Results of Using Re-washed Vials and Closures

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Introduction

When using glass autosampler vials in the laboratory, standard workflows require that vials and closures be considered consumable items and that they are discarded after each use. The use of laboratory dishwashers for the cleaning of laboratory glassware has sometimes been applied to autosampler vials. The cleaned vials are then utilized in the laboratory in hopes of reducing consumable costs.

This note shows that this cleaning is causing both physical damage to the glass vials and is also ineffective at removing contamination from the vial surface. This contamination is unpredictable and produces interference peaks in both LC and GC chromatography applications. Unpredictable contamination can be a major source of unexplained assay failures resulting in the need to repeat the entire analysis of complete sample runs.

The experimental work involved investigation of the physical effects of the washing process followed by the issues of contamination from the previous usage of both the vials and associated septa.

Physical Effects of Re-washing

Evaporation Variation

Sample 1: Re-washed 9 mm screw thread vials were provided in clear glass

Sample 2: Re-washed 9 mm screw thread vials were provided in amber glass

Sample 3: New clear glass 9 mm vials (C4000-1W) and new closures with PTFE/silicone septa (C5000-54B) were provided by Thermo Scientific™ National™ as baseline reference samples

Thermo SCIENTIFIC

Room Temperature Evaporation

- Vials filled with methanol were allowed to sit at room temperature for 1 hour
- Initial weight was taken on a four place analytical balance
- The vials were loaded into a sample rack and allowed to sit at room temperature for 7 days
- Final room temperature weight was taken and the difference calculated as sample loss in grams

High Temperature Evaporation

- The vials were returned to the rack and incubated at 40 °C for 24 hours
- The final high temperature weight was taken and subtracted from the final room temperature eight to yield the 40 °C sample loss in grams

The evaporation data is compared in Figures 1a–1c.

Evaporation levels are higher for all vials which have gone through the re-wash process.

Impact of using any Septa which has been Re-used

Figure 1d shows that any septa from the re-used vials and caps showed increased evaporative loss. This showed that the integrity had been lost due to the physical splitting of the PTFE and silicone layer.

Septa Re-use

Used Septum Evaluation

- 10 new 8-425 vials were filled with 100% methanol and capped with a new 8-425 closure with PTFE/Silicone pre-slit septum
- 10 re-used septa were hand inserted into a new 8-425 cap and attached to new 8-425 vials filled with 100% methanol

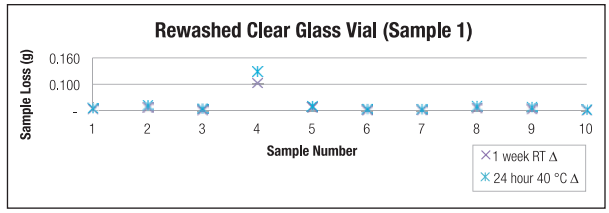

Figure 1a

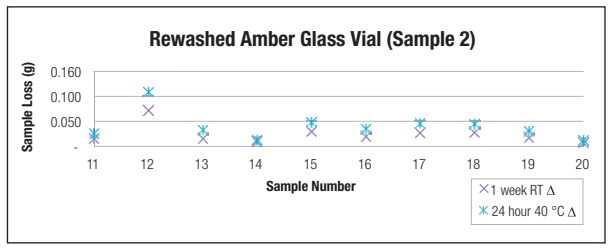

Figure 1b

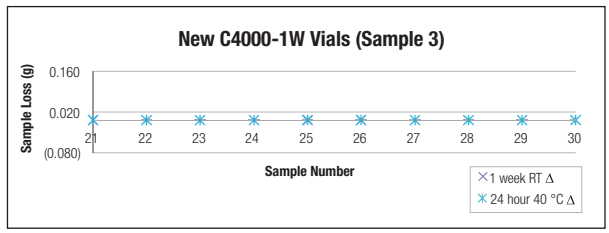

Figure 1c

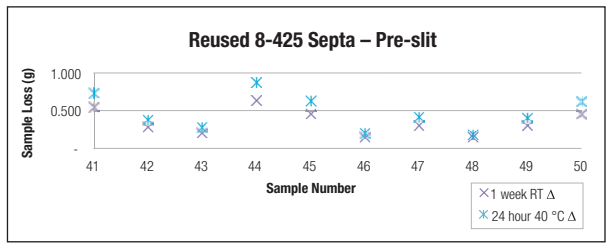

Figure 1d

Figure 2a: Typical amber and clear re-washed vials

Figure 2b: Typical re-wash screw thread damage

Figure 2c: Remains of identification patches on amber vials after re-washing

Surface Deterioration

Physically the vials exhibited damage to the threads and there was extensive abrasion on the vial surface. The extent of the abrasion was enough to have removed the write-on identification patches commonly found on this product. The removal was associated with the clouding of the glass limiting the transparency and visibility of sample levels (Figure 2a–c).

Glass Surface Microstructure

Chromatography vials are formed from high quality neutral borosilicate glass. Molten glass tubing is first drawn into long tubes of a specified outer diameter and wall thickness. The tubing is then reworked at high temperatures to form the closure profile and bottom shape of the vial. The vials are then annealed to remove stress and strengthen the glass before being packaged. The surface of the annealed glass is smooth and free of surface scratches and abrasions.

When vials are re-washed they become scratched, abraded and plaques deposit on the surface. The surface is no longer free of stress, but through the scratching areas of potential, breakage and cracking can be identified (Figure 3).

Post-annealing the neutral borosilicate glass may be considered to be a Type 1 glass with a homogenous surface chemically populated with siloxane bridges. Breaking of this surface both increase surface area and cleaves the siloxanes to release silanol groups on the glass surface. The increase in acidic silanols will increase the surface activity resulting in increased potential adsorption of any basic chemical species in the sample.

The effect of physical damage to the vial surface and the thread can have far reaching consequences.

- The changes in evaporation rate from the vial will introduce uncertainty into the concentration of standards and samples
- Loss of solvent from the vials will change concentration and have impacts on recovery and precision of measurement even when using internal standards
- The change in surface characteristics will increase the risk of permanent sample adsorbtion within the vial, particularly of basic solutes
- The glass is weakened and breakage of the vials during autosampler transport cannot be discounted

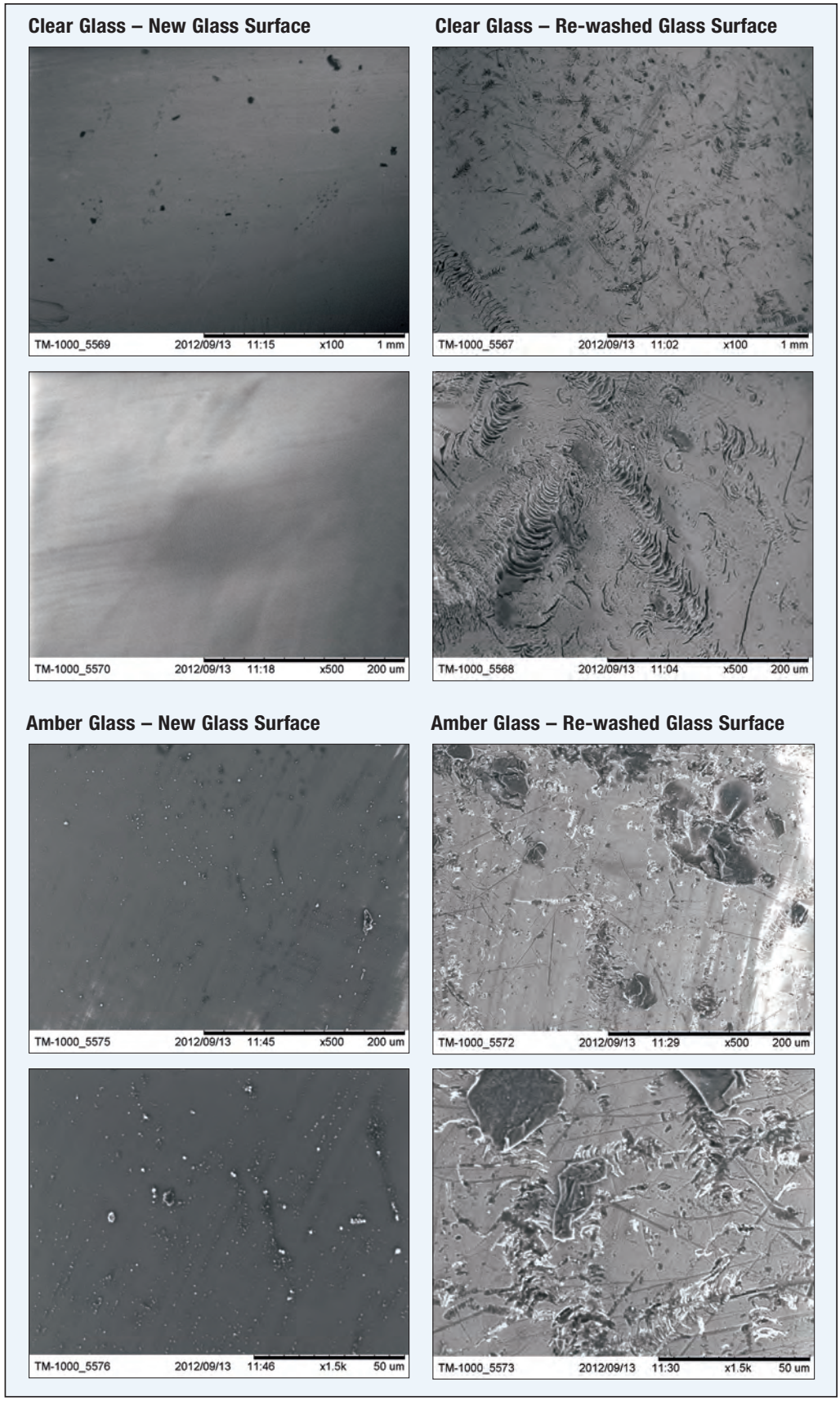

Figure 3: Comparison of new and re-washed glass vial surfaces

GC-MS Comparison of New Vials and Re-washed Products

One milliliter samples of methanol used as a blank solvent were stored in a number of standard glass vials. Samples stored in new vials were then compared to samples injected from randomly selected re-washed vials. Two types of vials were used, amber and clear. The clear vials were all manufactured from 33 expansion neutral borosilicate glass. Amber vials were 51 expansion neutral borosilicate Type 1. All the re-washed vials had been subjected to multiple wash cycles.

The new vial blank Total Ion Count (TIC) is compared to two amber vial blanks.

The profiles from the two vials , show that Vial A has cyclic polysiloxanes as a contaminant while Vial B has additional impurity peaks. The contamination is not predictable, but is dependent on the samples previously in the vials (Figure 4).

A similar blank comparison carried out with the clear 33 expansion vials also show great variation in both the intensity and profile of contaminants extracted from the vials. In this case, one vial showed that tailing species were extracted and distorted the GC baseline in a key part of the GC profile.

Another vial tested in the same sequence showed much lower responses for all peaks (Figure 5).

The use of such re-washed vials with GC-MS in TIC mode will lead to the production of significant impurity peaks that will interfere with analytical peaks or lead to poor quantification due to co-elution.

GC-MS Method

GC Instrument	Thermo Scientific™ TRACE™ GC
Oven	40 °C (0.5 minute), 15 °C/minute, 150 °C (1 minute), 10 °C/minute, 290 °C (5 minute)
SSL	Splitless (1 minute), 50 mL/minute split flow, constant septum purge
Left Carrier	Helium at 1.2 mL/minute, constant flow, Vacuum compensation ON
SSL Temp	250 °C
Columns	Thermo Scientific™ TraceGOLD™ TG-5MS 30 m × 0.25 mm × 0.25 µm
MS Instrument	Thermo Scientific™ ISQ™ single quadrupole GC-MS
Transferline Temp	290 °C
Ion Source Temp	230 °C
Ionization Source	EI
Electron Energy	70 eV
Scan Range	50–650 full scan, dwell time 0.2 seconds
Solvent Delay	3.5 minutes
Autosampler	Thermo Scientific™ TriPlus RSH™ autosampler
Sample Volume	1 µL

Table 1

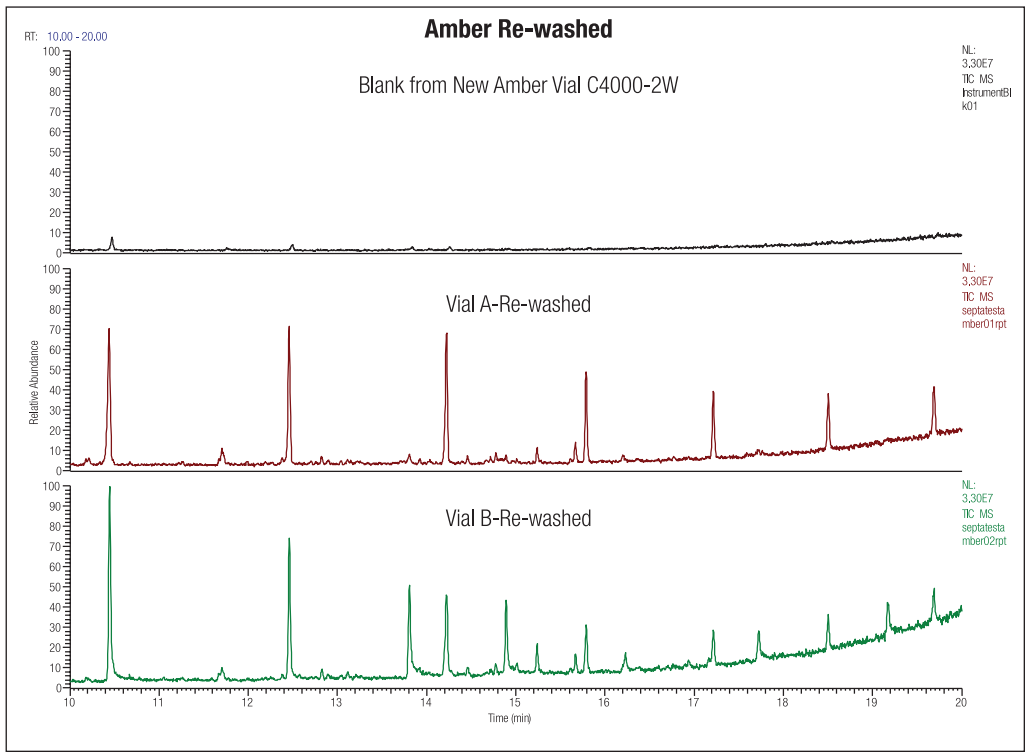

Figure 4: 10–20 minutes TIC screen

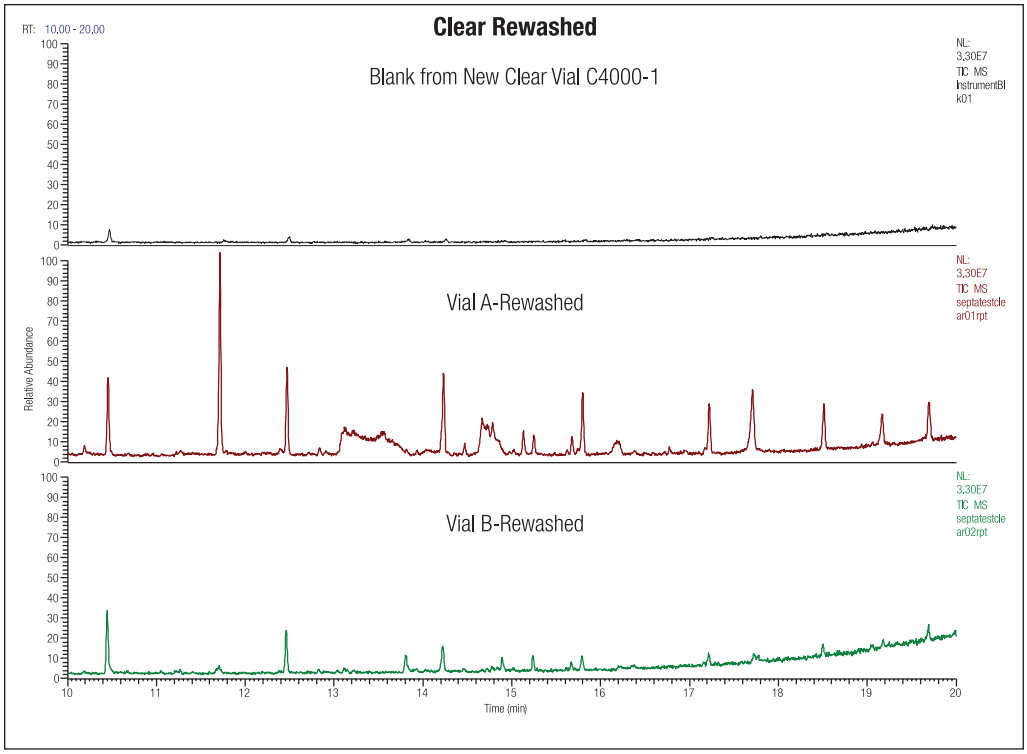

Figure 5

Impact of Re-using Septa

In addition to re-using vials the extraction and re-use of injection septa may also be encountered.

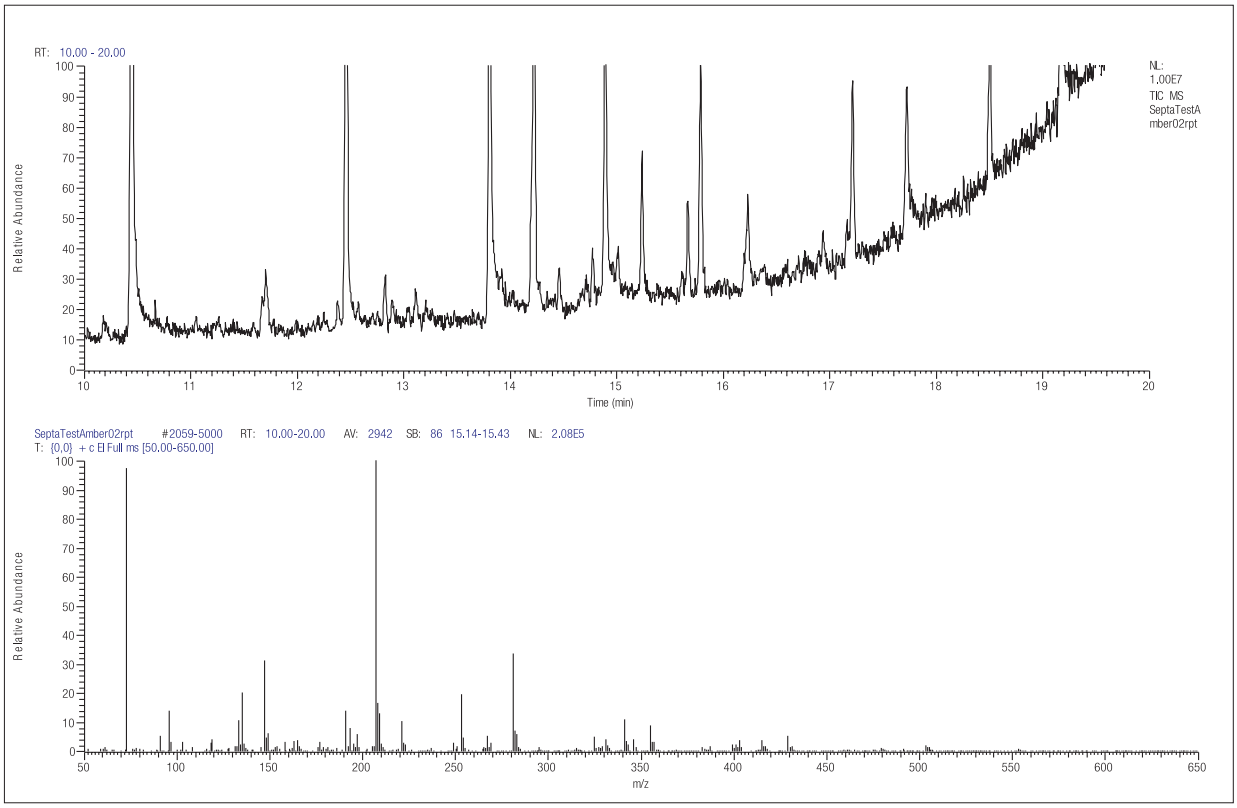

Figure 6: (Top) GC-MS and TIC trace showing components from siloxane extraction peaks. (Bottom) Siloxanes in particular are more easily extracted from damaged slit septa.

Effects on Instrumentation

After carrying out three separate runs with the re-washed vials and septa, the performance of the GC started to deteriorate even with the clean blanks.

The injection liner was inspected and deposits were found trapped in the liner. Some could be seen as black fragments which came from the discolored silicone layer.

Deterioration of the septa can increase instrument down-time required for injection liner replacement and also cause carryover into standards and blanks.

HPLC-UV Interferences Found in Gradient Profiling

HPLC Instrument	Thermo Scientific™ Accela™ 1250 PDA
Mobile Phase	A: Water/Formic acid 1% B: Acetonitrile A:B 5–95% 10 minutes
Flow Rate	0.5 mL/minute
Injection Volume	5 µL
Detection	PDA 210 nm, 220 nm, 254 nm

Table 2

A series of linear gradients were run and monitored by UV. Injections from the re-washed vials showed that a number of peaks were detected at 220 nm and 254 nm that were not present in the new vials samples.

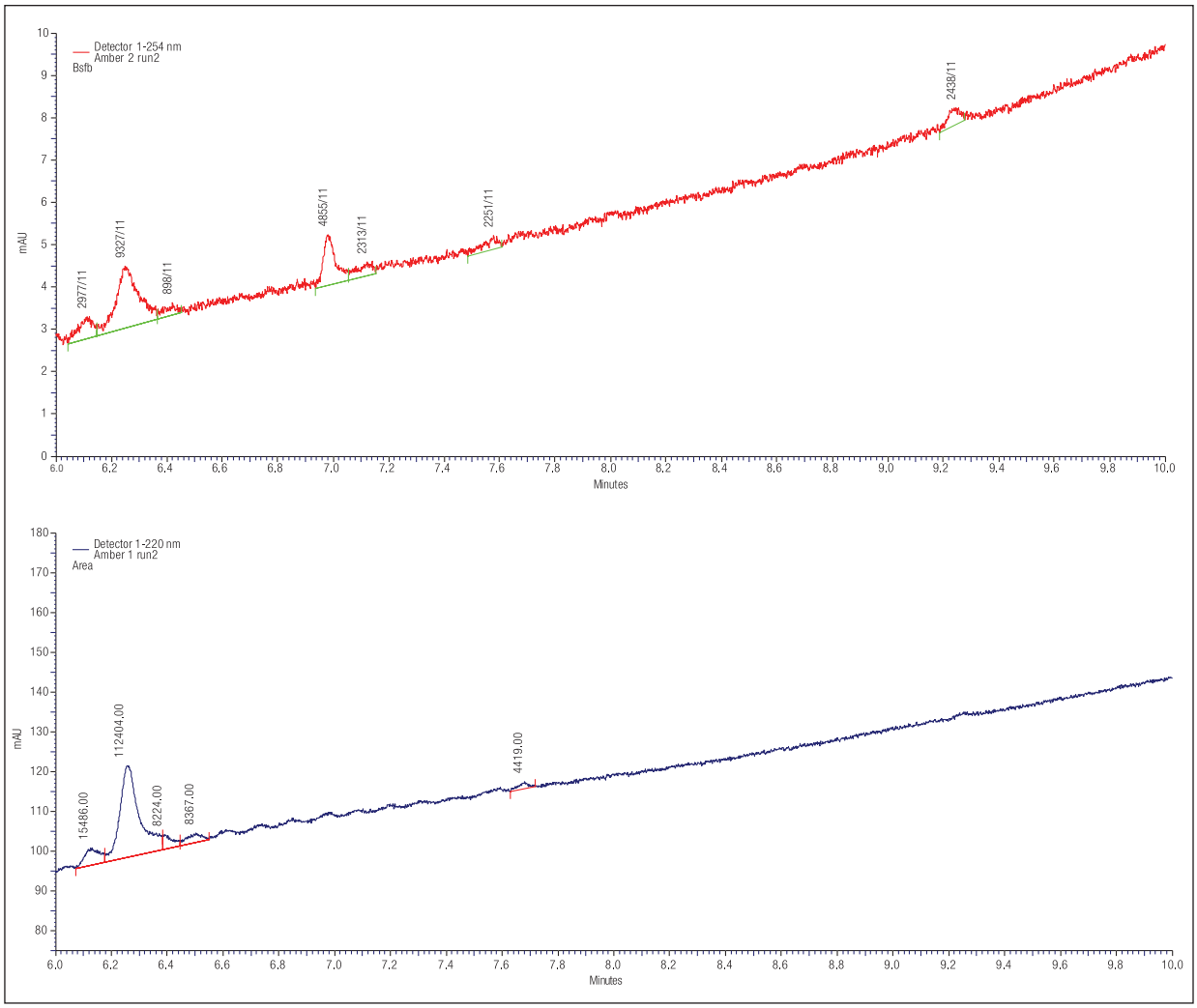

Figure 7: Extract from gradient profile from re-washed vial

HPLC Instrument	Thermo Scientific Accela 600 LCQ Deca XP	
Column	Thermo Scientific™ Hypersil GOLD™ 3 µm 100 mm × 2.1 mm	
Mobile Phase	A: Water/Formic acid 1% B: Acetonitrile A:B 10–100%; B in 25 minutes	
Flow Rate	0.3 mL/minute	
Injection Volume	10 µL	
Detection	+ESI MS	
	Probe position	C5
	ESI needle voltage	4.5 kV
	Capillary temperature	300 °C
	Capillary voltage	46 V
	Sheath gas	40
	Auxiliary gas	10
	Full scan	50 to 1500
	Microscans	3

Table 3: HPLC-MS

HPLC-MS Comparison of Background Signal from Re-used Septa against New Septa and Vial Combinations

The presence of siloxane contamination from re-used septa may be shown by the additional mass peaks shown in the scan from a gradient elution similar to the UV scans below.

Sample incubated with methanol at 30 °C for 12 hours with 1) Clear new vial and Silicone/PTFE septa
2) Clear re-washed vial with a recycled split septa.

Background MS signal increased by factor of 5 compared to new vial and septa combination when samples are compared using the instrument conditions shown in Table 3.

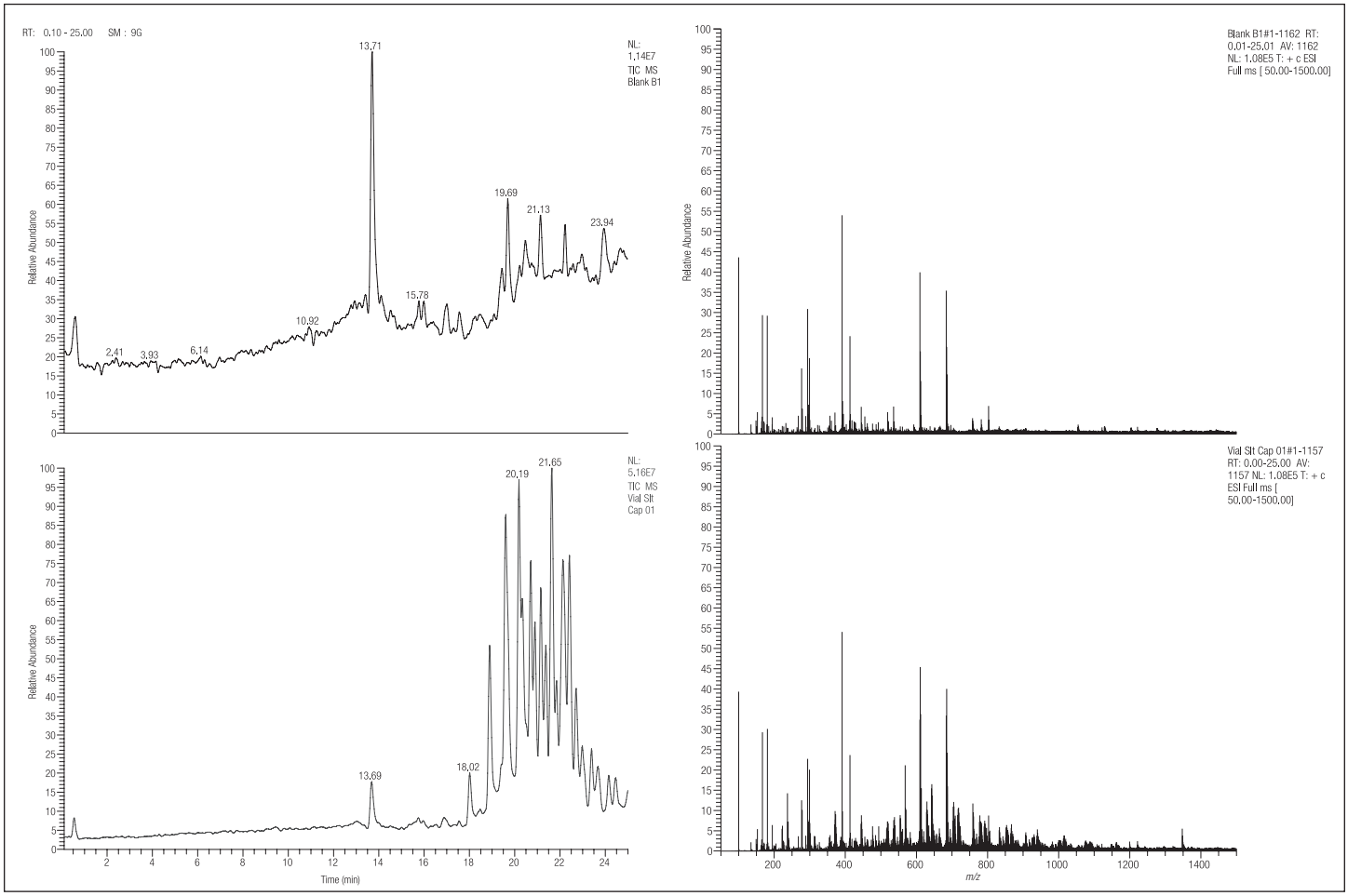

Figure 8: Comparison of blank with contaminated vial using the Silicone/PTFE septa

Summary

The use of re-washed vials and re-using septa is compared to the use of new vials.

- Comparison of blank injections from such re-washed vials was carried out with GC and HPLC instrumentation
- In all cases additional components were found in the GC-MS blanks. Interference with standards was found in the TIC mode.
- GC-Instrumentation down time was increased due to an increased rate of injection liner replacement
- In HPLC, the introduction of even small volume injections showed introduction of additional peaks in the UV mode
- Comparison of contaminated and new vials was clearly shown by a full mass range scan
- When re-used septa were added the contamination of the injection from the vials was increased

Conclusion

Optimal chromatography results can be compromised by the use of re-used/re-washed vials and septa. Re-used/re-washed vials and septa can introduce random non-reproducible interference peaks into otherwise stable chromatographic methods. This can be a major source of unexplained assay failures that often require retesting entire sample runs, sometimes from the initial prep stages. Time spent in retests and troubleshooting an assay failure that cannot be duplicated negates any perceived benefits of re-using vials and septa. Chromatographers requiring uncompromising sample integrity would be best advised to always use new vials and septa for each analysis.

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