On-Line Sample Preparation – A Quick and Easy Way to Improve Lab Productivity and Data Quality

Rebecca Veeneman

Agilent Technologies, Inc., Wilmington, Delaware, USA

ISCC 2010, C.27

Abstract

The advantages of automating a common sample-preparation step, namely sample derivatization, are presented. Increased productivity and reduced cost of analysis are highlighted. Examples including fatty acids, pentachlorophenol, and morphine are presented.

Introduction

Many sample preparation tasks, like sample derivatization, can be time consuming and resource intensive. Automating this procedure, therefore, is beneficial in many ways. By automating sample derivatization, lab productivity can be increased and the cost of analysis can be decreased.

Some of the simplest derivatizations involve silylation reactions. By silylating the analytes of interest, chromatographic performance is improved[1]. A typical protocol for this type of reaction involves adding a silyl donor to a sample solution. The solution is shaken and usually heated to 70°C for up to an hour before analysis. Agilent's 7693A Automatic Liquid Sampler (ALS) allows the operator to automate these tasks with little to no intervention. By programming the ALS to add the derivatizing reagent, mix the sample, and heat for the specified time, an operator is free to attend to other tasks such as experiment design and data processing. To further decrease the total time of analysis, derivatization can take place during injection in the hot inlet. The ALS is capable of making multi-layer (sandwich) injections (Figure 2) which enables this time and resource saving technique. By derivatizing the sample in the inlet, derivatization is complete within seconds during the injection cycle. In this case, the sample preparation and analysis take no longer than the GC cycle time. This is in contrast to the additional 15 minutes or more that is required for derivatization with conventional methods, before the injection takes place. Hot inlet derivatization can be accomplished with a number of analytes and yields reproducible results without operator involvement.

Figure 1. Agilent's 7693A automatic liquid sampler on a GC/MS.

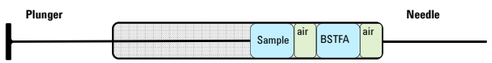

Figure 2. Diagram of multi-layer (sandwich) injections in the syringe.

Experimental

For conventional, in-vial derivatization, a stock solution of four fatty acids was made in hexane consisting of caprylic acid, capric acid, myristic acid, and palmitic acid at 5 mg/mL. To 0.5 mL, 100 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added. The solution was mixed for 30 sec, heated to 70°C for 15 min, and analyzed by an Agilent 7890 GC.

GC Inlet:	Split/Splitless Inlet in Split mode at 300°C with packed tapered liner (p/n 5183-4711)
Injection Volume:	1 µL
GC Oven:	85°C, 25°C/min to 200°C
Column:	20m x 0.10mm x 0.1µm DB-Wax (p/n 127-7022)
Carrier Gas:	Hydrogen, 3 mL/min constant flow
FID:	300°C, 40 mL/min H2, 400 mL/min air 50 mL/min constant column + make-up gas (N_2)

For derivatization in the hot inlet, 1 µL of the fatty acid stock solution and 1 µL of BSTFA were injected via sandwich injection (Figure 2). The carrier gas was slowed to 0.15 mL/min for 0.1 min to allow time for reaction[2].

For derivatization of pentachlorophenol in the hot inlet, 1 µL of the stock solution (1 mg/mL in hexane) and 1 µL of BSTFA were injected via sandwich injection. A programmed inlet flow was not required to achieve a complete reaction.

For derivatization of morphine and 6-acetylmorphine in the hot inlet, 1 µL of the sample (40 ng/mL in ethyl acetate) and 1 µL of BSTFA were injected via sandwich injection. The carrier gas was slowed to 0.1 mL/min for 0.2 min to allow time for reaction.

Typical GC Conditions for Hot Inlet Derivatization

GC Inlet:	Split/Splitless Inlet at 300°C with packed tapered liner (p/n 5183-4711)
Injection Volume:	2-layer sandwich with 0.5 µL air gap (Figure 2)
GC Oven:	70°C, 10°C/min to 280°C (2min)
Column:	30m x 0.25mm x 0.25µm HP-5MS (p/n 19091S-433)
Carrier Gas:	Hydrogen, ramped at 100 mL/min/min to the analytical flow rate
FID:	300°C, 40 mL/min H2, 400 mL/min air 50 mL/min constant column + make-up gas (N_2)

Results and Discussion

Automating Silylation Reactions with the ALS

A. Fatty Acid Analysis

Derivatizing fatty acids through silylation reactions improves peak shape and allows better resolution (Figure 3). Two methods of silylating fatty acids were used to demonstrate the advantages of automated derivatization on the GC. The ALS was programmed to add BSTFA, mix, and heat the sample just prior to injection. This procedure was repeated with average area RSDs of 0.5% for five samples of the four fatty acids, demonstrating typical injector performance[3]. Automated, in vial derivatization requires the same amount of time as "bench-top" derivatization, but frees the operator for other tasks and can reduce errors.

The second method of silylating fatty acids, hot inlet derivatization, achieved the same results of improved peak shape and resolution, but was complete within the injection cycle (Figure 3). With this method, derivatization and sample analysis are complete without adding additional cycle time.

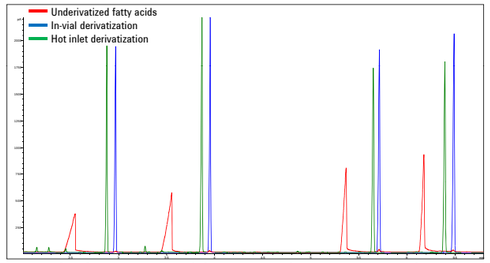

Figure 3. Chromatograms of fatty acids before derivatization, after in vial derivatization, and after hot inlet derivatization. The hot inlet derivatization chromatogram (green) is offset slightly for clarification.

B. Pentachlorophenol Analysis

Pentachlorophenol is another example where on-line derivatization can be beneficial. Analyzing pentachlorophenol is difficult due to tailing peaks and low sensitivity with many analytical techniques. This also reduces the accuracy and reproducibility of the analyses. Derivatizing pentachlorophenol with BSTFA addresses these problems and others, but can be time consuming, requiring nearly 1 hour for reaction time[4]. Modifying the derivatization procedure to an on-line (hot inlet) method allows the reaction to complete within the inlet, during injection, using only 1 µL of BSTFA per µL of pentachlorophenol (Figure 4).

C. Morphine Analysis

Morphine and its active metabolite, 6-acetylmorphine are shown in Figure 5. Prior to derivatization, the analytes also suffer from poor sensitivity and poor peak shape. After derivatization with BSTFA in the hot inlet, morphine and 6-acetylmorphine are easily separated and quantified.

Results and Discussion

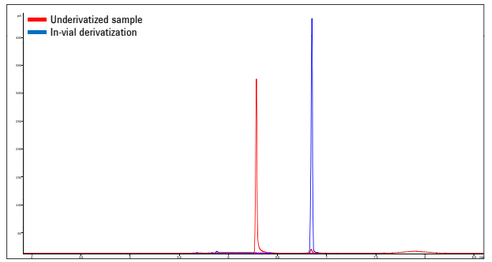

Figure 4. Chromatograms of pentachlorophenol before derivatization and after hot inlet derivatization. Prior to derivatization, the area RSD was 4.2%. After derivatization with BSTFA, the area RSD decreased to 1%

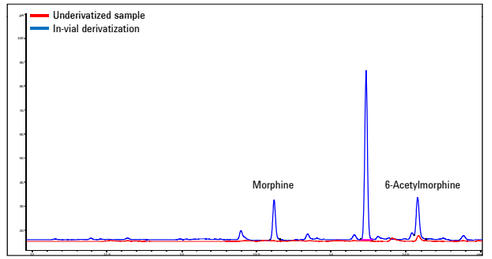

Figure 5. Chromatograms of morphine and 6-acetylmorphine underivatized and derivatized with 1 µL of BSTFA per µL of sample in the hot inlet.

Increased Lab Productivity and Decreased Cost with the ALS

The time savings in automating on-line derivatization is significant. Typical bench-top derivatization protocols can take up to an hour for the reaction to complete. However, by modifying the derivatization procedure to take place in the inlet, the reaction takes only seconds so derivatization and analysis are complete within the cycle time (Figure 6). Additionally, conventional derivatization methods use at least 100 µL of BSTFA for a 0.5 mL sample. On-line derivatization uses only 1 µL of BSTFA per injection – saving solvent and reducing exposure to hazardous chemicals.

Derivatizations can be further complicated by reversible reactions. While no examples were shown here, on-line derivatization is especially amenable to these sample types since the reaction occurs as the sample injection takes place and can be performed on each sample as needed.

Results and Discussion

Automating derivatization can eliminate errors by minimizing operator intervention, but achieves the same reproducibility, if not better, than derivatizing samples in the individual sample vials.

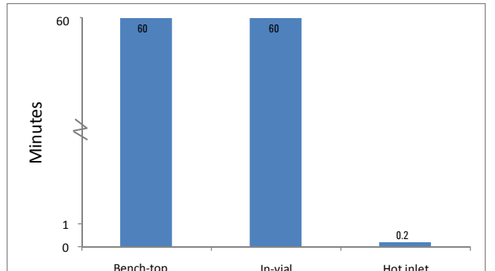

Figure 6. Time required to perform derivatization with bench-top methods, automated, in-vial methods, and hot inlet methods.

Conclusions

On-line derivatization can be easily automated with Agilent's 7693A Automatic Liquid Sampler.

- Derivatization can be done just in time for analysis, freeing an operator for other tasks, minimizing errors, and providing the same reproducibility, if not better, as manual methods.
- Hot inlet derivatization is a straight forward procedure with the new multi-layer (sandwich) injection capability of the ALS.
  - Hot inlet derivatization reduces the time for reaction and requires 100x less reagent.
  - This can significantly reduce the cost per analysis
- On-line derivatization can be especially useful for reversible reactions.

References:

1. Karl Blau and John Halket, Handbook of Derivatives for Chromatography, 2nd Ed., John Wiley & Sons: Chichester, England, 1993, pp51-63.

2. Kenneth Docherty and Paul Ziemann, "On-line, inlet-based trimethylsilyl derivatization for gas chromatography of mono- and dicarboxylic acids," JChrom A 921 2001 265-275.

3. Susanne Moyer, Dale Synder, Rebecca Veeneman, and Bill Wilson, "Typical Injection Performance for the Agilent 7693A Autoinjector," Agilent Technologies Publication 5990-4606EN.

4. Donghao Li and Jongman Park, "Silyl Derivatives of Alkylphenols, Chlorophenols, and Bisphenol A for Simultaneous GC/MS," Anal Chem 73 2001, 3089-3095.