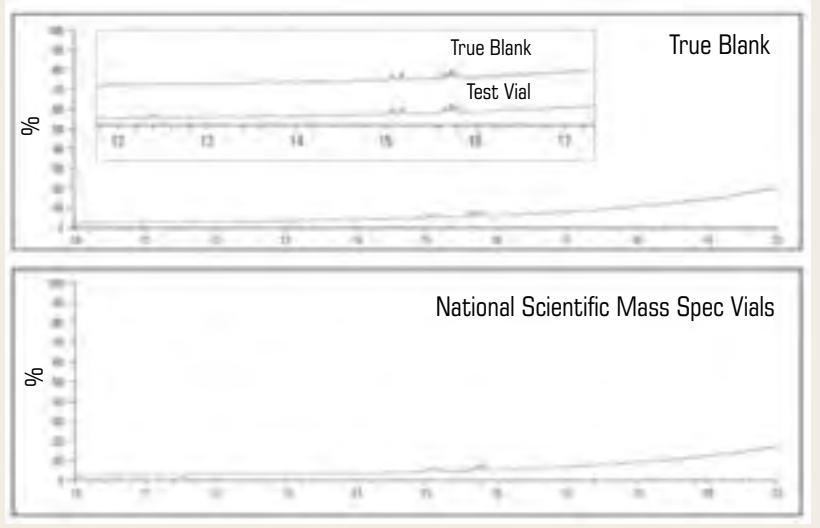


Figure 4 GC/MS background scan, TIC.



typical vial that has not been processed can exhibit particle counts exceeding 5000 particles per mL, with the highest counts occurring in the range below 0.5µm. This has traditionally been of little concern when GC inlet liners or HPLC guard columns are used. However, GC techniques employing on-column injection create the need for a sample vial with minimal background particulates in order to prevent an accumulation of foreign material at the head of the column that might adversely affect a separation. Similarly, newer techniques employing finely packed HPLC columns, capillary columns and direct connection of the analytical column to the sample valve also require the elimination of as much particulate matter as possible from the sample stream.

Low LC/MS Background

In order to further investigate the effectiveness of processed vials, samples of pre-cleaned Mass Spec vials and closures were exposed to acetonitrile at room temperature for two hours. Potential non-volatile organic compounds were determined using LC/UV and LC/MS with several different ionization techniques: positive electrospray, negative electrospray and positive atmospheric pressure ionization (APCI). Additional testing was conducted on samples exposed to acetonitrile for two hours at a temperature of 50°C to determine the effect of severe operating conditions. The results of the room temperature and 50°C appeared essentially the same, indicating that the background contribution from the processed vials is minimal over a wide range of conditions. Typical background scans for the room temperature exposure are shown in figures 1 and 2 using positive electrospray and negative electrospray ionization respectively. The top scan in each figure is the result of injecting the pure blank extracting solvent without exposure to glassware other than the original shipping container. The second scan for each figure represents an injection of an equal quantity of the extracting solvent after exposure to the precleaned sample vial.

Low GC/MS Background

A portion of the vial extracts prepared for LC/MS analysis was also taken for analysis by GC/MS. As with the LC/MS evaluation the vials were exposed at room temperature and 50°C. There was no significant difference between the room temperature and elevated temperature test results. A typical GC/MS chromatogram and mass scan are shown in figures 3 and 4 using positive electrospray ionization, with blank solvent in the upper scan and the vial extract shown in the lower scan.

Conclusion

In order to achieve improved levels of sensitivity, reliability, and reproducibility, today's

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high performance hyphenated techniques require the elimination of all possible contaminants from every stage. The sample vial is an important link between the prepared sample and final analysis, and as such must also meet this requirement.

Vials must, therefore, be produced under the strictest automated manufacturing and quality controls systems to ensure a reproducible performance is obtained with every use. Studies have shown that the new National Scientific Mass Spec Certified vials exhibit minimal background particulate levels and minimal LC/MS and GC/MS background contamination over a wide variety of conditions. Consequently, these new pre-cleaned vials are highly effective and offer the reassurance of meeting the rigorous demands of an extensive range of current highly sensitive chromatography applications.

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