The Forgotten Septum

How to Correctly Diagnose the Source of Bleed Contamination

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- Avoid lengthy inlet troubleshooting.
- Reduce interference with correct solvent-septum compatibility.

Septum bleed is not common, but when it occurs it is observed as sharp, repetitive peaks in high temperature portions of an analysis. Bleed peaks can come from either the injection port septum or the vial cap septum. Interfering peaks and inaccurate data can result, so it is important to correctly identify the source and understand how to eliminate or minimize the bleed.

Diagnose the Bleed Source

The bleed from either septum shows a similar pattern (Figure 1), but it is easy to determine the source with a simple test. Isolate the injection port by setting the instrument to perform a run without an injection. Perform an analysis; if the bleed disappears, then the vial cap septum was the source. Determining if the vial cap septum is the source of the bleed can save time by preventing unnecessary troubleshooting and maintenance of the injection port. If the vial cap septum is causing bleed, the problem can be eliminated or minimized with the following considerations.

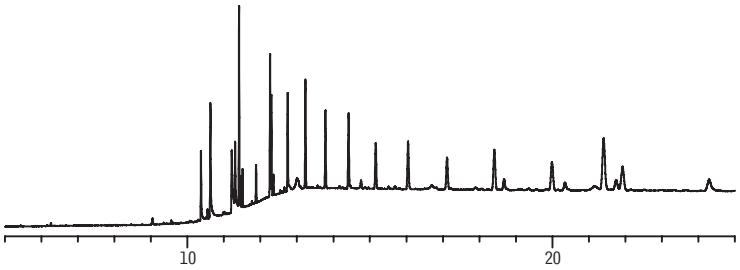
Check Solvent-Septum Compatibility

Most of the time, septum bleed is negligible. However, when a solvent and vial cap septum are incompatible, extreme contamination can occur. Figure 2 compares the first and fifth injections from a vial containing a derivatized amphetamine sample. In this case, the septum bleed peaks are almost as large as the analyte peaks. This level of bleed can interfere with analyses, especially those geared for trace levels. Reduce the risk of septum bleed by using a compatibility chart, such as the one in the on-line version of this article *(www.restek.com/general)* to determine which septum material is compatible with the sample solvent used.

Use Lined Septa

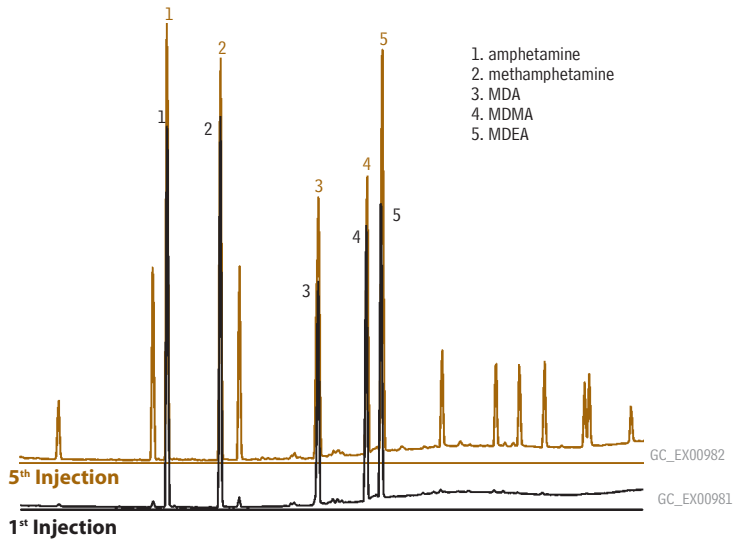
Most vial cap septa are lined with a protective layer of polytetrafluoroethylene (PTFE) to prevent solvent attack. As shown in Figure 3, PTFE effectively prevents septum breakdown due to solvent exposure. In comparison, unlined septa exhibit bleed after just 24 hours at room temperature. Bleed levels for unlined septa varied by material, but even a low level of bleed can interfere with integration and is of particular concern for trace analyses (Figure 4).

Figure 1 Sharp, repetitive peaks are typical of septum bleed from the vial cap or injection port.



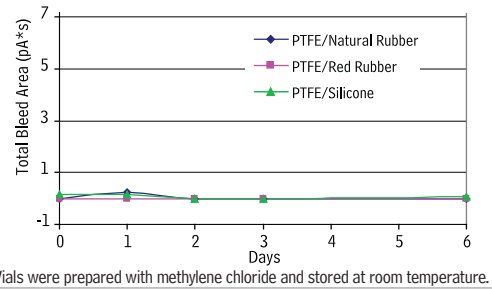
Columns:	Rtx®-5MS, 30m, 0.25mm, 0.25im (cat.# 12623)
Sample:	methylene chloride blank
Inj.:	1.0µL split (split ratio 10:1), 4mm split inlet liner w/ wool (cat.# 20782)
Inj. temp.:	240°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	70°C (hold 1 min.) to 290°C @ 20°C/min. (hold 13 min.)
Det.:	FID @ 250°C

Figure 2 Contamination from septum bleed can cause significant interference with target analytes.



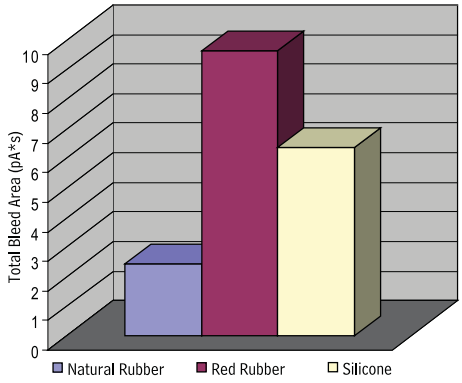
Column:	Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample:	100µg/mL each amphetamine, methamphetamine, MDA, MDMA, and MDEA extracted from methanol and HFAA derivatized
Inj.:	1µL, splitless (hold 0.5 min.), 3.5mm custom splitless inlet liner w/ IP deacitvated wool
Inj. temp.:	220°C
Carrier gas:	helium, constant flow
Flow rate:	1.25mL/min.
Oven temp.:	70°C (hold 1 min.) to 290°C @ 15°C/min. (hold 4 min.)
Det:	MS
Transfer Line temp.:	280°C
Scan range:	43-450amu
Ionization:	EI
Mode:	scan

Figure 3 PTFE lining prevents bleed due to solvent/septum interaction.



Vials were prepared with methylene chloride and stored at room temperature.

Figure 4 Unlined septa show bleed contamination within 24 hours.



Vials were prepared with methylene chloride, sealed with caps containing septa that were inserted upside down in order to expose the non-PTFE lined septum surface to the solvent, and stored at room temperature.

Figure 5 Bleed contamination increases over multiple injections.

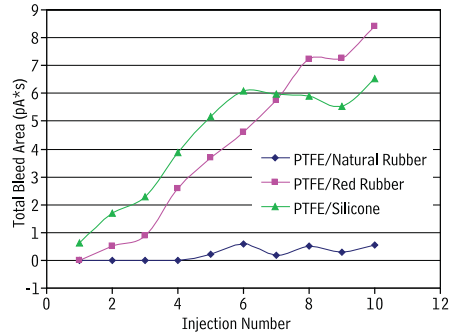
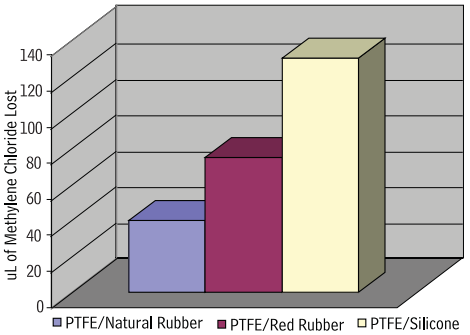


Figure 6 This bar graph shows the volume of solvent lost for three septum types.



Vials containing 300µL of methylene chloride were punctured and left at room temperature for 24 hours.

Consider Resealability

Multiple injections can core the vial cap septum and lead to significant bleed. Resistance to coring varies by septum material (Figure 5). Coring can be minimized by preparing separate vials for replicate injections, when feasible, and by carefully considering the type of septum material when multiple injections are necessary. Septum resealability also affects evaporative loss, which can be a significant source of error for low volume samples. For example, a relatively nonvolatile analyte in a volatile solvent can concentrate significantly due to evaporative loss (Figure 6). Vials should be recapped when necessary for extended runs or long term storage.

Conclusion

Septum bleed is not a very common occurrence, but when bleed does occur, it is easy to assume the injection port septum is the source because the vial cap septum often is not considered. However, correctly diagnosing the source of bleed contamination can save time and effort by preventing unnecessary injection port maintenance. Effectively and efficiently reducing interfering peaks by controlling septum bleed can significantly improve analytical performance, particularly for trace analyses.

Crimp-Top Vials, Snap Seal™ Style—12 x 32mm, 11mm Crimp

Description	100-pk.	1000-pk.
2.0mL Clear Glass Vial w/White Graduated Marking Spot	24383	24384
2.0mL Amber Glass Vial w/White Graduated Marking Spot	24385	24386
2.0mL Clear Glass Vial without Graduated Marking Spot	21152	21153

2.0mL, 11mm Aluminum Crimp Seals with Septa

Description	100-pk.	1000-pk.
Silver Seal, PTFE/Natural Rubber Septa	21174	21175
Red Seal, PTFE/Red Rubber Septa	24355	24356
Silver Seal, PTFE/Silicone Septa	24359	24360

Limited Volume Inserts for 2.0mL Crimp-Top & Short-Cap, Screw-Thread Vials

Description	100-pk.	1000-pk.
350µL Glass, Flat Bottom Insert w/ ID Ring	24692	24693

Rtx®-5MS—Low-bleed GC/MS Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623

Split Liners for Agilent GCs

ID* x OD & Length (mm)	qty.	cat.#
4mm Split w/ Wool		
4.0 ID x 6.3 OD x 78.5	ea.	20781
4.0 ID x 6.3 OD x 78.5	5-pk.	20782
4.0 ID x 6.3 OD x 78.5	25-pk.	20783

*Nominal ID at syringe needle expulsion point.