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Identification and Elimination of Polysiloxane Curing Agent Interference Encountered in the Quantification of Low-Picogram per Milliliter Methyl *tert*-Butyl Ether in Blood by Solid-Phase Microextraction Headspace Analysis

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Widespread use of the gasoline additive methyl *tert*-butyl ether (MTBE) and the subsequent human exposure that follows have led to the need to quantify MTBE in a variety of complex biological matrixes. In this work, we demonstrate our latest MTBE quantification assay for whole blood and uncover previously unidentified contamination sources that prevented routine quantification in the low picogram per milliliter (parts per trillion, ppt) range despite a sensitive and selective analytical approach. The most significant and unexpected sources of contamination were found in reagents and laboratory materials most relevant to sample preparation and quantification. In particular, significant levels of MTBE were identified in sample vial septa that use poly(dimethylsiloxane) (PDMS)-based polymers synthesized with peroxide curing agents having *tert*-butyl side groups. We propose that MTBE is one of the byproducts of these curing agents, which cross-link PDMS via the methyl side groups. Residual MTBE levels of ∼20 μg/septa are seen in septa whose formulations use these curing agents. Fortunately, these levels can be significantly reduced (i.e., <0.2 ng/septa) by additional processing. Performance achieved with this sample preparation approach is demonstrated using a mass spectrometry-based method to quantify blood MTBE levels in the low-ppt range.

Methyl *tert*-butyl ether (MTBE) is the second most-produced chemical in the United States with more than 200 000 barrels/day.[1] The high production rate is attributed to MTBE's use as a gasoline fuel oxygenate and octane enhancer.[2] Although the prevalence of automobile gasoline and the resulting emissions are the primary sources of MTBE in our environment, exposure can also occur indirectly through a variety of sources that include contaminated soil,[3] groundwater,[4,5] surface water,[6−8] and air.[9,10] The most common exposure sources for the general U.S. population are from automobile gasoline fumes, emissions, and contaminated underground water. For example, in urban areas that use reformulated or oxygenated gasoline, which can contain up to 15% MTBE by volume, environmental levels of MTBE in the air are typically in the low-ppb range with exposure levels reaching as high as 4.1 ppm during vehicle refueling.[11] Furthermore, leaking underground fuel storage tanks can quickly contaminate groundwater because of the significant solubility of MTBE in water (4.8 g/100 g[12]) and its stability under most anaerobic conditions.[13] By contrast, MTBE is less stable in surface water because of evaporation and microbial degradation.[14] Consequently, MTBE is found in 8.9% of tap water samples from the northeast and mid-Atlantic regions of the United States.[15] Because of the complexity and extent of these exposure sources and concentrations, the exposure profile among the general U.S. population is broad.

Although acute toxicological effects of MTBE have thus far proven to be relatively low in laboratory animals,[16−18] chronic

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exposure to high levels of MTBE and its metabolites have been shown to promote blood, renal, hepatic, and testicular cancers in laboratory rats.[19,20] However, the high doses of MTBE required to elicit disease in laboratory rats challenges the extrapolation of these results to low-dose exposure of humans.[21] For these reasons, the U.S. Environmental Protection Agency lists MTBE as a potential human carcinogen, and the Centers for Disease Control and Prevention measures MTBE internal dose in people and potential health effects, if any.

To help interpret acute and chronic effects of MTBE, researchers have mainly measured MTBE levels and its metabolites in blood,[22] urine,[22−24] and breath.[22,25,26] MTBE levels in humans and animals are relatively short-lived and are found to be correlated in blood, urine, and breath following exposure.[27] Among these sample matrixes, blood provides a more representative exposure profile because it is not as greatly influenced by the subjects' behavior such as fluid intake or breathing patterns, resulting in a more accurate assessment.[28] In humans, the terminal half-life of MTBE in blood is shown to be ∼1.8 h with a maximum concentration of 2 μM (176 ng/mL) when the subjects were exposed to 4.5 ppm MTBE in air for 4 h.[22] Terminal half-life following oral exposure has been shown to be somewhat longer at 3.7 h, achieving a 2 μM maximum concentration when subjects were administered 15 mg of MTBE in 100 g of water.[25]

In general, analyses of liquid samples with high concentrations of volatile analytes are performed by headspace (HS) gas chromatography (GC) analysis delivered by either an automated HS sampler or a gastight syringe. Likewise, direct GC injection approaches are typical for high concentrations of volatile analytes in breath and urine samples. In those studies in which analyte concentrations are low, preconcentration approaches are necessary. Among the approaches most used for blood and urine samples are purge and trap (P&T); however, solid-phase microextraction (SPME) with the use of labeled or unlabeled internal standards is emerging as an alternative.[29]

In this work, we combine SPME with use of a labeled internal standard to quantify MTBE in blood. When this approach is combined with GC/mass spectrometry (MS) analysis using selected-ion monitoring (SIM), detection limits readily extend into the low-ppt (i.e., pg/mL) range. However, one of the primary obstacles that we have encountered in quantifying low concentrations has been from contamination interference. Until recently, the sources of contamination have not been well understood, thus making this contamination difficult to control. Interestingly, the most significant of these sources was found to be certain polysiloxane materials that come in close contact with our samples. Our research has revealed that many polysiloxane materials are formulated with *tert*-butyl peroxide curing agents that react to produce significant quantities of MTBE as a byproduct. The chemistry involved with producing this MTBE residue is identified as well as the necessity and approach taken to minimize it. Here we describe the identification and minimization of MTBE contamination sources relevant to our analysis of MTBE in human whole blood.

EXPERIMENTAL SECTION

Reagents and Materials. MTBE (99%) was purchased from Sigma-Aldrich Corp. (Milwaukee, WI), MTBE-d_{12} from Cambridge Isotope Laboratories, Inc. (Andover, MA), HPLC grade water from Mallinckrodt Baker, Inc. (Phillipsburg, NJ), and P&T grade methanol from Honeywell Burdick & Jackson (Muskegon, MI).

Commercially available water often contains unacceptable levels of volatile organic contaminants. To remove these contaminants, HPLC grade water was helium purged and distilled in-house. The equipment for the distillation and helium purge system consisted of a Fuchs continuous distiller fitted with a bubbler to allow helium stripping during the distillation. The equipment and procedure, which are described in previous work,[30] involved purging the water for 17 h with ultra-high-purity helium (i.e., 99.9999%) followed by 4 h of reflux before the water was collected by distillation. The water was then immediately transferred while hot with a 10-mL serological pipet to either 5- or 25-mL glass ampules and sealed with an oxygen/natural gas flame.

Standard crimp-top 10-mL headspace vials were purchased from MicroLiter Analytical Supplies, Inc. (Suwanee, GA). The 20-mm headspace vial septa used are from Supelco (PN 2-7539 manufactured by Specialty Silicone Products, Inc. (Ballston Spa, NY)) or MicroLiter (PN 20-0055 Level 4 produced by Integrated Liner Technologies (Albany, NY)). These septa were nominally 20-mm diameter, between 1 and 1.3 mm thick, and were composed of a PDMS-based polymer with a poly(tetrafluoroethylene) (PTFE) barrier layer between 0.1 and 0.15 mm thick. The MicroLiter septa were cleaned by the producer to meet our minimal specifications equivalent to 17 h at 100−120 °C and either vacuum below 1.3 kPa or nitrogen purging above 100 mL/min. Prior to use, the septa were then reprocessed in-house for 17 h at 100 °C under vacuum below 1.3 kPa to remove any residue or postprocess contaminants from packaging, shipping, and storage. The headspace vials were sealed by backing the septa with a zinc-plated steel washer and capping the two with a standard 20-mm-diameter aluminum crimp top.

Sample Preparation. Internal standard was prepared from neat MTBE-d_{12}, which was serially diluted with methanol (P&T grade) to 3.6 μg/mL. This stock solution was aliquoted and flame-

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sealed in glass ampules and stored at −60 °C. A working internal standard solution (~18 ng/mL) was prepared weekly by dilution of the intermediate stock solution with methanol and then stored at 4 °C in 2-mL borosilicate glass vials with PTFE/silicone septa screw caps. The internal standard working solution (i.e., 40 μL) was aliquoted into all unknowns, blanks, standards and quality control specimens prior to analysis. All glassware was triple-rinsed with methanol and baked at 150 °C for at least 17 h prior to use.

MTBE standards were prepared in a similar manner as the internal standard by creation of intermediate solutions with methanol (P&T grade) that were stored at −60 °C in flame-sealed glass ampules. Aqueous calibration standards were prepared fresh weekly by transferring 40 μL of each intermediate standard solution into 25 mL of water. Final MTBE concentrations for standards ranged from 0.0008 to 2.005 ng/mL. A standard blank was prepared along with calibration standards to assess contamination during standard preparation. Following addition of internal standard, calibrators were aliquoted into headspace vials, crimp-sealed, and stored at 4°C until analysis.

Quality control (QC) samples were used to verify performance of the analytical assay. These samples were prepared by adding concentrated standards of MTBE into fetal bovine serum (Hyclone Laboratories, Logan, UT). After thorough mixing to allow for MTBE to equilibrate into the sera, aliquots were flame-sealed into glass ampules and stored at −60 °C. On the day of use, an aliquot of QC serum was thawed, mixed, and sampled as though it were an unknown. Two QC pools were prepared (at approximately 109.5 and 565.6 pg/mL) and quantified by 20 separate determinations. Blind quality control samples were evaluated using Westgard QC rules[31] by an independent QC officer. If a quality control sample exceeded QC limits for MTBE, then all results for MTBE in that run were rejected.

Blood samples were collected in 10-mL Vacutainers that were reprocessed to minimize residue contamination.[32] This procedure included removing and processing the Vacutainer stopper for 17 days under 1.3 kPa vacuum at 150 °C to reduce any interfering residue. Vacutainers that underwent this procedure delivered no detectable levels of MTBE (i.e., <0.2 pg/mL) to water stored horizontally for 6 days at 5 °C. Otherwise, unprocessed stoppers were found to deliver up to 20 pg/mL MTBE in water stored under these conditions.

To prepare the blood samples for analysis, we first placed the samples on a rotating mixer in a level 1 (type II class A/B3) biological safety cabinet at room temperature for a minimum of 15 min. Blood was sampled with a 5-mL glass barrel gas tight syringe (Hamilton). Once 3 mL of blood was drawn into the syringe, the blood was dispensed into a tared 10-mL headspace vial. Internal standard working solution (40 μL of 18 ng/mL MTBE-d_{12} in methanol) was added, and then the vial was capped and weighed. Weights of samples were used to correct the final concentrations. Quality control fetal bovine serum samples and standard water blanks were prepared using the same technique as blood samples.

Method regents and materials were quantified for MTBE using the same sample preparation and analysis approach as QC and blood samples. Reagents were analyzed in a 10-mL headspace vial as a neat solution or diluted with redistilled HPLC water to yield 3 mL and spiked with internal standard. MTBE concentrations in the septa were quantified by performing consecutive 5-mL methanol extractions. These extractions were analyzed both separately and combined. A final extract was taken to verify extraction completion. Each extraction was performed in a sealed 10-mL headspace vial that was placed for 15 min in an ultrasonic bath. For analysis, 40 μL of the methanol extract and 40 μL of the internal standard working solution were added to 3 mL of water.

Instrumentation and Setup. Solid-phase microextraction of each sample headspace was performed using an autosampler (Combi-Pal, Leap Technologies, Carrboro, NC). Samples were maintained at 15 °C until they were analyzed. During analysis, the samples were transferred to an agitating incubator (350 rpm, 40 °C) and sampled with a 75-μm Carboxen−PDMS SPME fiber (Supelco, Bellefonte, PA), which was inserted through the headspace vial septum into the headspace for 6 min. The SPME fiber was then inserted into the GC injection port fitted with a 1-mm-i.d. glass liner maintained at 250 °C. The volatile analytes were thermally desorbed and introduced onto a DB-VRX (Agilent Technologies, Palo Alto, CA) column (40 m × 0.18 mm × 1 μm film) by pulsed splitless injection at 50 psi. After 1 min, the injection port pressure was then decreased to maintain a constant flow of ~1 mL/min helium. Volatile analytes were refocused on the GC column using a cryogenic trap (Scientific Instrument Services, Ringoes, NJ) that was maintained at −100 °C for 1 min and then rapidly heated to 225 °C (~13 °C/s). The GC oven temperature was ramped from 0 °C (1.5 min hold) at 7 °C/min to 140 °C and then 40 °C/min to 220 °C (4.5 min hold). Response was measured using a quadrupole MS (5973N, Agilent) in SIM mode where the primary quantification ion was at m/z 73, the confirmation ion was at m/z 57, and the internal standard ion was at m/z 82 using 25-ms dwell times for each. Qualitative analysis was performed using full mass scan from m/z 21 to 500. Confirmation of MTBE was established by GC retention time and mass spectral data.

Quantification. All standards were prepared in water rather than in serum or whole blood because of the broad variation of VOC levels found in these biological media. Standard addition analyses indicated that water-based calibrators yield similar slopes as serum- and blood-based calibrators, thus validating the use of water-based calibrators for quantifying VOCs in whole blood.[33] An appropriate standard set of seven calibrators, one ranging from 0.8 to 500 pg/mL or one from 9.4 to 9400 pg/mL, was analyzed with each sample set. The low-concentration standard set was used to help estimate trace levels of MTBE residue in reprocessed septa, water, and methanol. The high-concentration set was used to quantify MTBE in septa that had not been reprocessed and in whole blood. It should be noted that, for our blood analysis assay, the formal limit of detection is conservatively set at 10 pg/mL to ensure adequate signal from both quantification ion (m/z 73) and confirmation ion (m/z 57). In this paper, MTBE contamination of laboratory reagents at levels below the lowest standard are not

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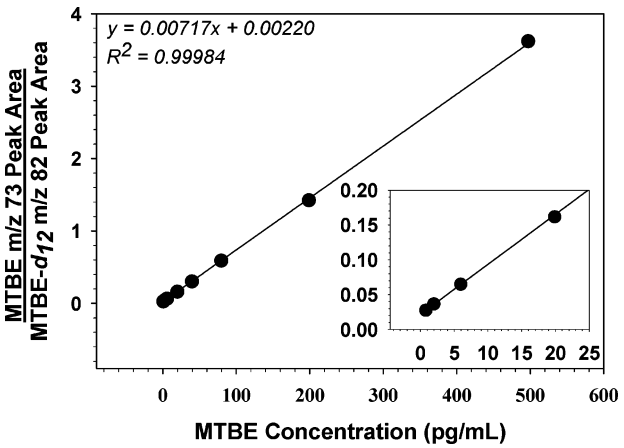

Figure 1. Typical MTBE calibration fit for standards in water matrix inset with expanded view of lowest four concentration standards. The fit shown is weighted $1/x$.

extrapolated but rather designated as <0.8 pg/mL. Formal quantification of MTBE levels in blood below the 10 pg/mL level were accomplished by a separate assay, which quantified from 0.8 to 500 pg/mL using magnetic sector MS, as described in other work.[34]

Samples were quantified by their analyte ion-to-internal standard ion ratio, which compensates for variable loss throughout sample preparation and analysis. Isotopically labeled internal standards are particularly useful for correcting the effects of variable partitioning and SPME extraction efficiency. Peak integration, instrument response calibration, and sample quantification was performed using Xcalibur Quan software (ThermoFinnigan, San Jose, CA) because of enhanced data processing and archiving capabilities.

Instrument response was calibrated with every sample batch. Samples were analyzed beginning with the first four lowest concentration standards in order of increasing concentration, then a low-concentration QC, a high-concentration QC, the unknowns, a second low-concentration QC, a high-concentration QC, and ending with the remaining standards in order of increasing concentration. As shown in Figure 1, a weighted, $1/x$, least-squares model was fit to the calibration data, where x is the concentration of the standards.

RESULTS AND DISCUSSION

The most challenging aspect often encountered in monitoring environmental pollutants in biological and environmental samples is interference from background contamination. This difficulty arises because most of the VOCs monitored are common environmental pollutants that pervade laboratory space, materials, and reagents. This prevalence is especially true for MTBE, which is one of the most widely used chemicals in the United States. Successful measurement of trace levels of MTBE in blood involves minimizing significant sources through decontamination of the laboratory environment as well as careful selection and processing of materials and reagents. Once interfering residue or contamination in materials, reagents, and surrounding environment has been identified, it may be effectively eliminated, controlled, or quantified.

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Identification of the Primary Source of MTBE Contamination. In the case of MTBE, we found that materials and reagents that were used to collect and prepare unknown samples, standards, blank samples, and QC samples presented a greater contamination threat to the assay than did secondary sources such as the laboratory air. In fact, those materials and reagents that came in closest contact with the samples were found to be the most significant sources of contamination. Once these sources were identified and minimized, we were able to achieve improved precision and sensitivity in our analysis of MTBE in human blood. The most significant and perplexing sources of MTBE were the polysiloxane headspace vial septa.

To demonstrate the significance and variability of the levels found in these septa, we evaluated MTBE among four different lots (BK017, CD025, CF027, BH022) for the 20-mm 0.005-in. PTFE/0.030-in. silicone septa (PN 2-7539) sold by Supelco. In our initial survey, we first compared relative MTBE response from five septa from each lot. Analyses were performed by placing a septum in a vial capped with a septum that had been suitably cleaned so as not to contribute to the response as described below in this section. Among the different lots of the unprocessed septa, we found MTBE response to vary ~13% from septum to septum within the same lot and 33% from lot to lot. This variability includes both experimental precision in monitoring absolute response and heterogeneity of MTBE among the septa and lots.

We quantified MTBE concentration with septa from lot CD025 whose MTBE levels fell in the middle of the lot-to-lot comparison. The MTBE measured using this lot was between 20.91 and 23.65 μg/septum. These results were obtained by quantifying MTBE in consecutive methanol extracts of the septa material.

These high-MTBE residue levels found in the headspace vial septa can cause significant contamination in standard and in blood samples by as much as 80 pg/mL MTBE. It should be noted that other concomitant compounds are present at significant levels, which will be discussed later. Nevertheless, this contamination problem prompted developing a cleaning procedure that involved processing the septa in a vacuum oven. MTBE levels measured following this procedure drop significantly by at least a factor of 100 to 0.15 ng/septum. The significance of this cleaning procedure can be deduced from the study discussed below in which septa from lot CD025 were processed from 1 to 17 h at 1-h increments at 110 °C under ~1.3 kPa vacuum. The septa were measured for MTBE by sealing them in appropriately processed 10-mL headspace vials immediately after removal from the oven. After all the septa had been processed, we performed analyses by sampling in order of those septa that experienced the longest processing time to the shortest. Plotted in Figure 2 is the log of the relative peak areas for MTBE at m/z 73 measured in SIM mode. The majority of the MTBE residue is removed after ~7 h. The septum-to-septum variability of 13% seen in unprocessed septa may explain the fluctuation in MTBE levels at the longer processing times.

Even though the MTBE levels are still detectable after 17 h of processing, the remaining levels become insignificant for two reasons. First, the polysiloxane side of the septum does not come in direct contact with the vial headspace until the PTFE barrier is punctured, and second, MTBE partitions into the aqueous standards and blood samples. This reduction in effective MTBE residue level can be seen by comparing MTBE response from a

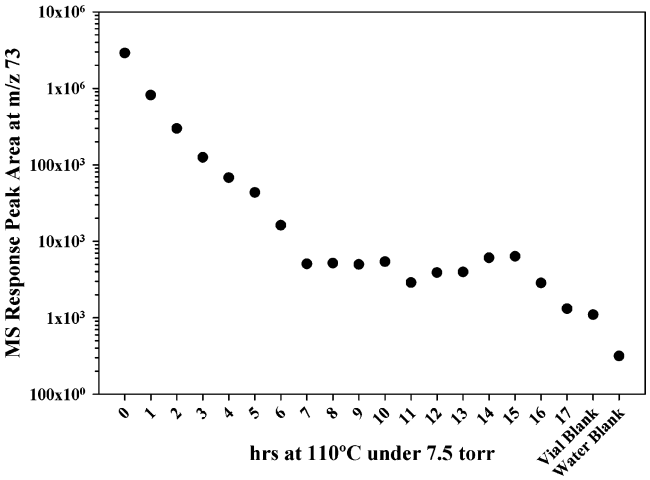
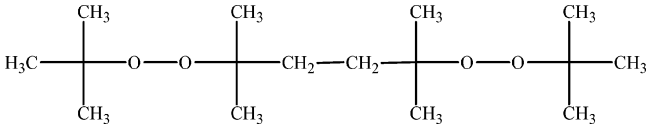


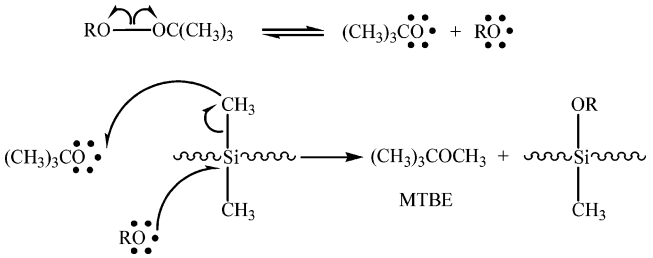
Figure 2. Decreased MTBE response as a function of increased processing time from headspace vial septa processed in a vacuum oven at 110 °C under 1.3 kPa.

blank vial containing a cleaned septum, a blank vial, and a blank vial with 3 mL of water, where relative responses were 1318, 1100, and 317, respectively. Thus, with suitable processing of the septa, the corresponding MTBE response from a blank vial containing 3 mL of water falls below our lowest standard of 0.8 pg/mL.

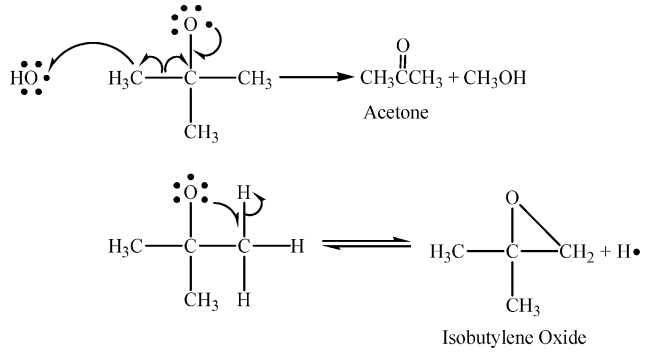
Proposed MTBE Formation Mechanism in Headspace Vial Septa. Because the quantities of MTBE extracted from the septa were too high to be attributed to adsorption from the surrounding environment, we concluded that the MTBE, as well as other residues, are somehow incorporated during polymer processing or synthesis. Material residue such as this is often overlooked and needs to be identified if one is to avoid or eliminate its interference. Thus, our investigations in this area led to the identification of a class of polymer peroxide curing agents with *tert*-butyl peroxy side groups that are commonly used to promote cross-linking between methyl side groups. Although polysiloxane formulations vary by incorporating monomers with different side groups such as hydrogen, phenyl, and vinyl, PDMS is typically the primary polymer component. For this reason, many polysiloxane synthesis routes use peroxide curing agents to upwardly adjust cross-link density. In the case of the Supelco septa, either 2,5-dimethyl-2,5-di(*tert*-butyl peroxide)hexane (BAB [78−63−7]) as shown below or a similar peroxide having a *tert*-butyl side group was used as a curing agent:[35]



In using BAB, oxide radicals are formed as a strong reducing agent intended to abstract methyl hydrogen atoms forming *tert*-butyl alcohol as a byproduct. However, we believe that the oxide radicals also attack other side groups as well, producing a number of different byproducts. These and other byproducts might interfere with sensitive analytical measurements, such as trace level quantification of MTBE. MTBE can be formed upon radical attack of the PDMS methyl side group as shown:



Upon analysis of the polysiloxane septa from Specialty Silicone Products, high levels of both *tert*-butyl alcohol and MTBE were found, consistent with radical attack of the methyl hydrogen as well as the proposed attack of the methyl carbon. Furthermore, using this same scheme, one might expect the formation of other less volatile byproducts that include 2,5-dimethyl-2,5-hexanediol ([110−03−2], bp 214−215 °C) and 2,5-dimethoxy-2,5-dimethylhexane ([53273−13−5], 166 °C). However, the relatively low vapor pressures of these compounds likely prevent their detection using our gas-phase collection approach. Nevertheless, formation of MTBE as a reaction byproduct of a *tert*-butyl peroxide has been reported for di-*tert*-butyl peroxide [110−05−04].[36] In this work, byproducts of the peroxide curing agent found in their formulation were reported as 90% acetone, 7.5% MTBE, 0.4% isobutylene oxide, and trace levels of *tert*-butyl alcohol. The formation of acetone likely results from loss of a methyl from the *tert*-butyl oxide radical and the formation of isobutylene oxide from oxide radical attack of the α-carbon:



These and other *tert*-butyl peroxide-related residues are identifiable in the septa. Shown in Figure 3 is a mass chromatogram of volatile residue typical of that found in polysiloxane septum cured with the aid of a *tert*-butyl peroxide curing agent. Components were identified by matching full scan mass spectra with spectral library entries. The most intense responses are from cyclic PDMS residue, which is shown off scale, where hexamethylcyclotrisiloxane is designated as D3, octamethylcyclotetrasiloxane as D4, decametheylcyclopentasiloxane as D5, and so forth. Nevertheless, significant response is also seen for compounds that are associated with the *tert*-butyl peroxide curing agent and include acetone, *tert*-butyl alcohol, isobutylene oxide, MTBE, ethyl *tert*-butyl ether, *tert*-amyl alcohol, and 6-methyl-2-heptanone. A number of these compounds are relatively common and may originate from other sources and side reactions.

(35) Valade, J. Scientific Specialty Products, Inc., personal communication, 2002.

(36) Val'kovskii, D. G.; Sosin, S. L.; Korshak, V. V. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1963**, *7*, 1319−1327.

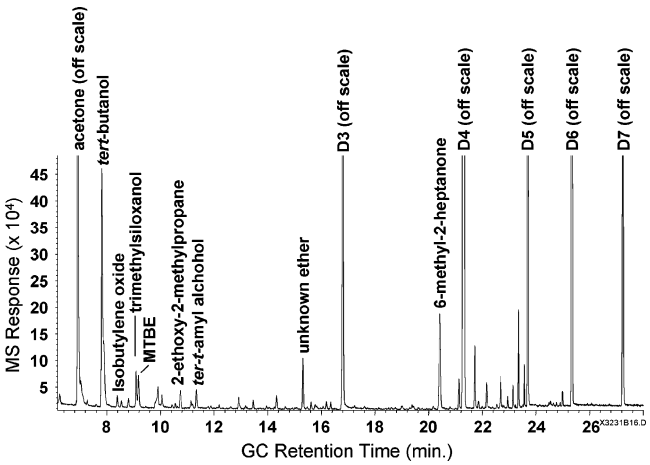


Figure 3. Mass chromatogram of volatile residue collected from a polysiloxane/PTFE barrier headspace vial septum. Apart from PDMS residue, significant response is seen for those compounds derived from *tert*-butyl oxy containing peroxide curing agents.

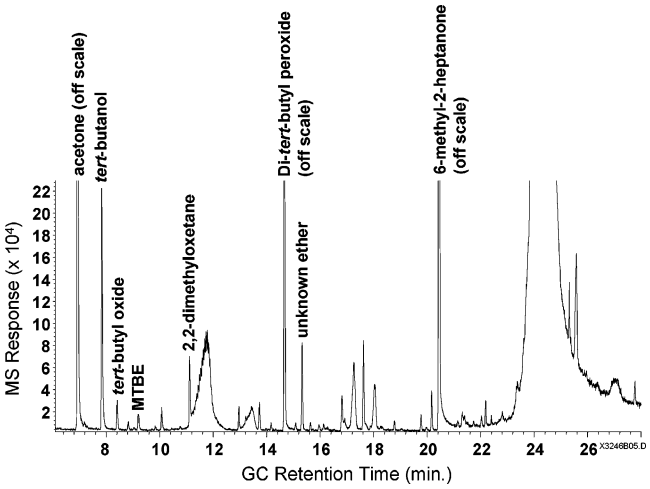


Figure 4. Mass chromatogram of volatile residue from Verox DBPH-40. Identified are those peroxide residue compounds that carry over as significant polysiloxane volatile residue.

To help verify the source of this residue, we analyzed the curing agent itself. A striking similarity can be seen upon comparison of VOCs emitted from neat Verox DBPH-40 curing agent (3M Corp.) as shown in Figure 4 with those VOCs from septa material. In addition to MTBE, we identified a number of other compounds that are distinct congeners of the curing agent and include *tert*-butyl alcohol, isobutylene oxide, 2,2-dimethyloxetane, and di-*tert*-butyl peroxide. Those compounds that produced broad mass chromatographic peaks could not be identified and are possibly reactive congeners of BAB that are apparently unstable under the analysis conditions. Other residue compounds that are unrelated to BAB (e.g., 6-methyl-2-heptanone) apparently carry through in the formulation.

Processing and Handling of Water and Methanol Solvents. Other sources of MTBE contamination in HPLC-grade water and methanol are believed to be from accumulation of MTBE from the surrounding air environment during production, storage, and handling. When analyzed, methanol generally had higher MTBE levels than did commercially available water. This difference in relative MTBE levels is expected on the basis of MTBE's lower solubility in water, which is 4.8 g/100 g, compared with its miscibility with methanol. Nevertheless, because water is used as the solvent for the final standard dilution, we found it necessary to distill, helium purge, and sequentially flame seal the water into glass ampules to ensure its highest purity, not only for MTBE but for other target compounds.[30]

Table 1. Comparison of Amounts of MTBE in Standard Water and Methanol Matrixes

sample formulation	est MTBE amt (pg/mL)
3-mL aliquot of a. 25 mL of redistilled HPLC-grade water b. 333.5 μL of 18 μg/mL MTBE-d_{12} in methanol	<0.8
3-mL aliquot of a. 25 mL of nonredistilled HPLC-grade water b. 333.5 μL of 18 μg/mL MTBE-d_{12} in methanol	<0.8
a. 3 mL of redistilled HPLC-grade water b. 40 μL of 18 μg/mL MTBE-d_{12} in methanol	<0.8
a. 3 mL of redistilled HPLC-grade water b. 40 μL of 18 μg/mL MTBE-d_{12} in methanol c. 40 μL of HPLC-grade methanol	1.7 ± 0.5
a. 3 mL of redistilled HPLC-grade water b. 40 μL of 18 μg/mL MTBE-d_{12} in methanol c. 40 μL of P&T-grade methanol	0.8 ± 0.3

Shown in Table 1 is a comparison of MTBE levels measured in water and methanol. For comparison of these different water and methanol samples, we used a standard set ranging from 0.8 to 500 pg/mL. MTBE was typically not detectable in the HPLC-grade water. More significant levels of MTBE were measured in the HPLC- and P&T-grade methanol at 89.7 and 21.5 pg/mL, respectively. Although only a small quantity of methanol (i.e., ~45 μL of methanol/3 mL of H_2O) is used in the standard solution, the amount of MTBE in the HPLC-grade methanol still exceeds that which can be tolerated for producing a 0.8 pg/mL standard; whereas, the concentration in the P&T-grade methanol falls below this limit and does not require reprocessing or hermetic storage if shelf life and handling are minimized. To minimize MTBE adsorption, we limit the shelf life of unopened bottles of methanol to ~6 months, and once a bottle is opened, it is used in only one application.

Blood Analyses. Identification of the primary sources of MTBE residue is significant because the identification allowed us to effectively target MTBE's reduction in the background to values well below our lowest standard of 0.8 pg/mL. This ability to measure, minimize, and control MTBE contamination significantly improves the precision and accuracy of our blood screening assay as well as the method detection limits. The high level of performance is demonstrated in our fetal bovine serum QC sample results that we use to monitor precision and accuracy of our blood screening assay. Fetal bovine serum is used instead of whole blood for QC purposes because it can be stored up to 2 years at −70 °C with no noticeable analyte partitioning effects. Results from these analyses accumulated over a 9-month period on three different instruments are plotted in Figure 5. QC samples were analyzed in two pairs consisting of a low and high level and were analyzed before and after each set of blood samples. Experimentally determined concentrations for these QC samples are 114 ± 9 and 568 ± 31 pg/mL for the low- and high-QC samples, respectively. Accuracy in a given run can be deduced by comparing the

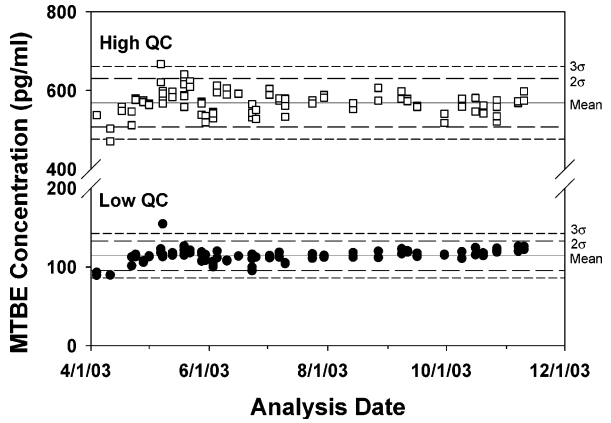


Figure 5. QC sample levels determined with each blood screening run over a 9-month period on three instruments. Low-QC mean concentration is 114.3 ± 9.4 and high mean level is 568.5 ± 30.9 pg/mL.

Table 2. Quantification of MTBE Added into Blood

calculated added concn (pg/mL)	measured total concn (pg/mL)	measured backgrd adjusted concn (pg/mL)	% diff
0	12.5 ± 1.1		
3.90	17.0 ± 2.7	4.5 ± 2.9	15.8
12.3	25.8 ± 1.6	13.4 ± 3.1	8.6
38.9	52.4 ± 4.2	40.0 ± 4.5	2.6
123	137.5 ± 4.5	125.1 ± 6.2	1.7

deviation from these established mean values. If the QC results fall outside our acceptance criteria, which includes deviation greater than two standard deviations, the data from that run is rejected. Overall, this approach and method yield good long-term precision (i.e., 9 months) as is apparent from the small relative standard deviation (RSD) of 8.2 and 5.4% for the low- and high-QC samples, respectively. Furthermore, using regression methodology, no statistically significant differences between instruments were apparent.

Because differences in the composition of fetal bovine serum and whole blood may potentially bias our measurements, any precision and accuracy differences between these matrixes should be identified. For this evaluation, we added a known amount of MTBE in whole blood from one donor. Four sample replicates were prepared at four different concentrations by aliquoting into each blood sample internal standard working solution and 40 μL of a standard stock solution of either 299, 945, 2990, or 9450 pg/mL MTBE in water. Samples were organized into four replicate sets and analyzed from lowest to highest concentration. Water blanks, standards ranging in concentration from 9.45 to 945 pg/mL, and low- and high-concentration QC samples were included in the analysis run.

The results from this MTBE-fortified blood experiment are compared in Table 2 for the calculated and average measured concentrations that range from 3.90 to 123 pg/mL. Added MTBE concentrations were calculated by first determining the internal standard dilution factor for the blood samples. These dilution factors were deduced by dividing the weight of the calibration standard formulation (3.00 mL of water, 40 μL of standard stock solution, and 40 μL of the internal standard working solution = 3.07 mL) by the weight of the MTBE−fortified blood mixture (∼ 3 mL of blood, 40 μL of standard stock solution, 40 μL of internal standard stock solution). Blood concentrations quantified from the standard response curve were multiplied by the appropriate dilution factor to determine the measured total concentrations. Because the blood collected had a measurable amount of MTBE, which was determined at 12.5 ± 1.1 pg/mL, we subtracted the background level to obtain the measured background-adjusted concentration. No further corrections were made as MTBE levels were not detectable in any of the water blanks. The percent difference between calculated and measured values decreased from 15.8 to 1.7% as the concentration of the added MTBE increased. However, this percent difference is insignificant relative to the deviation among the four replicates. Before background subtraction, the deviation for the lowest added concentration is 15.9% and decreases to 3.3% for the highest added concentration. High relative standard deviation for the lowest MTBE concentration is attributed mainly to the blood background level of ∼12.5 pg/mL. Otherwise, in the absence of background subtraction, this method demonstrates a high degree of accuracy and precision in the low ppt range for the analysis of MTBE in blood.

CONCLUSION

The presence of residue and contamination in standard laboratory materials can have significant impact on sensitive analytical measurements of MTBE in many different matrixes. In this method, common silicone septa were found to be the most significant source of MTBE interference. Removal of this MTBE contamination was required for accurate quantification of low-picogram per milliliter levels of MTBE in whole blood. The source of this contamination was identified as a *tert*-butyl peroxide-type curing agent commonly used to cross-link methyl side groups in polysiloxane materials. This curing agent, which forms a bond between two polysiloxane methyl groups and forms *tert*-butyl alcohol as a byproduct, may also electrophilically attack a methyl side group to form MTBE. In common PDMS/PTFE barrier headspace vial septa, MTBE levels were found to be ∼20 μg/septum and contributed as much as 80 pg/mL to the standard and blood samples. This high-background level masks the low-picogram per milliliter levels of MTBE typically found in blood samples from the U.S. population.

Ideally this contamination could be avoided altogether through selection of a polysiloxane material that does not use a *tert*-butyl peroxide-type curing agent. However, the potential for interference from other synthesis residues make it necessary to preclean these materials when quantifying in the low-picogram per milliliter range. In this work, we demonstrate the ability to lower the MTBE level below our lowest standard of 0.8 pg/mL by baking the septa at 110°C for 17 h under a 1.3-kPa vacuum.

Identification and reduction of significant background contamination sources allowed us to lower detection limits significantly and provided a higher degree of precision and accuracy for our blood screening assay. Long-term and instrument-to-instrument performance as deduced from accumulation of QC sample results collected on three different instruments during a 9-month period yielded RSDs of 8.2 and 5.4% for 114.3 and 568.5 pg/mL MTBE in fetal bovine serum, respectively. Similarly, MTBE-fortified whole blood experiments yielded good accuracy

and precision where percent differences between the calculated and measured values ranged from 1.7 to 15.8% and RSDs for four replicates ranged from 3.3 to 15.9%.

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