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Gas Chromatography-Mass Spectrometry Paired with Total Vaporization Solid-Phase Microextraction as a Forensic Tool

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TITLE:

Gas Chromatography-Mass Spectrometry Paired with Total Vaporization Solid-Phase Microextraction as a Forensic Tool

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KEYWORDS:

GC-MS, total vaporization, SPME, controlled substances, GHB, GBL

SUMMARY:

Total Vaporization Solid Phase Microextraction (TV-SPME) completely vaporizes a liquid sample whilst analytes are sorbed onto a SPME fiber. This allows for partitioning of the analyte between only the solvent vapor and the SPME fiber coating.

ABSTRACT:

Gas Chromatography – Mass Spectrometry (GC-MS) is a frequently used technique for the analysis of numerous analytes of forensic interest, including controlled substances, ignitable liquids, and explosives. GC-MS can be coupled with Solid-Phase Microextraction (SPME), in which a fiber with a sorptive coating is placed into the headspace above a sample or immersed in a liquid sample. Analytes are sorbed onto the fiber which is then placed inside the heated GC inlet for desorption. Total Vaporization Solid-Phase Microextraction (TV-SPME) utilizes the same technique as immersion SPME but immerses the fiber into a completely vaporized sample extract. This complete vaporization results in a partition between only the vapor phase and the SPME fiber without interference from a liquid phase or any insoluble materials. Depending upon the boiling point of the solvent used, TV-SPME allows for large sample volumes (e.g., up to hundreds of microliters). On-fiber derivatization may also be performed using TV-SPME. TV-SPME has been used to analyze drugs and their metabolites in hair, urine, and saliva. This simple technique has also been applied to street drugs, lipids, fuel samples, post-blast explosive residues, and pollutants in water. This paper highlights the use of TV-SPME to identify illegal adulterants in very small samples (microliter quantities) of alcoholic beverages. Both gamma

hydroxy butyrate (GHB) and gamma butyrolactone (GBL) were identified at levels that would be found in spiked drinks. Derivatization by a trimethylsilyl agent allowed for conversion of the aqueous matrix and GHB into their TMS derivatives. Overall, TV-SPME is quick, easy, and requires no sample preparation aside from placing the sample into a headspace vial.

INTRODUCTION:

Solid-Phase Microextraction (SPME) is a sampling technique in which a liquid or solid sample is placed into a headspace vial and a SPME fiber, coated with a polymeric material, is then introduced into the sample headspace (or immersed in a liquid sample). The analyte is sorbed onto the fiber and then the fiber is placed inside the GC inlet for desorption[1,2]. Total Vaporization Solid-Phase Microextraction (TV-SPME) is a similar technique as immersion SPME but completely vaporizes a liquid sample before analytes are adsorbed onto the fiber. This allows for partitioning of the analyte between only the solvent vapor and the coating of the fiber, allowing for more of the analyte to be adsorbed onto the fiber and resulting in good sensitivity[3]. There are various SPME fibers available and the fiber should be chosen based on the analyte of interest, solvent/matrix, and derivatization agent. See **Table 1** for established TV-SPME analytes.

TABLE 1

To perform TV-SPME, analytes are dissolved in a solvent and an aliquot of this mixture is placed into a headspace vial. Samples do not need to be filtered because only the solvent and volatile analytes will vaporize. Specific volumes of liquid samples must be used to ensure total vaporization of the sample. These volumes are determined by using the Ideal Gas Law to calculate the number of moles of a solvent multiplied by the molar volume of the liquid (Equation 1).

$$V_o = \left(\frac{PV_v}{RT}\right)\left(\frac{M}{\rho}\right) \qquad \text{Equation 1}$$

where V_o is the volume of the sample (mL), P is the vapor pressure of the solvent (bar), V_v is the volume of the vial (L), R is the ideal gas constant (0.083145 $\frac{(L*bar)}{k*mol}$), M is the molar mass of the solvent (g/mol), T is temperature (K), and ρ is the density of the solvent (g/mL).[3]

In order to use the correct vapor pressure, the Antoine equation (Equation 2) is used to account for the influence of temperature:[4]

$$log_{10}P = A - \left(\frac{B}{T+C}\right) \qquad \text{Equation 2}$$

where T is temperature and A, B, and C are the Antoine constants for the solvent. Equation 2 may be substituted into Equation 1, yielding:

$$V_o = \left(\frac{\left(10^{A-\frac{B}{T+C}}\right)V_v}{RT}\right)\left(\frac{M}{\rho}\right) \qquad \text{Equation 3}$$

Equation 3 gives the volume of the sample (V_o) that can be completely vaporized as a function of the temperature and solvent used.

To perform derivatization with TV-SPME, the SPME fiber is first exposed to a vial containing the derivatization agent for a predetermined amount of time depending on the analyte. The SPME

fiber is then exposed to a new vial containing the analyte of interest. This vial is heated inside of a heated agitator. The analyte is then adsorbed onto the fiber with the derivatization agent. The derivatization of the analyte and/or the matrix takes place on the fiber before being inserted into the GC inlet for desorption. **Figure 1** shows a depiction of the TV-SPME process with derivatization.

FIGURE 1

TV-SPME is beneficial because it allows for the analyte to be derivatized during the extraction process which reduces analysis time. Other methods, such as liquid injection, require that the analyte react with the derivatizing agent in solution prior to being injected into the GC. TV-SPME also requires little to no sample preparation. A matrix containing an analyte may be placed directly into the headspace vial and analyzed. Many compounds of interest are compatible with TV-SPME. Compounds must be soluble in a solvent and sufficiently volatile to allow for vaporization. Additionally, compounds must be thermally stable to be analyzed by GC-MS. TV-SPME has been used to analyze drugs and drug metabolites, racing fuels, polycyclic aromatic hydrocarbons, and explosive materials[3,5-10].

PROTOCOL:

1. General TV-SPME sample preparation and GC-MS analysis

NOTE: If the sample is already dissolved in a matrix, skip to Step 1.2.

1.1. Extract or dissolve the solid sample in enough solvent (water, methanol, acetone, etc.) to reach the desired concentration. Liquid samples can be used “as is”.

NOTE: The amount of solid sample used depends on the desired concentration of the sample. Concentrations below 1 ppm (w/v) are recommended to avoid overloading the GC column. Analyte should be soluble in chosen organic solvent.

1.1.1. Ensure that the sample has fully dissolved.

1.2. Calculate the volume needed to fully vaporize the sample using Equation 3 at the chosen temperature. For example, if the experiment is to be performed at 60 °C, calculate the volume needed to completely vaporize the solvent at 60 °C.

1.2.1. Transfer this sample volume into a headspace vial and secure the cap. Acceptable methods for transferring liquid samples at the microliter scale include manually via glass syringe, an electronic glass syringe, or an autosampler robot capable of liquid transfers for sample preparation.

1.3. If derivatizing the sample, prepare the proper derivatization agent by placing ~1 mL of the agent into a headspace vial.

1.3.1. Choose the derivatization agent based on the type of derivatization needed: alkylation, acylation, or silyation. In this case, the recommended derivatization agent for the carboxylic acid and alcohol functional groups found on GHB is O-bis(trimethylsilyl) trifluoroacetamide (BSTFA). The derivatization agent may be used "as is" and does not require dilution. One mL of derivatization agent is enough to ensure complete saturation of the SPME fiber.

CAUTION: Derivatization agents are toxic and should be handled in a fume hood.

1.4. Set the proper incubation/extraction temperature based upon the calculation in step 1.2. This temperature ensures total vaporization, sufficient sample extraction, and complete derivatization (if necessary).

1.4.1. Select GC-MS parameters (oven temperature program, flow rate, inlet temperature, etc.) based on the class of the compound(s) of interest. See Step 3 for an example parameter set.

1.4.2. Ensure that the proper inlet liner (e.g., 2 mm inner diameter or less) is in the GC inlet.

1.5. Ensure the SPME fiber has been properly conditioned and is in good working condition before beginning the analysis.

1.5.1. Vary the conditioning parameters based on the type of SPME fiber being used. Please see SPME fiber instructions for proper conditioning temperature and time. In general, analyzing several SPME fiber blanks until they are reproducible is sufficient to characterize a SPME fiber as fully conditioned.

2. Gamma Hydroxy Butyrate (GHB) and Gamma-butyrolactone (GBL) sample preparation

2.1. Prepare a sample of GHB and/or GBL in water with a concentration of less than 1 ppm.

2.2. Transfer 1 µL of this sample to a 20 mL headspace vial using one of the methods described in 1.2.1.

2.2.1. Note that that the analysis of aqueous samples requires the lowest sample volumes. For example, one µL of water will completely vaporize into a 20 mL headspace vial at 60 °C.

2.2.2. Cap the vial immediately.

2.3. Place ~1 mL of BSTFA + 1% trimethylchlorosilane (TMCS) into a separate 20 mL headspace vial and cap.

NOTE: GBL does not derivatize. A derivatization step is still required, however, is to ensure the water solvent derivatizes and does not interfere with the sample.

CAUTION: BSTFA is toxic and should be handled in a fume hood.

3. GC-MS parameters and setup for GHB and GBL in water

3.1. Create a method using the following GC-MS parameters:
Initial oven temperature: 60 °C held for 1 minute.
Oven program: 15 °C/minute.
Final oven temperature: 280 °C, held for 1 minute.
Flow rate: 2.5 mL/minute (speed optimized flow for a 0.25 mm i.d. column).
Inlet temperature: 250 °C.
Transfer line temperature: 280 °C.

3.2. Ensure a narrow (2 mm i.d. or less) SPME inlet liner has been placed inside the GC inlet.

3.3. Ensure the PDMS/DVB SPME fiber has been properly conditioned and is in good working order prior to analysis.

NOTE: PDMS/DVB SPME fibers should be conditioned in the GC inlet at 250 °C for 30 minutes. PDMS/DVB SPME fibers should be an off-white color.

3.4. Run the GC-MS on the sample.

REPRESENTATIVE RESULTS:

A GBL volume study was performed to demonstrate the sensitivity of TV-SPME compared to headspace and immersion SPME. A 100ppm$_v$ sample of GBL in water was prepared and placed into 20 mL headspace vials with volumes of 1, 3, 10, 30, 100, 300, 1000, 3000, and 10,000 µL. The phase ratio of the samples allowed for TV-SPME (1-3 µL), Headspace SPME (10 – 3,000 µL) and Immersion SPME (10,000 µL). All samples were analyzed in triplicate and the average peak area was plotted against the sample volume. Overall, sample volumes that allowed for TV-SPME demonstrated more sensitivity than headspace or immersion SPME for GBL in water as shown in **Figure 2**. A comparison of the chromatograms for each method is shown in **Figure 3.**

FIGURE 2

FIGURE 3

Realistic samples of wine spiked with an effective dose of GHB and GBL are shown in **Figure 4** and **Figure 5,** respectively. These samples also show the interconversion of GBL and GHB. When TV-SPME is performed properly, a sharp, abundant peak will result as shown in **Figure 6**. TV-SPME has good sensitivity and therefore proper concentrations should be used as to not overload the column. When high concentrations are present, peak asymmetry will result as shown in **Figure 5** and **Figure 7**. In these cases, diluting the sample or using a split injection can improve peak shape.

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

When derivatizing, the analyst should ensure that the method allows for the analyte(s) to be fully derivatized prior to being desorbed into the GC. Partial derivatization can result in a peak representing the derivatized analyte and a peak representing the underivatized analyte. Partial derivatization will also result in a lower sensitivity for the analyte as less of it may adsorb onto the fiber.

FIGURE AND TABLE LEGENDS:

Figure 1: Depiction of the TV-SPME process with derivatization. The SPME fiber first enters the derivatization vial where the derivatization agent (yellow circles) sorb onto the fiber. The fiber is then introduced to the sample (blue circles) and heated. Formation of the derivative (green circles) takes place on the fiber during the extraction time.

Table 1. Recommended SPME fibers with established TV-SPME analytes.

Figure 2: Graph of average peak area versus sample volume for GBL in water. A GBL volume study was performed to demonstrate the efficacy of TV-SPME compared to headspace and immersion SPME. A 100ppm$_v$ sample of GBL in water was prepared and placed into 20 mL headspace vials with volumes of 1, 3, 10, 30, 100, 300, 1000, 3000, & 10000 µL. All samples were analyzed in triplicate and error bars correspond to standard deviation of the mean.

Figure 3. Total Ion Chromatograms for GBL in water. (100 ppm) for 3 µL (blue), 300 µL (red), and 10,000 µL (green).

Figure 4. Realistic sample of GHB in wine with an 8 mg/mL concentration. Peaks: 1) GBL, 2) hexanoic acid-TMS, 3) GHB-TMS$_2$, 4) benzoic acid-TMS, 5) octanoic acid-TMS, 6) glycerol-TMS$_3$, * denotes a cyclic siloxane (fiber/column bleed).

Figure 5. Realistic sample of GBL in wine with a 10 mg/mL concentration. Peaks: 1) GBL, 2) hexanoic acid-TMS, 3) Siloxane, 4) trimethyl(2-phenylethoxy) silane, 5) GHB-TMS$_2$. TIC shows GBL converting to GHB.

Figure 6: Total Ion Chromatogram for GBL in water with a 0.1-ppm concentration. Results following the TV-SPME method previously described for GBL in water.

Figure 7: Total Ion Chromatogram for GBL in water with a 10-ppm concentration. Results following the TV-SPME method previously described for GBL in water.

DISCUSSION:

TV-SPME has some benefits over liquid injection GC in that large sample sizes (e.g., 100 µL) may be used without instrument modifications. TV-SPME also has some of the same benefits as headspace SPME. Headspace SPME does not require any extraction or filtration because any nonvolatile compounds will remain in the headspace vial and will not be adsorbed onto the fiber, yielding a clean sample. This method also helps to eliminate matrix effects due to this being a two-phase system (headspace and fiber) as opposed to a three-phase system (sample, headspace, and fiber) like standard headspace SPME. TV-SPME is like immersion SPME in that immersion SPME is also a two-phase system. With immersion SPME, a fiber is immersed into a liquid (typically aqueous) sample containing the analyte as opposed to extracting the analyte from its vapor. TV-SPME differs from immersion SPME because immersion SPME requires a polar/aqueous matrix in order to generate sufficient driving force for analytes to leave the solution phase and sorb to the fiber coating. In addition, immersion SPME requires much larger sample volumes (e.g., mL).

Many solvents may be used with TV-SPME including methanol, acetone, water, and acetonitrile. SPME fibers should not be exposed to or immersed in chloroform as these solvents can damage the fiber coating. Twenty mL screw cap glass headspace vials have been found to have the best performance with TV-SPME methods. It is recommended to use an autosampler with TV-SPME. While many parameters of the TV-SPME method may be adjusted as desired, the proper volume and extraction temperature must be used for each solvent. Sample volume and extraction temperature are proportional with one another and must be adjusted accordingly. For example, the extraction temperature of a method may be reduced, but the sample volume must also be reduced. This volume may be found by adjusting Equation 3.

Modifications to the derivatization procedure may be made. Derivatization may be performed pre- or post-extraction, at room temperature or heated in the agitator, by exposing the fiber to the vapor of the derivatization agent or by direct immersion of the fiber in the derivatization agent.

There are limitations to the TV-SPME method including the need for compounds to be soluble, thermally stable, and volatile. TV-SPME requires expensive SPME fibers which can have their coating stripped or broken during analysis. These limitations are outweighed by benefits such as large sample volumes relative to typical GC injection volumes, high sensitivity, and no need for filtration. TV-SPME is preferred to headspace SPME because more of the sample is extracted onto the fiber and matrix effects are reduced. TV-SPME is also preferred to immersion SPME because immersion SPME consumes much more sample than TV-SPME. TV-SPME allows for derivatization during the extraction process which reduces analysis time compared to methods such as liquid injection that require that the analyte to be derivatized prior to injection. TV-SPME also requires little to no sample preparation. TV-SPME is simple, efficient, and sensitive for the analysis of a wide variety of samples, including drugs, explosive materials, and racing fuels.

ACKNOWLEDGMENTS:

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DISCLOSURES:

The authors have nothing to disclose.

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Figure

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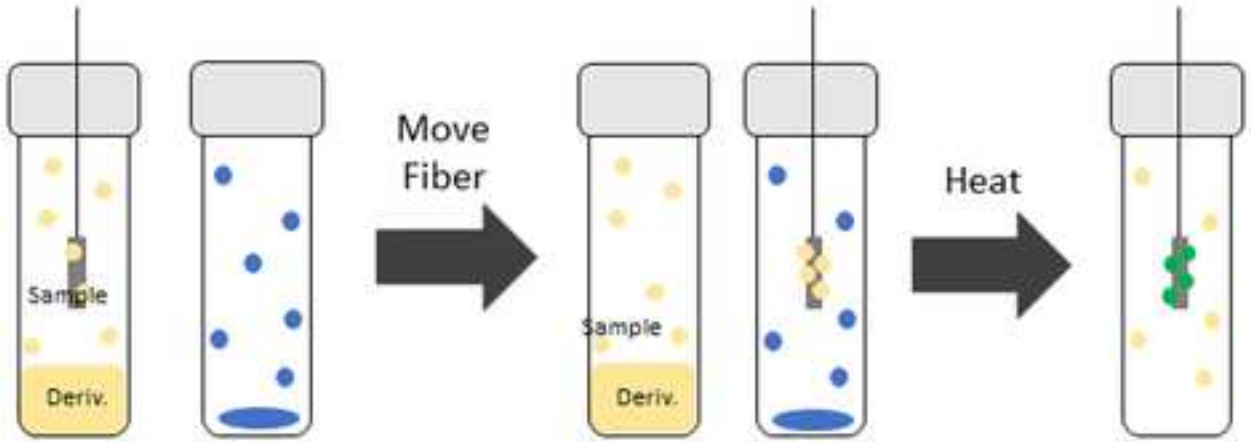

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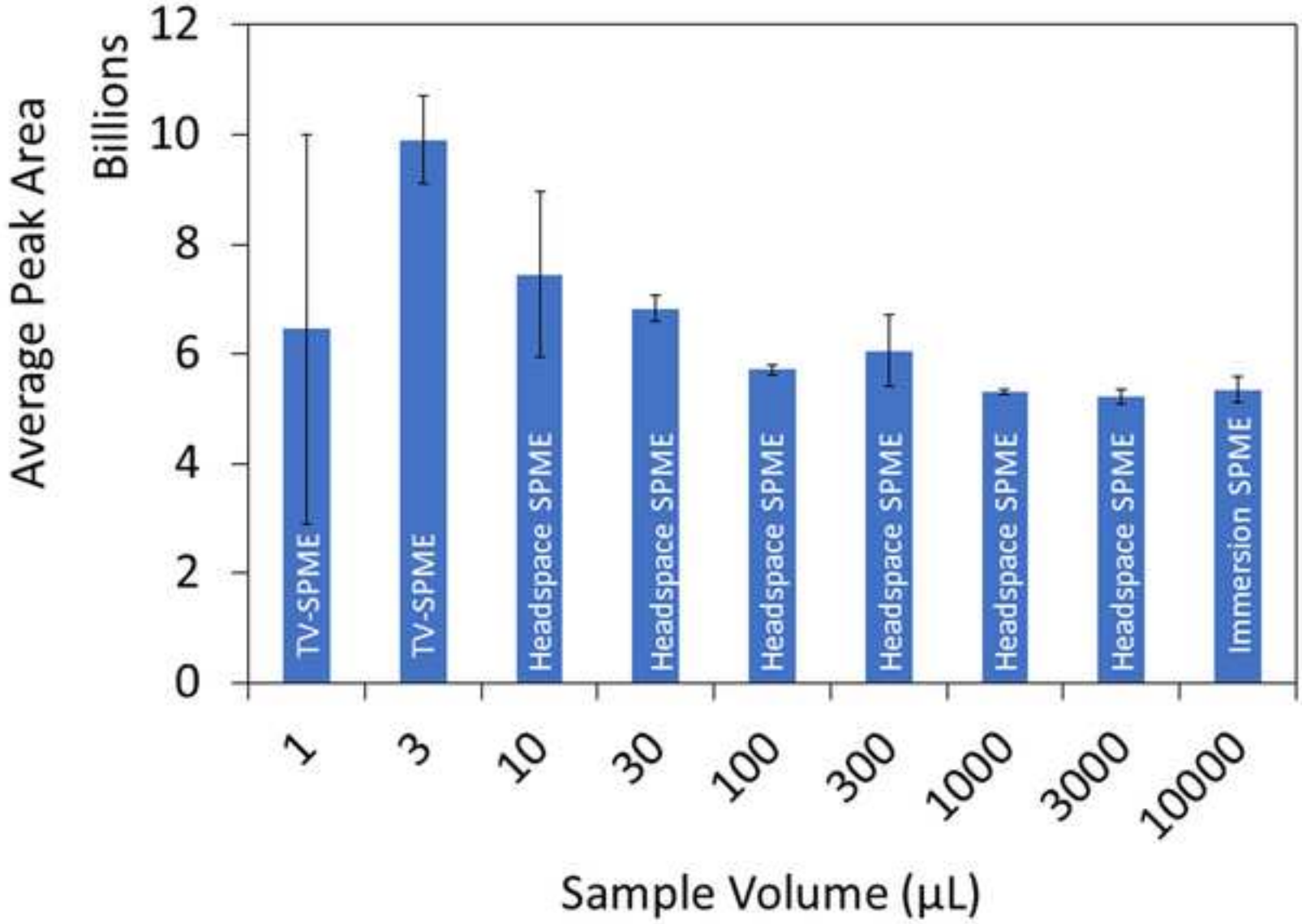

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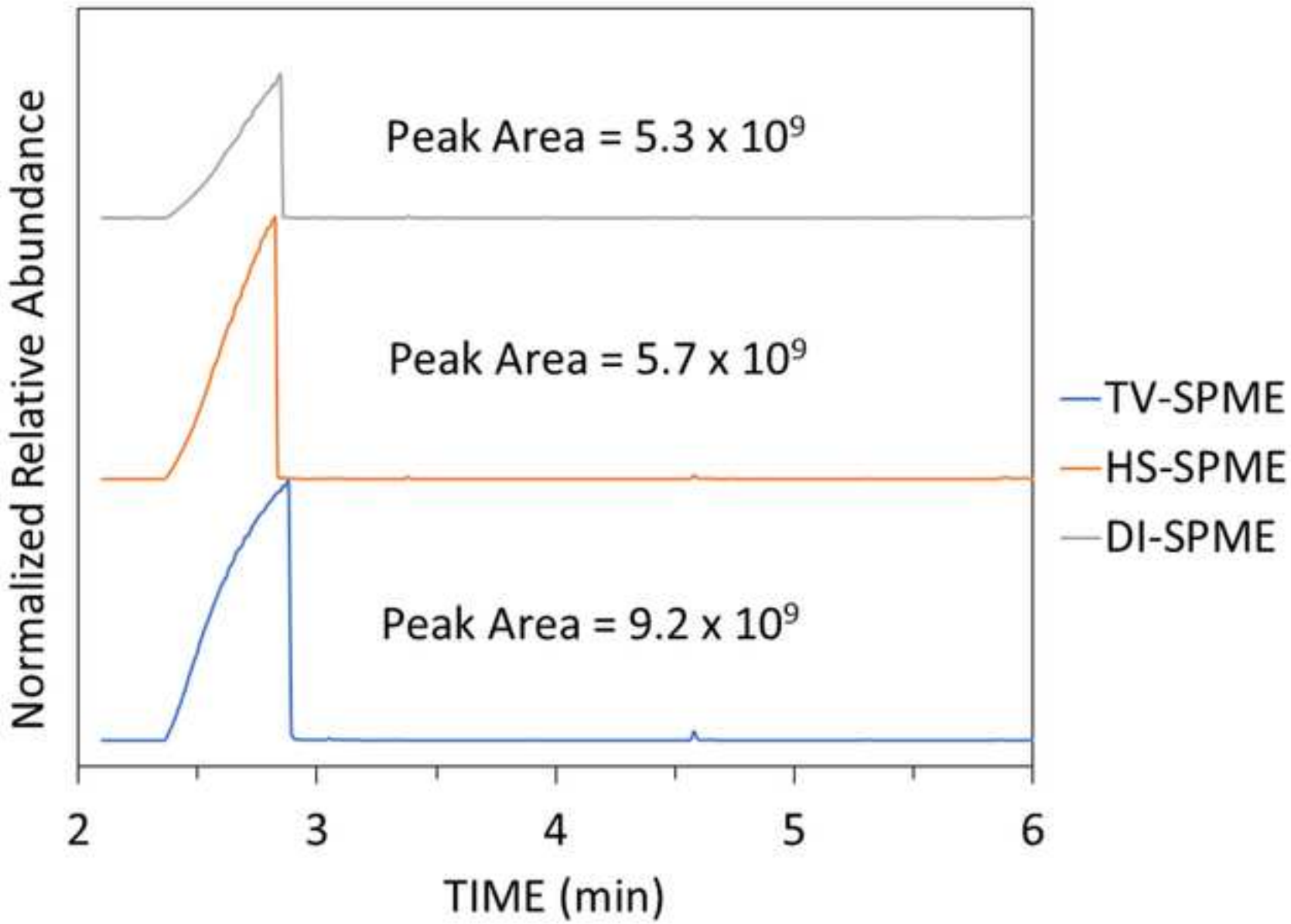

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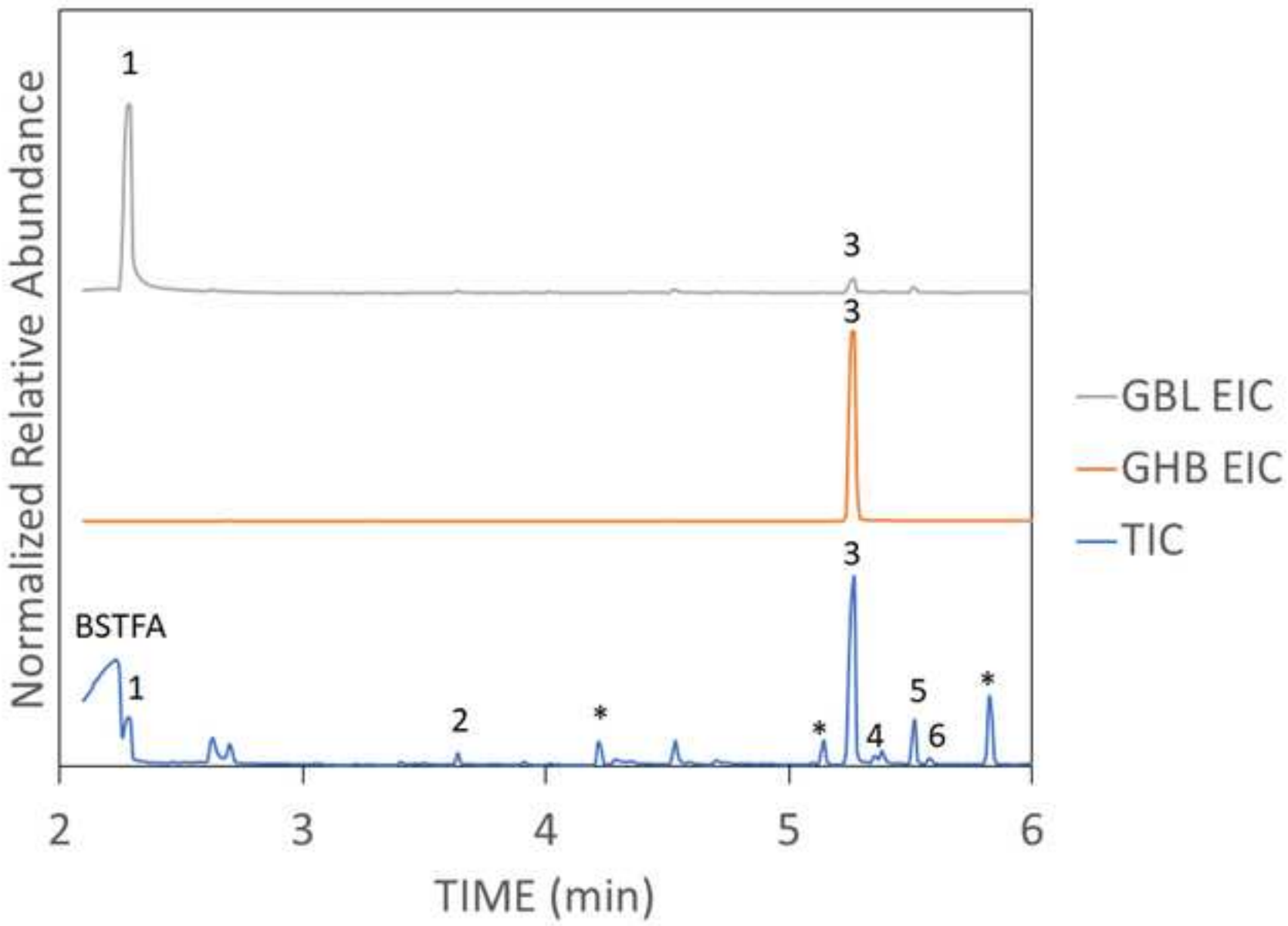

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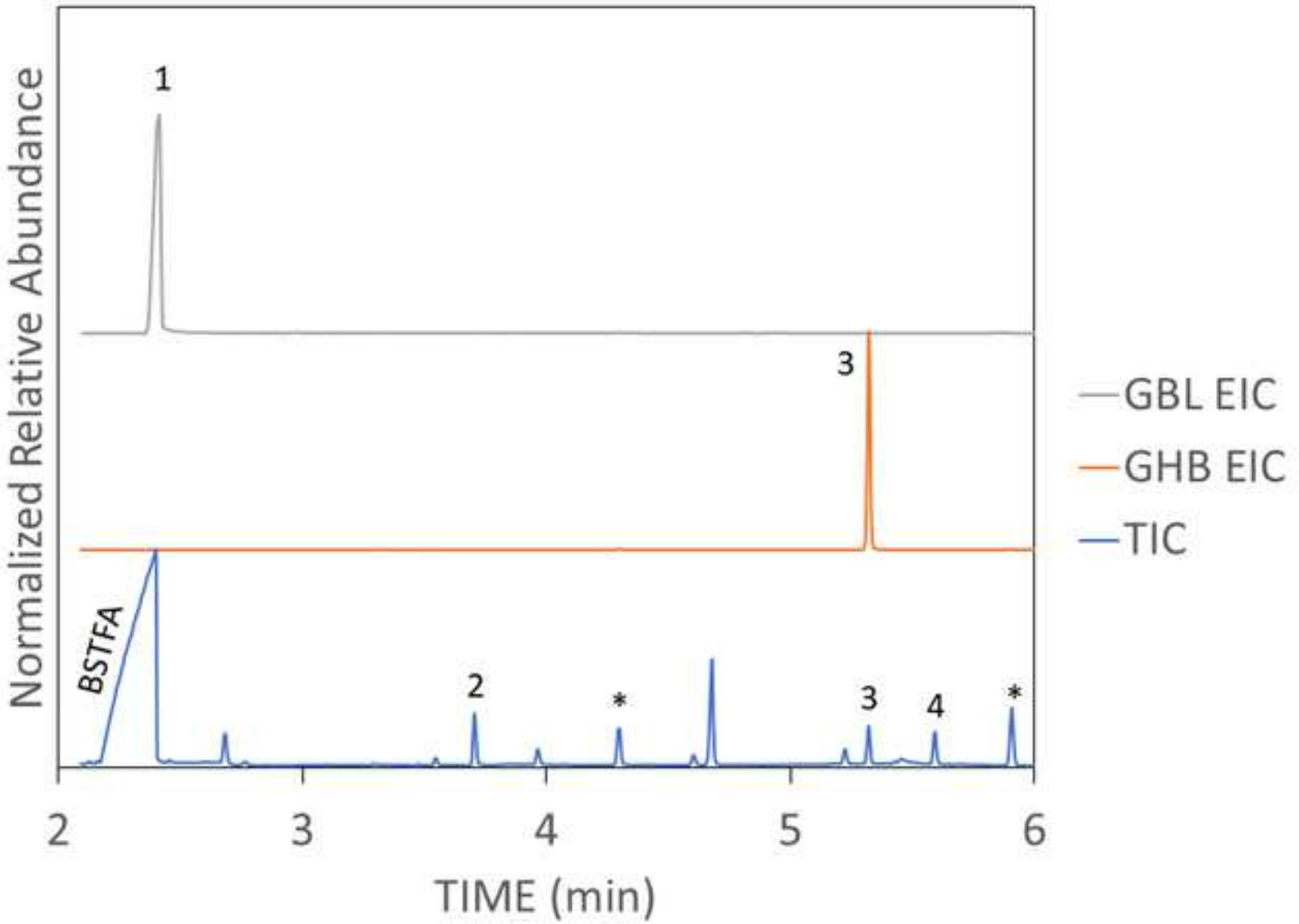

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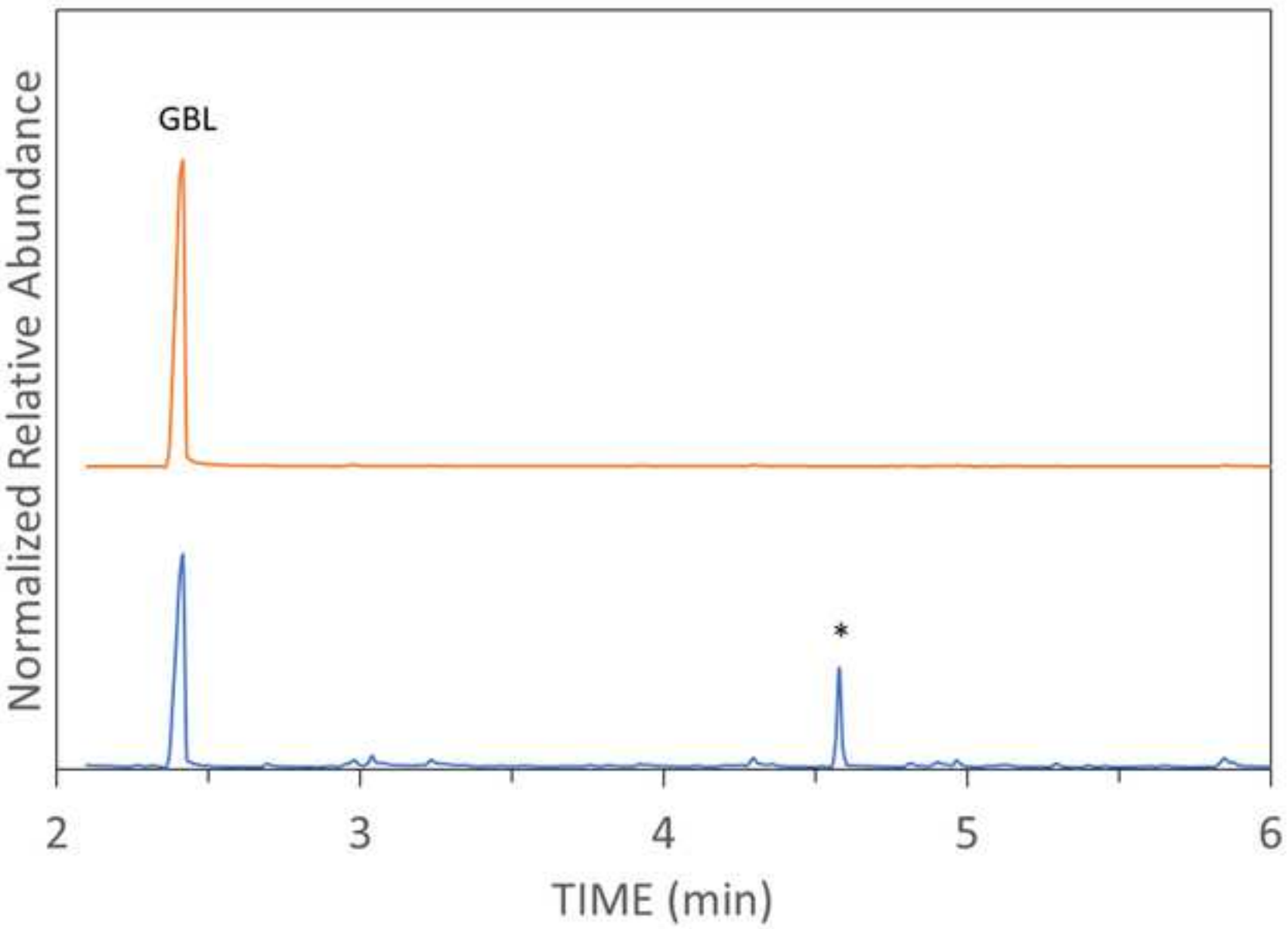

Figure

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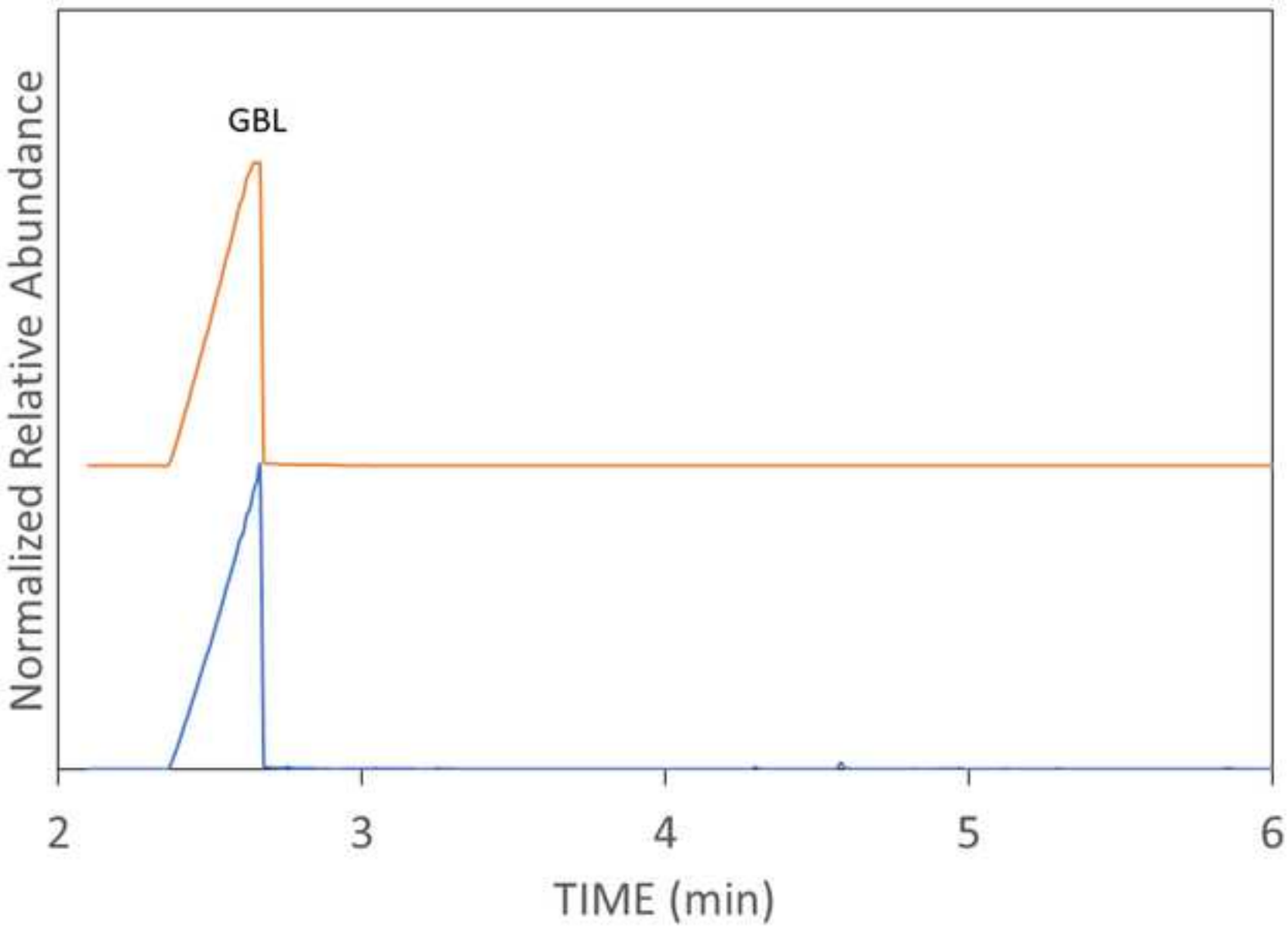

Table 1. Recommended SPME fibers with established TV-SPM

Sample	Analyte(s)
Human Hair	Nicotine, cotinine
Smokeless Powder	Nitroglycerin, diphenylamine
Racing fuel	Methanol, nitromethane
Water	Polycyclic aromatic hydrocarbons
Beverages	ɣ-Hydroxybutyric acid, ɣ-butyrolactone
Solid Powder	Methamphetamine, amphetamine

ME methods. See Supelco SPME fibers selection guide for additional recommendations.

Recommended SPME Fiber	Reference(s)
Polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA)	3
Polydimethylsiloxane (PDMS), polyethylene glycol (PEG)	7, 8
PEG	9
PDMS	10
PDMS	This Work
PDMS/DVB	Unpublished

TABLE OF MATERIALS

Name of Material / Equipment	Company
10 µL Syringe	Gerstel
BSTFA + 1% TMCS (10 x 1 GM)	Regis Technologies Inc.
eVol XR Sample Dispensing System Kit	ThermoFisher Scientific
ɣ-Butyrolactone (GBL)	Sigma-Aldrich
ɣ-Hydroxy Butyric Acid (GHB)	Cayman Chemicals
Headspace Screw-Thread Vials, 18 mm	Restek
Magnetic Screw-Thread Caps, 18 mm	Restek
Optima water for HPLC	Fisher Chemical
SPME Fiber Assembly Polydimethylsiloxane (PDMS)	Supelco
SPME Fiber Assembly Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)	Supelco
Topaz 2.0 mm ID Straight Inlet Liner	Restek

Catalog Number
100111-014-00
50442882
66002-024
B103608-26G
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23083
23091
W71
57341-U
57293-U
23313

RESPONSE TO REVIEWER COMMENTS

1. **Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues and define all abbreviations at first mention.**
   We have noticed some minor typos and corrected them.

2. **Please submit each figure individually as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps.).**
   Each figure is now converted to an PSD file format at 300 dpi.

3. **In Fig.1, what do the green circles represent on the fiber? Does heat change the composition of the derivatized fiber or the adsorbed analyte?**
   This has been answered (green circles) within the caption for Fig. 1.

4. **Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.**

   For instance,
   1.1: How much of the solid sample do you recommend should be dissolved in how much of solvent? What is a proper solvent: volatile and non-polar?
   This has been clarified

   1.2: What do you mean by "given temperature": should this be near the temperature where the solvent vaporizes? Please specify the temperature used.
   This has been clarified

1.3: 1 mL is the volume, what should the concentration of the derivatization agent be? What are the characteristics of a "proper derivatization agent". Please help readers/viewers decide what they could use.

This has been clarified

1.4 Please provide details that will help film a specific protocol and help readers/viewers understand the video. If the parameters in step 3 are relevant here, please reference them in 1.4.

This has been clarified

1.5 & 3.3: Please briefly describe how to decide if the SPME fiber has been properly conditioned.

This has been clarified

2.3: Please define BSTFA at first mention; how much of BSFTA should be taken?
Note after 2.3: Please clarify what you mean by water derivatizing.

We corrected all of these. The comment about water derivatizing has been clarified.

5. **Please remove the embedded Table from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.**

   We have done this.

6. **JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents, and in the case of the Supelco SPME fibers selection guide, this could be referenced as a PDF document and the**

reference cited.

We removed a sentence referencing Supleco.

7. **For Fig. 2, please specify whether error bars depict standard error or standard deviation.**

   We specified the errors bars as standard deviation.

8. **Please upload the Materials Table separately and sort the entries alphabetically by the name of the material.**

   We have done this and sorted the entries.

9. **In the discussion, line 300: do you mean small or large sample volumes?**

   We clarified that TV-SPME uses larger sample volumes than typical injection protocols, which adds sensitivity. That being said, TV-SPME consumes much less sample than does immersion SPME.

Minor Concerns:

maybe at line 163, 3.3. Is there a word missing in the sentence?

This has been revised.

1. Provide temperature of vaporization for 1 µL of GHB and GBL sample in the experimentation part.

This has been revised.

2. Provide a comparative chromatogram of TV-SPME, HS-SPME and DI-SPME at similar concentration levels of analytes.

We have included a figure, although the concentration of GBL is high – resulting in peak asymmetry. We report the peak areas for each (TV 8.6×10^9, HS 5.8×10^9, DI 5.4×10^9)