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Article

Septum Bleed during GC–MS Analysis: Utility of Septa of Various Makes

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The significant presence of septum-related ghost peaks, causing interference in routine gas chromatography–mass spectrometry analyses at sample injection port temperatures above 100°C, is demonstrated. A comparative study with commonly employed septa of various types and makes under varying analytical conditions, e.g., injection port temperature, carrier flow rate, capillary column type and oven heating rate reveal that long-chain hydrocarbons, substituted phthalate derivatives and silanes (silicon compounds) are responsible for such interferences, which is confirmed from their respective peak fragmentation patterns after comparison with standard mass spectrometry library data. Consequently, prior blank studies at actual analysis conditions may become mandatory for quantification and reduction of such interferences, ignoring septa quality and performance claims.

Introduction

In past gas chromatographic (GC) analyses, it has been reported that septa of various makes release volatile organic compounds (VOCs) when subjected to high temperatures (1–9).These reports also revealed the frequent presence of contaminants such as finger oils from handling, or organic contaminants picked up during storage of these septa. A recent study also describes the detection of a mixture of hydrocarbons, alcohols and ketones, found at lower temperatures from septa made from butyl rubber (9). After release, part of such organics primarily collect in the analytical column downstream, subsequently creating either baseline disturbances or irrelevant peaks, first due to on-column focusing at lower analysis (oven) temperatures; these later appear as ghost peak(s) in the chromatogram after elution, thus interfering with identification and quantification of the target analytes. Frequently, any inconsistent septum bleed also affects the reproducibility in repeat analysis (3, 4). The problem was reported to be quite common in temperature-programmed analyses, as the released VOCs routinely collected in the column during the oven cool-down event, and initial hold periods, even with the septum purge mode on (3). Because capillary columns require much lower gas flow rates (typically ≤ 2 mL/min) than packed columns ($\sim$25 mL/min or even more), the VOCs released from septa become more concentrated in the former, and thus bleed problems become pronounced, especially when used with the highly sensitive mass spectrometry (MS) detector. It is generally believed that the septa bleed may be reduced by injecting samples in split mode (6) and/or by using a septum purge.

In our recent measurements with a newly acquired GC–MS equipment, while analyzing samples consisting of a mixture of benzene or cyclohexane reaction products at injector port (IP) temperature above 200°C, we always observed the presence of a number of strong peaks in the chromatograms at retention times (t_R) beyond 14 min, irrespective of the nature and amount of samples used. After taking into account all standard protocols and precautions, even repeat measurements without any sample injection revealed the persistence of these peaks. After systematically eliminating all other possibilities, we concluded that the septum in use was the source of such ghost peaks. The latter was confirmed when the septum was isolated with a clean aluminium foil, preventing any contact with the carrier gas. Although a change of septum of the same make did not alter the results, using septa of other makes only marginally changed the results. We therefore realized that global advancements in septum manufacture have failed to address the vital issue of persistent bleeding at high temperatures. Our efforts to find appropriate reference details about ghost peak interferences also revealed only a few studies (1–9). Therefore, we carried out a systematic evaluation in this direction, and our results suggest that mere selection of high quality septa is not sufficient for correct or accurate analyses. Instead, in each case regular comparative blank studies (without any sample injection) at actual measurement conditions (IP temperature, septum purge flow and split ratio) are mandatory for the elimination of ghost peak interferences.

Experimental Details

The current measurements are conducted using a recently procured Thermo Scientific Ceres 800 GC coupled to a DSQ II MS system from Thermo Scientific (Nasik, India). The split/splitless IP system in use is shown in Figure 1. It consists of a cylindrical steel body fitted at the inlet, with a carrier gas line, and at the exit, with split and septum purge gas lines. The body is welded to a support, which is fixed to the gas chromatograph by two screws. A glass pre-column or liner is inserted into the body: a reinforced graphite seal and a steel washer ensure isolation between the upper and lower parts of the IP body. The upper part contains a spring and an internal ring through which the carrier flows. The IP is closed by an external nut, which holds the introduction septum. The IP is heated directly by an aluminium block, which contains the heating resistance and the temperature probe.

Different septa shown in Figures 2A–2D used in this study were obtained from various sources, including: SGE Analytical Science (Australia, part number 041904; suggested application maximum temperature of 400°C); yellow PTFE coated (part number 041827 suggested application maximum temperature of 200°C); Thermo Scientific BTO (part number THC31303230 no suggestion of application maximum temperature) supplied

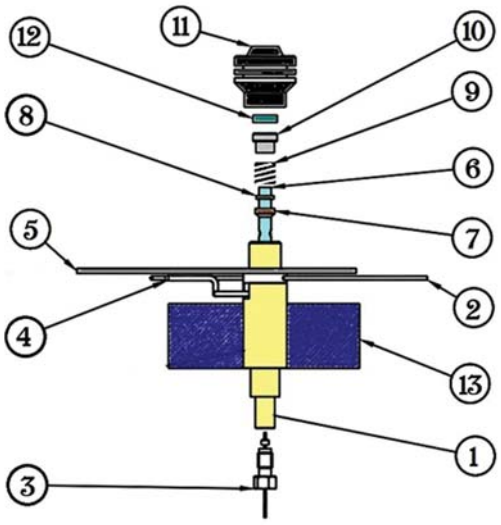


Figure 1. Schematic presentation of injector port assembly of GC–MS system in use: cylindrical steel body (1); carrier gas line (2); split gas line (3); septum purge gas line (4); support (5); glass pre-column or liner (6); reinforced graphite seal (7); steel washer (8); spring (9); internal ring (10); external nut (11); septum (12); aluminium block (13).

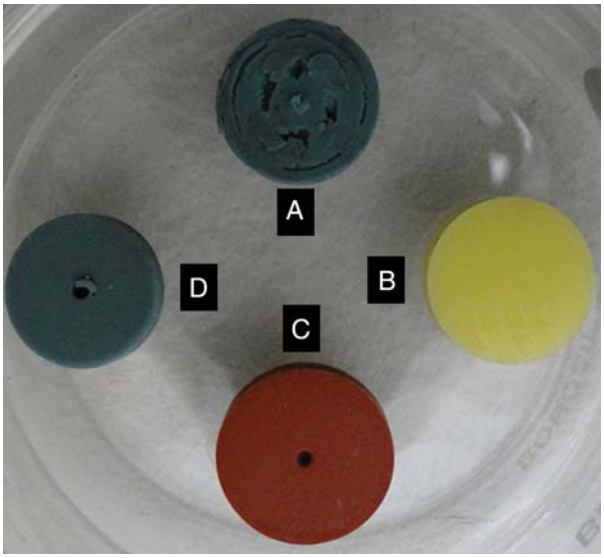


Figure 2. Photographs of various septa used in this study. Septa B and C are shown without syringe injections. Septum A was also found to become damaged after 25 syringe injections at 250°C, unlike Septum D.

with the GC–MS instrument system; and CNW Technology (Germany), ultra-low bleed septum (code number 1.442077.0050 suggested application maximum temperature of 350°C), obtained locally from National Analytical Corporation (Mumbai, India). Septa for measurements were selected at random from different lots received and used after standard conditioning steps. The following capillary columns were used in this evaluation study: Agilent DB-5MS, 30 m × 0.25 mm, 0.25 μm coat; Thermo TR-5MS, 15 m × 0.25 mm, 0.25 μm coat; and SGE BP 10, 30 m × 0.32 mm, 0.25 μm coat; however, no significant differences were noticed except in the change in t_R of various ghost peaks. Thus,

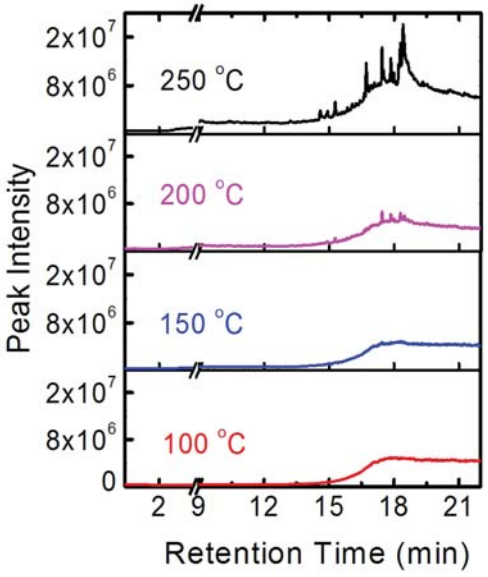


Figure 3. GC–MS chromatograms obtained using Septum A at different IP temperatures.

although the GC–MS analyses reported herein were conducted using a DB-5MS column, similar conclusions would also hold for others not included here. Carrier (helium) flow was fixed at 0.8 mL/min, oven temperature programming was as follows: 50°C (hold 3 min) at a rate of 20°C/min to 150°C (hold 5 min); then a rate of 20°C/min to 230°C (hold 8 min), totaling a 25 min run. The septum purge was fixed at 3 mL/min in all analyses. Because with an increase in carrier (helium) flow (from 0.6 to 1 mL/min), a corresponding shift of each peak's t_R to a lower magnitude was observed, for sake of consistency, further measurements were made at a fixed carrier flow of 0.8 mL/min. We analyzed 3–5 septa in each category/batch and more than five runs (even more than 10) for each septum at the selected measurement condition. All MS measurements were conducted at a fixed source temperature of 220°C and transfer line temperature at 250°C. The MS fragmentation patterns at peak t_R were checked and compared with the available standard listings of NIST and Wiley Mass Libraries.

Results and Discussion

Typical GC–MS chromatograms obtained using Septum A (sans sample injection) at increasing IP temperatures 100, 150, 200 and 250°C are shown in Figure 3. At 100 and 150°C, the chromatograms remained free from ghost peaks. However, at IP temperature >170°C, many peaks began to appear, and their respective intensities increased with further increase of IP temperature. Because the other experimental parameters like the oven-heating program and various gas-flow rates remained unchanged (keeping the column bleed or other system hardware performance identical), the observed peaks were ascribed to septum bleeding. Therefore, for any sample analysis using Septum A, either the IP temperature must be less than 170°C (thus severely restricting the particular septum use), or the

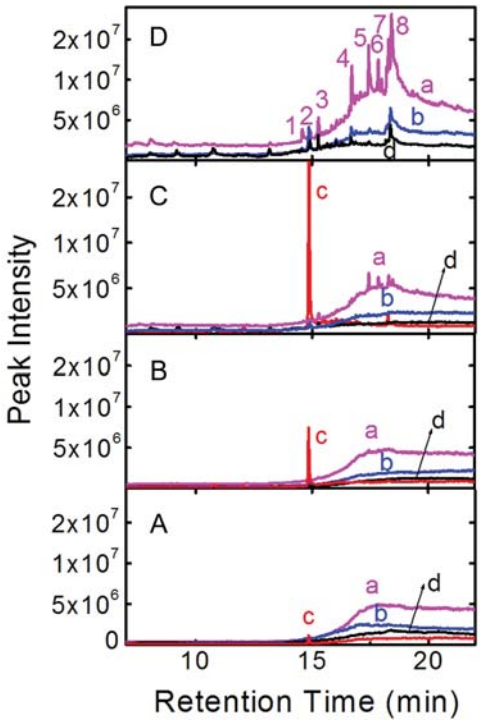


Figure 4. GC–MS chromatograms observed at IP temperatures: 100°C (A); 150°C (B); 200°C (C); 250°C (D). Traces for four different septa are represented as follows: a: Septum A; b: septum B; c: Septum C; d: Septum D. Individual Y-axes are shown differently for clear presentation.

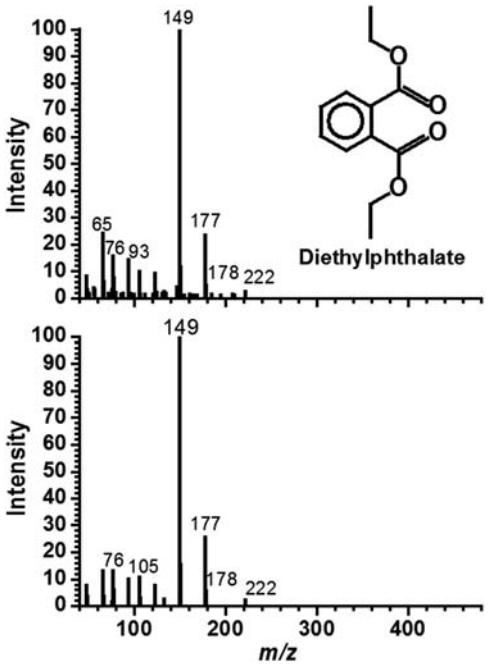


Figure 5. Comparison of MS fragmentation pattern of compound giving rise to the 14.88 min t_R peak in Figure 4 with standard fragmentation pattern of diethyl phthalate in standard libraries.

observed set of ghost peaks must be accounted for in each analysis. The latter option may, however, introduce unknown errors in routine analysis, especially if any genuine sample peak t_R overlaps with any of these ghost peaks' t_R.

The other three septa (B, C and D) were also tested separately in a similar manner, and their respective chromatograms are compared with that of Septum A in Figures 4A–4D at fixed injection port temperatures 100, 150, 200 and 250°C. All other septa except Septum C showed satisfactory performance (negligible ghost peaks) at IP temperature ≤150°C shown in Figures 4A and 4B. In case of Septum C, it was subsequently identified (Figure 5) that diethyl phthalate released from the septum was responsible for the sharp peak at t_R of 14.88 min. As shown in Figures 4A–4C, this peak intensity increased proportionately with a further increase in IP temperature. Because the bleeding from Septum C near IP temperature of 200°C was quite severe, further analyses at higher IP temperatures were not attempted. Therefore, Figure 4D at 250°C IP temperature includes only three chromatograms obtained with the remaining septa. In Figure 5, comparison of the MS fragmentation pattern for 14.88 min t_R peak with the standard library listings (NIST and Wiley) confirms the chemical responsible to be diethyl phthalate.

The yields of diethyl phthalate from four septa with respect to the peak area (× 10e6 mV/s) at t_R 14.88 min are listed in Table I. The peak at 14.88 min was chosen for comparison because it appeared even at lower IP because of Septum C bleeding. At IP temperature ≥200°C, the recorded chromatograms (Figures 4C and 4D) exhibit many new peaks. Amongst

Table I
Relative Peak Areas (10e6 mV × s) for Diethyl Phthalate (t_R 14.88 min) Released from Different Septa

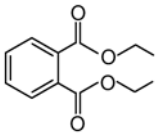
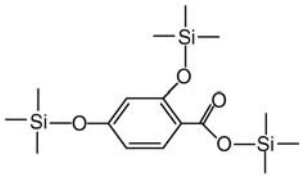
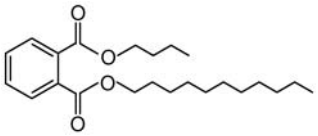
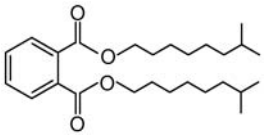
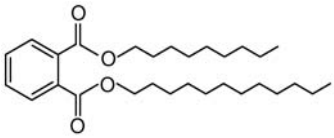
Injection port temperature (°C)	Septum A	Septum B	Septum C	Septum D
100	No peak	No peak	3.3	No peak
150	No peak	1.6	23.9	No peak
200	No peak	4.9	68.4	—
250	No peak	13.8	N/A*	—
With ethanol wash and baking at 150°C				
100	No peak	No peak	1.1	No peak
150	No peak	No peak	2.0	1.6
200	0.7	Not used	13.5	3.7
250	1.4	Not used	14.6	5.7

*The test was not performed due to high bleeding observed.

these, the chromatogram obtained for Septum D at 250°C was found to be extraordinarily crowded with many peaks. However, the individual intensity (area) of any of these peaks remained lower than that of the diethyl phthalate peak obtained from Septum C at 200°C and shown in Figure 4C. In Table II, all compounds responsible for various ghost peaks when Septum A was used at 250°C IP are listed along with their respective t_R.

To reveal the severity of interference from septum bleeding while analyzing unknown samples at elevated IP temperature, a typical analysis result using Septum D is shown in Figure 6. The chromatogram relates to the identification of various high boiling phenolic and aromatic hydrocarbon products obtained in gas-phase benzene oxidation, which were preconcentrated in ethanol solvent. The comparable areas of the two types of peaks (analyzed products in sample and septum related ghosts)

Table II
List of the Compounds Indentified in GC–MS Analysis using Septum A at 250°C Injection Port Temperature (Figure 4D)

Peaks	t_R (min)	Compounds	Structures
1	14.59	Dodecanoic acid	
2	14.88	Diethyl phthalate	
3	15.28	2,4, Bis ((tri-methylsilyl) Oxy)-benzoic acid	
4	16.71	Octadec-9-enoic acid	
5	17.43	Phthalic acid butyl undecyl ester	
6	17.82	Phthalic acid bis (7 methyl octyl) ester	
7	18.28	Phthalic acid dodecyl nonyl ester	
8	18.41	Hexadecanoic acid	

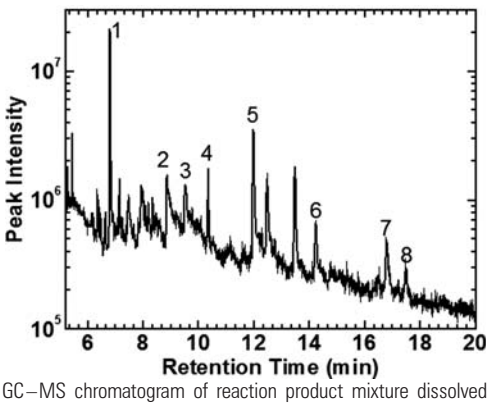


Figure 6. GC–MS chromatogram of reaction product mixture dissolved in ethanol. Septum D was used; sample injected: 5 μL; IP temperature: 150°C; column: Thermo TR-5MS, 15 m × 0.25 mm, 0.25 μm film; helium flow: 0.8 mL/min; septum purge: 3 mL/min; split ratio 1:20; oven temperature programming ramp 50°C (hold 5 min) at a rate of 20°C/min) to 150°C (hold 10 min) totaling a 25 min run. Peaks are due to phenol (1) at 6.8 min; 1,2 dihydroxybenzene (2) at 8.86 min; 1,4 dihydroxybenzene (3) at 9.53 min; biphenyl (4) at 10.35 min; 2-phenylphenol (5) at 11.97 min; benzophenone (6) at 14.24 min; 4-phenylphenol (7) at 16.79 min; 4-phenoxyphenol (8) at 17.5 min.

clearly reveal the extent of the interference due to the latter (unnumbered), which remained even at higher split ratio or septum purge flow.

Conclusions

The current results reveal that other than Septum C, the remaining three are suitable for GC–MS analysis if used at IP temperature $\leq$150°C. Moreover, Septum D performance is generally superior to the other septa. Septum C bleeds even at 100°C, suggesting its unsuitability in routine high-temperature GC–MS studies. Significantly, all septa used in this study bleed phthalate compounds at much lower IP temperature, contrary to the respective manufacturers' claims on quality and usage. Therefore, it is suggested that the user should verify such technical claims first with an on-site analysis under actual working conditions and check the presence of ghost peaks, especially at higher IP temperature. These comparative studies also reveal that other sections of the GC hardware do not contribute in the presence of ghost peaks.

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