APPLICATIONS COMPENDIUM FOR AGILENT 7696A SAMPLE PREP WORKBENCH

MAINTAIN CONSISTENT, ACCURATE, AND SAFE SAMPLE PREPARATION

The Measure of Confidence

Agilent Technologies

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SEARCH

Maintain sample prep consistency and reproducibility, run after run. Visit agilent.com/chem/workbench

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SEARCH

ENHANCE THE RELIABILITY OF YOUR MANUAL SAMPLE PREPARATION AND STANDARDS DEVELOPMENT

Manual sample prep is tedious and inherently variable, which can lead to time-consuming rework, wasted supplies, and the lingering uncertainty that your samples are not prepared the way your protocols – or regulatory requirements – dictate.

You can ease these frustrations with the Agilent 7696A Sample Prep WorkBench – the industry's *only* standalone sample preparation instrument

The Agilent 7696A Sample Prep WorkBench automates repetitive and error-prone steps in your sample prep workflow. It combines precise automation with intuitive software to ensure consistent sample processing, eliminate analyst-to-analyst variability, and allow chemists to work on other, more critical, tasks.

In addition, the 7696A Sample Prep WorkBench can reduce your costs by reducing waste and eliminating the need for re-injections due to missed steps. It also fits into most fume hoods, minimizing exposure to hazardous chemicals and reagents.

(Continued)

FOR MORE INFORMATION

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- **7696A Sample Prep WorkBench Webpage**
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  - Automating Sample Preparation For The GC Analysis Of Biodiesel Using Method EN14105:2011
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  - 7696A Sample Prep WorkBench: How it works
  - 7696A WorkBench Testimonial: Research Institute of Chromatography
  - 7696A WorkBench Testimonial: Institute of Food Safety & Health

Expanded capabilities eliminate *more* mundane steps that slow your productivity

We've enhanced the 7696A Sample Prep WorkBench with new automation technologies that prevent errors associated with weighing, diluting, and making derivatives. These include:

- **A high-precision weigh station** (up to five decimal places) that lets you weigh exact amounts of material directly into a GC or LC vial. Meets ASTM and EN petrochemical analysis standards.
- **LC vial racks** that allow you to place vials directly onto the autosampler – eliminating vial placement and transcription errors.
- **A dilution wizard** that speeds up method development, minimizes mouse clicks, and automatically creates standards for calibration curves and other repetitive dilutions.

Here's what you'll find inside...

This compendium highlights the application of the Agilent 7696A Sample Prep WorkBench across a range of industries, sample types, and sample prep techniques. You'll also find tips and techniques to help you:

- Maintain precision at the lowest volumes
- Minimize variability errors between analysts
- Reduce the need for costly rework
- Lower health and safety risks
- Improve accuracy, precision, and reproducibility through gravimetric weighing techniques
- Ensure data security and seamless traceability for GC and LC sample preparation

ENERGY AND CHEMICALS

Ensure precise sample weighing and dilution for both volatile and non-volatile samples (gasoline through heavy oils)

Precision in sample preparation minimizes the chance for errors in the final results. Automation delivers that precision regardless of the time of day. The following applications demonstrate the use of WorkBench to automate some of the mundane tasks associated with dilutions of samples with a wide range of volatilities, derivatization of FAMEs in jet fuel, and biodiesel analysis using method EN14105:2011.

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ENERGY AND CHEMICALS

Maintain sample prep consistency and reproducibility, run after run. Visit agilent.com/chem/workbench

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Automation of a Complex, Multi-Step Sample Preparation Using the Standalone Agilent 7696A WorkBench

Application Note

Biofuels and Alternative Energy

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Abstract

The Agilent 7696A Sample Prep Workbench was used to automate a multi-step sample preparation. We chose ASTM method D6584 as a test case to demonstrate the capabilities of the WorkBench. This method requires a complex derivatization of non-volatile contaminants before analysis by gas chromatography. The WorkBench was used to prepare several different types of biodiesel and the calibration standard used to quantify the target contaminants. The results with the WorkBench prepared samples were nearly identical to those prepared manually. Analysis precision was very high and well within industry specifications for the WorkBench prepared samples. To further test the WorkBench, multiple groups of chemists developed an automated sample preparation for a biodiesel sample. The analysis results obtained between each group were also nearly identical with very high analysis precision.

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Introduction

In analytical chemistry, sample preparation can be as simple as adding a solvent or as complex as performing chemical reactions to improve the instrumental measurements that follow. While sample preparation is a critical component to any chemical measurement, chemists rarely look forward to performing this job, especially if it is complex, boring and involves handling unpleasant chemicals. As a result, manual sample preparation can be the source of many errors and poor precision. To help reduce errors and improve precision, many manual sample preparations are done using large amounts of chemicals and expensive volumetric glassware to make handling, dispensing, and measuring easier.

A good example of a difficult manual preparation is ASTM method D6584. This method measures the free and total glycerin content in B100 biodiesel to assure good product quality [1]. Since the various glycerins found in biodiesel are not volatile, they cannot be measured using gas chromatography (GC). Method D6584 describes a sample preparation protocol to derivatize these compounds with a trimethylsilation reagent so they can be analyzed with GC. The steps for this sample preparation are complex, time consuming, and use pyridine, a toxic solvent with a distinctly unpleasant odor. This explains the unpopularity of this sample prep among chemists working with biodiesel.

The Agilent 7696A Sample Prep WorkBench is a standalone instrument specifically designed to perform automated sample preparation [2,3]. It uses two 7693A injection towers to volumetrically transfer liquids between 2-mL vials. The vials containing various chemical resources, standards and samples are housed in three 50-position trays. The sample tray compartment houses a robotic arm to move vials, a vortex mixing station and a sample heating station.

Designing the 7896A WorkBench Procedure

The ASTM D6584 preparation procedure can be completely described in six individual steps as shown in Table 1. When done manually, this prep consumes large amounts of standards, reagents, solvents and disposable glassware. Since the Agilent WorkBench uses smaller 2-mL vials, this procedure can be scaled down by a factor of 10. The WorkBench also uses two pipetting syringes to transfer liquids, thus eliminating the expense of disposable glassware. Table 1 also shows how each step was scaled to accommodate the 2-mL vials used by the WorkBench.

Before building a WorkBench sample prep, we first defined the chemical resources needed to prepare the biodiesel samples and the position of those resources in the WorkBench trays. Table 2 shows each resource, their tray positions and the pipetting syringe parameters used to dispense each resource. The WorkBench software also provides a graphic, overhead view of the resources in the sample trays as shown in Figure 1. In this example, we show 10 samples in tray positions 1 to 10 and 10 n-heptane resource vials that will be used with each sample. The n-heptane vials are stored in tray positions 101 to 110.

Table 1. ASTM Method D6584 uses a six step derivatization of Glycerins in Biodiesel to prepare the samples for analysis by high temperature GC. Since the Agilent 7696A Sample Prep WorkBench uses 2-mL vials, the manual sample must be scaled down 10:1

Steps	Manual Sample Prep in 15-mL Vials	10:1 Scaling →	WorkBench Sample Prep using 2-mL Vials
1	Add 100 mg B100 to a 15-mL vial with PTFE screw cap		Add 10 mg B100 to a 2-mL vial with PTFE screw cap
2	Add 100 µL ISTD1 solution (butanetriol) to the vial		Add 10 µL ISTD1 solution (butanetriol) to the vial
3	Add 100 µL ISTD2 solution (tricaprin) to vial		Add 10 µL ISTD2 solution (tricaprin) to vial
4	Add 100 µL derivatization reagent (MSTFA) to vial and mix		Add 10 µL derivatization reagent (MSTFA) to vial and mix
5	React at room temperature for 15 minutes		React at room temperature for 15 minutes
6	Add 8 mL n-heptane to vial and mix		Add 800 µL n-heptane to vial and mix

Table 2. Four chemical resources are needed to completely derivatize Glycerins in Biodiesel. The resources, tray positions and syringe parameters are set in the Workbench Software. The syringe draw speeds are used to load each resource into the syringe. The syringe dispense speeds are used to transfer the resource into the 2-mL sample vials

Chemical resource	Tray position	Syringe size (µL)	Syringe draw speed (µL/min)	Syringe dispense speed (µL/min)
ISTD1 (1000 µg/mL butanetriol in pyridine)	51	100	250	500
ISTD2 (8000 µg/mL tricaprin in pyridine	52	100	250	500
MSTFA derivatization reagent	53	100	250	500
n-Heptane	101–110	250	500	2000

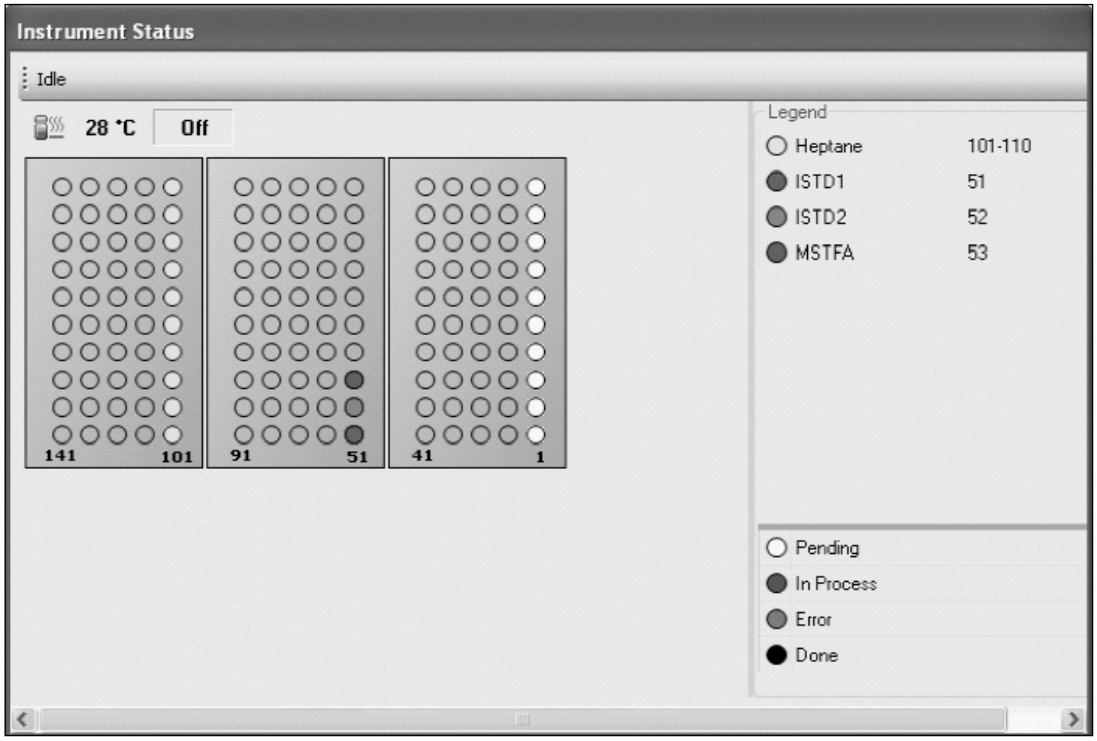

Figure 1. The WorkBench software provides an overhead view of each chemical resource in the sample trays. For this example, in addition to the chemical resources, 10 samples were placed in tray positions 1 to 10.

Sample weighing cannot be performed using the WorkBench because there is no analytical balance. Since weighing 10 mg of biodiesel can be very challenging, an Eppendorf Reference Adjustable-Volume Pipettor (10–100 µL) was used to transfer the sample. Weighing 10 mg of biodiesel was done by manually pipetting 11.4 µL of biodiesel into tared 2-mL vials and recording the weight to the nearest 0.1 mg.

To mimic the manual sample prep workflow, individual WorkBench methods were created for each step outlined in Table 2. For instance, we created a method called ADD_ISTD1.M to add the first internal standard solution (ISTD1) to every sample before adding the second internal standard (ISTD2) using method ADD_ISTD2.M. With this approach, we only needed to wash the syringe with solvent after switching to a different resource. This greatly reduces the amount of wash solvent needed and allows more samples to be prepared before refilling the wash solvent reservoirs. The final "script" for the WorkBench sample prep, including the syringe wash steps, is shown in Table 3. To run the complete sample prep, each method is run by the WorkBench sequence queue as shown in Figure 2.

Table 3. A final "Script" showing each step in the sample prep protocol and the corresponding Workbench Methods needed to perform each action

Steps	Biodiesel preparation protocol	Method name	Comments
1	Add 10 µL ISTD1 solution to every sample vial	ADD_ISTD1.M	Uses 100-µL syringe in rear tower
2	Wash 100-µL syringe	Wash_Back.M	Solvent reservoirs in rear tower
3	Add 10 µL ISTD2 solution to every sample vial	ADD_ISTD2.M	Uses 100-µL syringe in rear tower
4	Wash 100-µL syringe	Wash_Back.M	Solvent reservoirs in rear tower
5	Add 10 µL MSTFA reagent to every sample vial and mix	ADD_MSTFA.M	Uses 100-µL syringe in rear tower
6	Wash 100-µL syringe	Wash_Back.M	Solvent reservoirs in rear tower
7	React at room temperature for 15 minutes	Reaction.M	One 15 minute wait time is used for all samples
8	Add 800 µL n-heptane to every sample vial and mix	ADD_Heptane.M	Uses 250-µL syringe in front tower

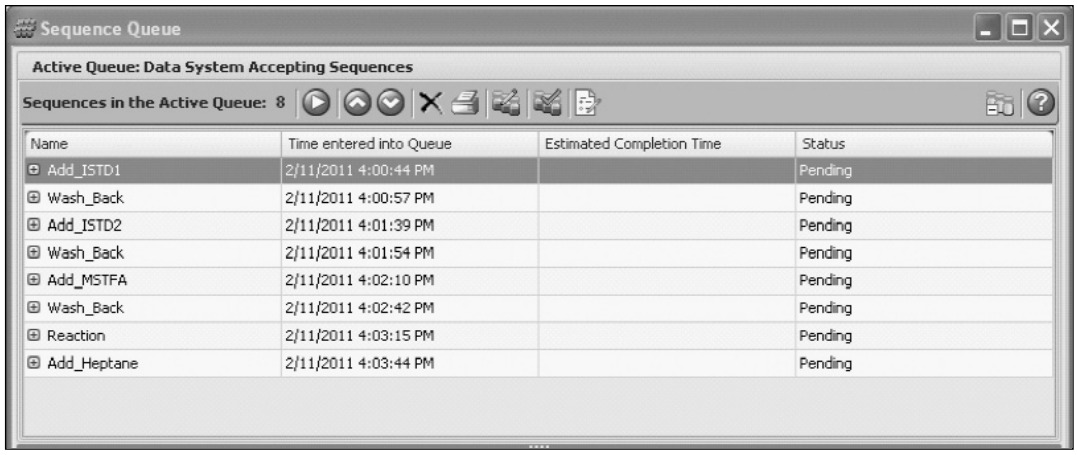

Figure 2. The WorkBench Sequence Queue is used to run the WorkBench methods described in Table 3.

Experimental

An Agilent 7890A GC was configured to run ASTM D6584. This configuration is outlined in Table 4. The GC conditions used to analyze the biodiesel samples and standards are shown in Table 5.

Preparation of GC calibration standards

ASTM D6584 also requires the derivatization of five calibration standards with the same preparation used for the samples. After running the standards by GC, the resulting calibration curves were evaluated for linearity before running any samples. The calibration standards were prepared both manually and with the WorkBench with the same protocol used for the samples. The calibration curves resulting from the manual prep were used to quantify the manually prepared biodiesel samples. The calibrations resulting from the WorkBench prepared standards were used to quantify the WorkBench prepared samples.

Comparison of manual sample prep and WorkBench sample prep

The first question many users will ask is "does a scaled WorkBench sample prep produce the same results as the manual sample prep?". To help answer that question, two different types of biodiesel samples were prepared using the manual ASTM protocol and the WorkBench. The first biodiesel sample came from a small local producer using canola oil as the feedstock. The second sample was supplied by a national producer using a soybean oil feedstock. For both the manual and WorkBench protocols, each biodiesel sample was prepared and analyzed in duplicate to evaluate the repeatability (single user precision) according to the ASTM method.

Multiuser precision - reproducibility

In order to evaluate multi-user precision, four different chemists were provided with a soybean biodiesel sample, calibration standards and a WorkBench with the chemical resources shown in Table 2. Each chemist was given the list of sample preparation steps outlined in Table 3 and asked to develop and use a WorkBench protocol. Duplicates of a soybean biodiesel sample were prepared using their WorkBench followed by GC analysis.

Table 4. Gas Chromatographic Instrument configuration used to analyze samples using ASTM Method

Standard Agilent 7890A GC	
Hardware	
G3440A	Agilent 7890A Series GC
Option 122	Cool-On-Column Inlet with EPC control
Option 211	Capillary FID with EPC control
G4513A	Agilent 7693A ALS
Columns	
Analytical Column	Select Biodiesel for Glycerides 15 m x 0.32mm id x 0.1 µm film (p/n cp9078)
Data System	Agilent Multi-Technique Chemstation
Consumables	
5181-1267	10 µL PTFE fixed autoinjector syringe
Standards and Reagents	
5190-1408	Biodiesel D6584 Calibration Standards Kit
5190-1407	Biodiesel MSTFA Kit
	Reagent grade n-heptane

Table 5. GC Instrument Conditions for ASTM Method D6584

Cool-on-column inlet	
Initial temperature	50 °C
Temperature program	Oven track mode
Column flow	Helium at 3 mL/min constant flow mode
Column Temperature	
Initial	50 °C for 1 min
Rate 1	15 °C/min to 180 °C, hold 0 min
Rate 2	7 °C/min to 230 °C, hold 0 min
Rate 3	30 °C/min to 380 °C, hold 10 min
Flame ionization detector	380 °C

Results

Preparation of GC calibration standards

The 5-level calibration curves for glycerin, monoolein, diolein and triolein are shown in Figure 3. The five standards used to create these curves were prepared with the Agilent WorkBench. The glycerin curve was used to quantify free glycerin in the biodiesel samples. The monoolein curve was used for the monoglycerides, the diolein curve for all diglycerides and the triolein curves for all triglycerides found in the samples. The same calibration standards were also prepared manually and used to construct calibration curves. In Table 6, we compared the calibration models for all four compounds from the manually prepared standards and the WorkBench prepared standards. The manually prepared standards and the WorkBench prepared standards yielded nearly identical calibration curves and the correlation coefficients (r^2) from the WorkBench prepared standards exceeded the ASTM specification of at least 0.99 or greater.

Table 6. The calibrations curves resulting from manual and WorkBench Preparation Protocols were very similar as shown by the respective slopes and intercepts for each compound. Both preparation Methods met the ASTM requirement for Correlation Coefficient Values (r^2) of 0.99 or greater

	Manual Prep			WorkBench		
Compound	**Slope**	**y-int**	**r^2**	**Slope**	**y-int**	**r^2**
Glycerin	1.0433	0.0028	0.9997	1.1027	0.0049	0.9995
Monoolein	1.3446	–0.0171	0.9997	1.3786	0.0044	1.0000
Diolein	1.2176	–0.0010	0.9999	1.2086	–0.0014	0.9999
Triolen	0.8303	–0.0018	0.9965	0.8703	0.0030	1.0000

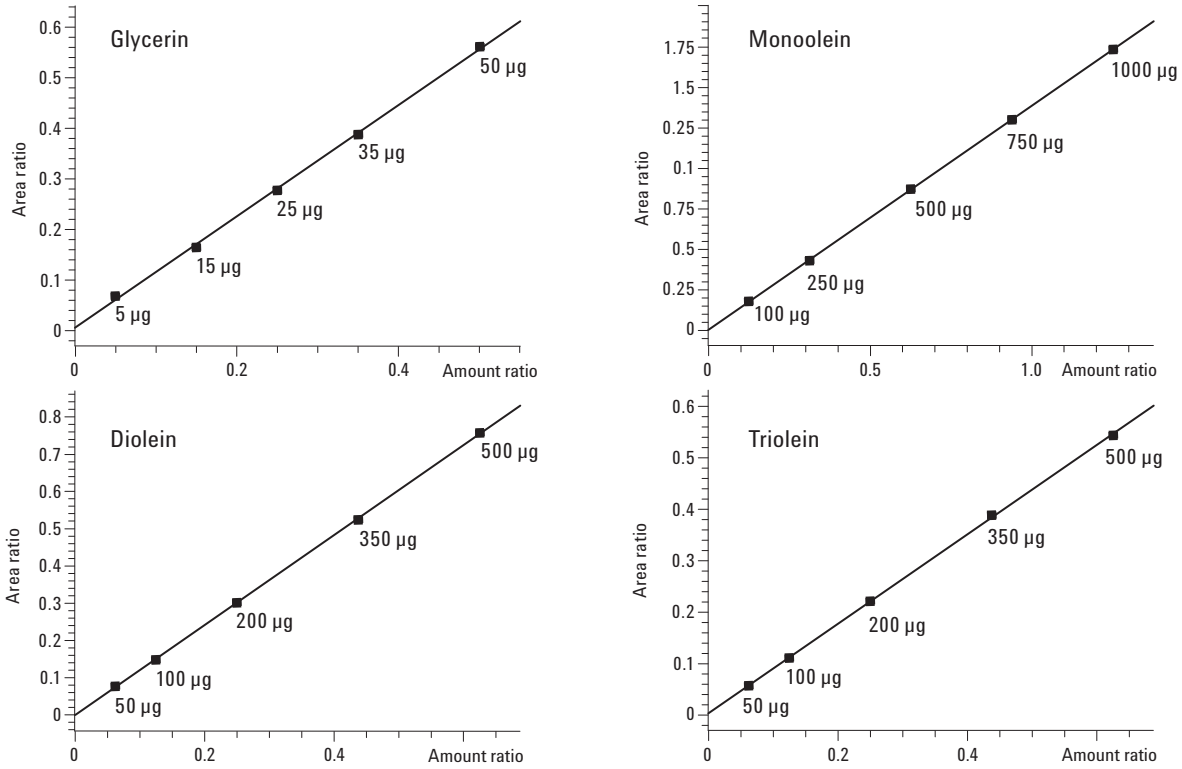

Figure 3. Calibration curves from standards prepared using the WorkBench.

Comparison of manual sample prep and WorkBench sample prep

The biodiesel samples prepared manually and with the WorkBench were analyzed according to ASTM method D6584. Figure 4 shows a comparison of biodiesel sample 1 (canola) chromatograms resulting from the manual prep and the WorkBench prep. In the regions where the various glycerins elute, both chromatograms look identical. For all samples, the free and total glycerins were quantified and the results are listed in Table 7. The WorkBench sample prep yielded results that were identical to those prepared manually. Both samples were prepared and analyzed in duplicate to determine the repeatability of the sample preparations. Repeatability (r) is used to measure the precision for a single operator by taking the difference between duplicate analyses of each sample. As seen in Table 7, the samples prepared using the WorkBench exceeded minimum repeatability specification set by ASTM for this analysis. This shows that after a 10-fold reduced scale, samples prepared with WorkBench can easily provide the same precise results as manually prepared samples using much larger amounts of chemicals, reagents and solvents.

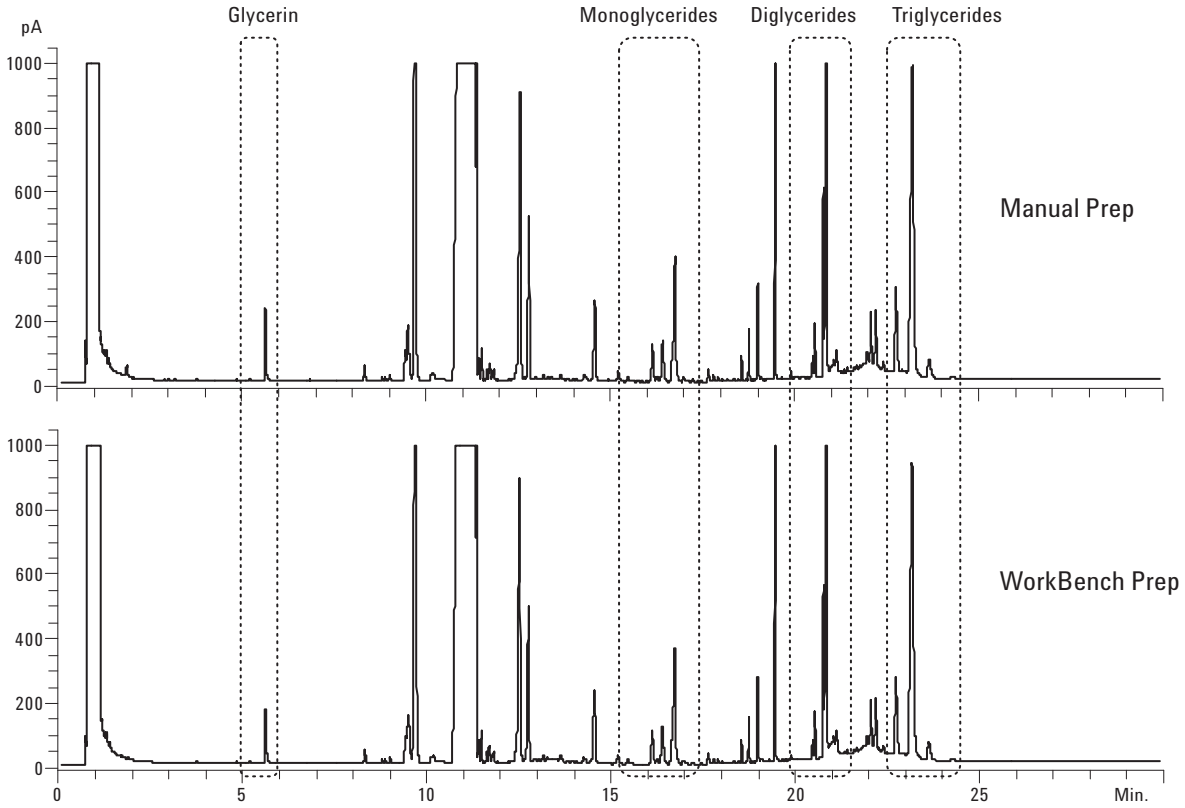

Figure 4. A comparison of data from a canola biodiesel sample prepared manually and using the Agilent WorkBench. These chromatograms show remarkable similarity in the four regions where glycerin, monoglycerides, diglycerides and triglycerides are separated.

Table 7. For two different types of Biodiesels, the WorkBench sample results were nearly identical to the samples prepared manually. The precision (repeatability) observed for the WorkBench samples were well within ASTM Specifications

Biodiesel Sample 1 (canola)

	Manual Prep			WorkBench			Reproducibility (r)
	Run 1	**Run 2**	**r**	**Run 1**	**Run 2**	**r**	**Specification**
Free Glycerin	0.000	0.000	0.000	0.000	0.000	0.000	2.58E-04
Monoglycerides	0.169	0.169		0.168	0.163		
Diglycerides	0.282	0.286		0.291	0.286		
Triglycerides	0.533	0.536		0.565	0.554		
Total Glycerin	0.984	0.991	0.007	1.023	1.003	0.020	0.083

Biodiesel Sample 2 (soybean)

	Manual Prep			WorkBench			Reproducibility (r)
	Run 1	**Run 2**	**r**	**Run 1**	**Run 2**	**r**	**Specification**
Free Glycerin	0.008	0.008	0.000	0.008	0.008	0.000	0.002
Monoglycerides	0.138	0.144		0.141	0.140		
Diglycerides	0.022	0.023		0.022	0.021		
Triglycerides	0.009	0.009		0.006	0.005		
Total Glycerin	0.177	0.184	0.007	0.176	0.174	0.002	0.046

Multiuser precision - reproducibility

Figure 5 shows the same soybean biodiesel sample independently prepared by four different chemists on four different days. The chromatography between each chemists is nearly identical. The quantitative results obtained by each chemist are shown in Table 8 along with an evaluation of the precision between groups (reproducibility). These results show a very high level of precision when several chemists develop an automated WorkBench protocol for preparing the same sample.

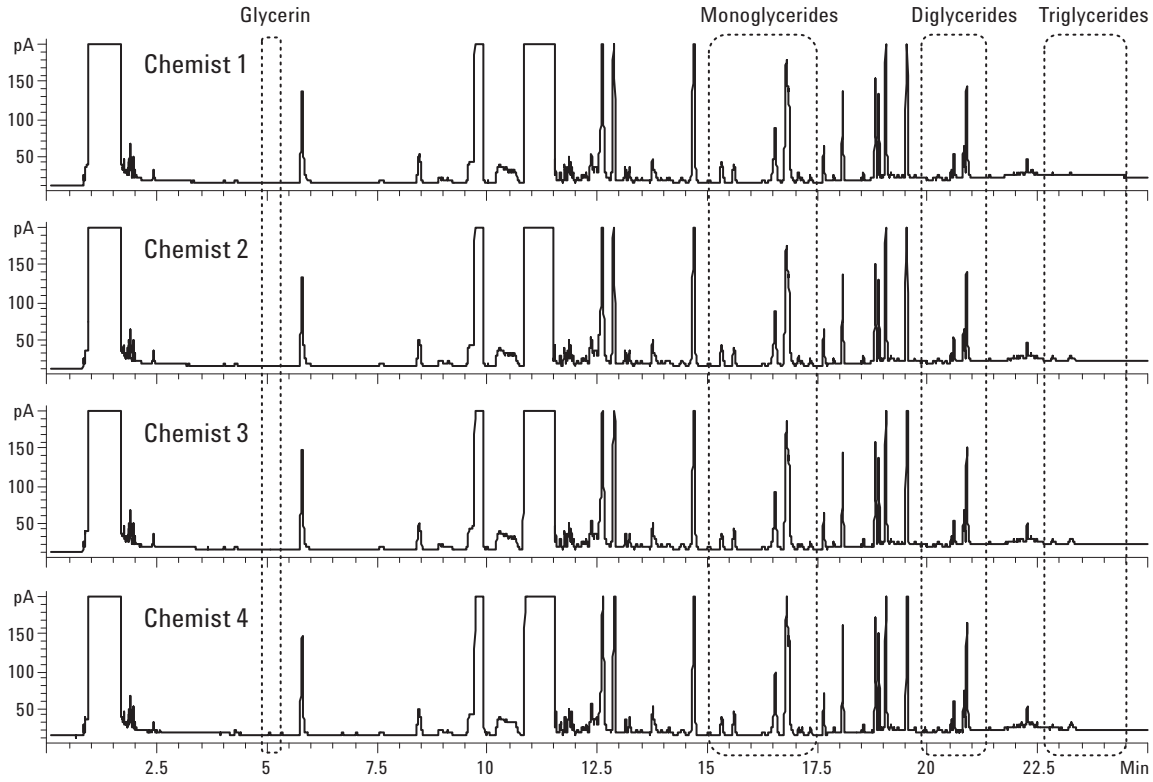

Figure 5. A comparison of data from a soybean biodiesel sample prepared by four different chemists working on four different days. Each chemist developed a WorkBench sample preparation protocol and then analyzed the samples using ASTM method D6584. The results are nearly identical.

Table 8. Each chemist obtained nearly the same results when using the Agilent WorkBench for Automated Sample Preparation. The precision (reproducibility) was well within the ASTM Specification for multiple operators

	Chemist 1			Chemist 2			Reproducibility	ASTM R
	Run 1	Run 2	Average	Run 1	Run 2	Average	(r)	Specification
Free Glycerin	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.007
Monoglycerides	0.107	0.114	0.111	0.109	0.118	0.113		
Diglycerides	0.032	0.034	0.033	0.033	0.036	0.034		
Triglycerides	0.009	0.009	0.009	0.008	0.009	0.008		
Total Glycerin	0.152	0.161	0.156	0.154	0.166	0.160	0.005	0.094

	Chemist 3			Chemist 4			Reproducibility	ASTM R
	Run 1	Run 2	Average	Run 1	Run 2	Average	(r)	Specification
Free Glycerin	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.007
Monoglycerides	0.116	0.114	0.115	0.113	0.114	0.113		
Diglycerides	0.033	0.033	0.033	0.032	0.033	0.032		
Triglycerides	0.007	0.007	0.007	0.006	0.006	0.006		
Total Glycerin	0.160	0.157	0.159	0.155	0.157	0.156	0.004	0.091

Conclusion

This paper demonstrates that a complex, multi-step sample preparation protocol can be automated with the Agilent 7696A WorkBench. Analytical results obtained with WorkBench prepared samples were the same as those obtained using a traditional manual sample preparation. Even after scaling the preparation steps for the 2-mL vials, the quantitative precision was very high with WorkBench prepared samples. Reducing the sample prep scale with the WorkBench also used 10 times less solvents, reagents, and calibration standards. Additionally, there was no need to use disposable glassware and expensive volumetric glassware.

References

1. "D6584 Test Method for Determination of Free and Total Glycerine in B-100 Biodiesel Methyl Esters by Gas Chromatography", ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2010.
2. "Agilent 7696A Sample Prep WorkBench", Agilent Technologies, Publication Number 5990-6908EN, January 28, 2011.
3. Rebecca Veeneman and Dale Snyder, "Improved Data Quality through Automated Sample Preparation", Agilent Technologies, Publication Number 5990-6974EN, December 10, 2010.

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5990-7525EN

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Automating Complex Standards and Sample Preparations Using the Agilent 7696A WorkBench

In analytical chemistry, sample preparation can be a simple dilution or a multi-step derivatization to improve the instrumental measurements. While sample preparation is a critical component to any chemical measurement, chemists rarely look forward to performing this job, especially if it is complex, boring and involves handling toxic chemicals. A good example of a difficult manual preparation is ASTM method D6584; which is used to measure glycerins in B100 biodiesel.[1] The method requires derivatization of the non-volatile glycerins prior to GC analysis. This sample preparation is complex, time consuming, and uses pyridine; a toxic solvent with a very unpleasant odor.

The Agilent 7696A WorkBench is a standalone instrument specifically designed to automate sample preparation.[2,3] Its ability to perform complex sample preparations, like ASTM D6584, is demonstrated using commercially available biodiesels. The analysis results obtained with the WorkBench were identical to those prepared manually (Figure 1). Using the WorkBench, a lab can also get consistent and precise results with less experienced technicians. Figure 2 shows excellent results obtained by four different chemists with no prior biodiesel experience. Full details for this application are contained in Agilent Publication No. 5990-7525EN.

Key Benefits

- Obtain the same results as manual preparation regardless of experience
- Fewer consumables
- Use less of your expensive reagents
- Less exposure to toxic chemicals
- More time for key tasks (that is, method development, data review)

[1] "D6584 Test Method for Determination of Free and Total Glycerine in B-100 Biodiesel Methyl Esters by Gas Chromatography," ASTM International: 100 Barr Harbor Drive, West Conshohocken, PA, USA, 2010.

[2] Agilent 7696A Sample Prep WorkBench, Agilent Technologies, Publication Number 5990-6908EN, January 28, 2011.

[3] "Improved Data Quality through Automated Sample Preparation," Rebecca Veeneman and Dale Synder, Agilent Technologies, Publication Number 5990-6974EN, December 10, 2010.

Automating Complex Sample Preparations Using the Agilent 7696A WorkBench

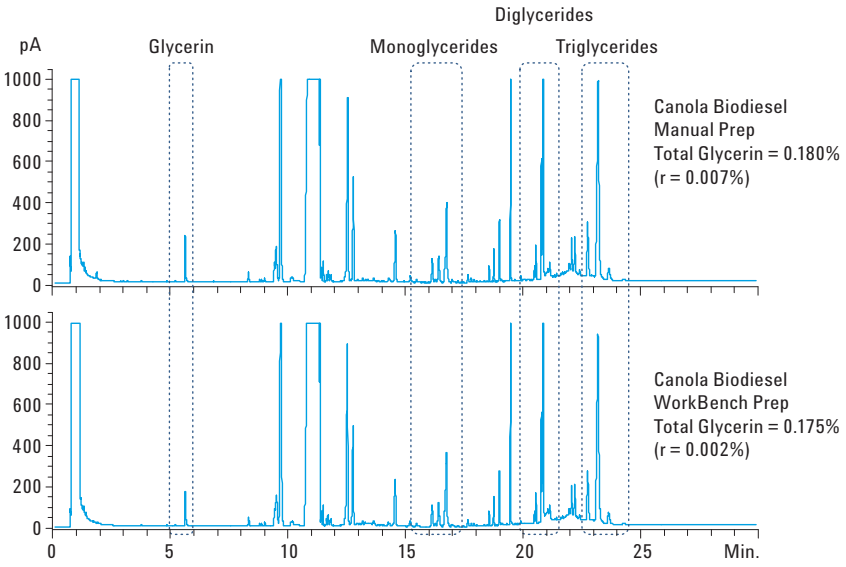

Figure 1. *A WorkBench prepared sample gave the same result, with higher precision, when compared with manual preparation. Each sample was prepared in duplicate to determine repeatability (r).*

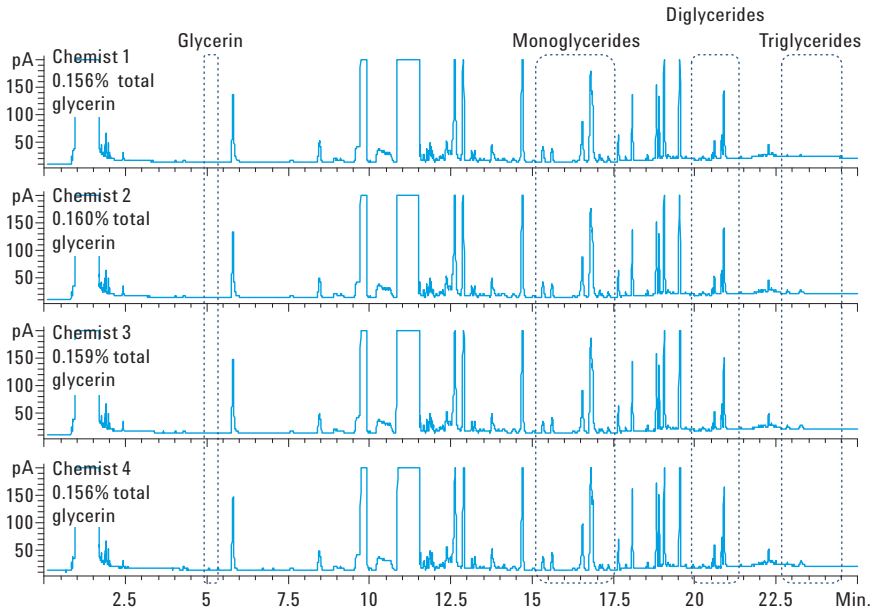

Figure 2. *A comparison of data from a soybean biodiesel sample prepared by four different chemists using the Agilent WorkBench.*

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Printed in USA, September 8, 2011
5990-8204EN

Agilent Technologies

Automated Standard and Sample Preparation Using the Agilent 7696A WorkBench for GC/MS Analysis of FAME Contamination in Jet Fuel

Application Note

Fuels

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Abstract

The Agilent 7696A Sample Prep WorkBench was used to prepare calibration standards and samples for the GC/MS analysis of total FAME in jet fuel using the IP585 method. The WorkBench needed 10 times less reagents and standards to achieve better analysis results when compared to manual sample preparation techniques. The GC/MS calibration using WorkBench prepared standards meet all performance criteria without any re-work, saving considerable time in the laboratory. WorkBench prepared jet fuel samples exceeded the method's precision requirements for several different levels of FAME contamination. The analysis results obtained from the WorkBench samples provided better recovery of the known FAME concentrations compared to the manually prepared samples.

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Introduction

The Energy Institute method, IP585, uses GC/MS to measure trace fatty acid methyl esters (FAME) in commercial jet fuel.[1] FAME contamination occurs when multiproduct pipelines (MPP) are used to transport both biodiesel and jet fuel. A limit of 5 mg/kg of total FAME content has been established by the Joint Inspection Group (JIG), a consortium of jet fuel producers and users. A recent Agilent paper describes the operation and performance of the Agilent 5975C Series GC/MSD system when running method IP585.[2]

As with most instrumental measurements, successful preparation of calibration standards and samples plays a significant part to achieving quality results. For the IP585 method, 1-mL volumes of calibration standards are made using graduated microliter pipettes. Using a microliter syringe, an expensive internal standard solution containing 1000 mg/mL of methyl heptadecanoate-d33 (C17:0-d33) is added to every calibration standard and sample. Due to the small volumes being measured, these procedures require considerable skill to correctly prepare standards and samples. A better approach would be to automate the sample preparation using an instrument specifically designed to dispense and mix liquids in microliter volumes with high accuracy and precision.

The Agilent 7696A Sample Prep WorkBench is a standalone instrument specifically designed to perform automated sample preparation. It uses two Agilent 7693A injection towers to volumetrically transfer liquids between 2-mL vials. Vials containing various chemical resources, standards, and samples are housed in three 50-positions trays. The sample tray compartment contains a robotic arm, a vortex mixing station, and a sample heating station. Calibration standard preparation using the Agilent WorkBench have been shown to provide better calibrations compared to manually prepared standards. Additionally, samples prepared in 2-mL vials using the WorkBench were shown to give the same quantitative results as manually prepared samples.[3] In this application note, the Agilent 7696A Workbench was used to prepare 11 calibration standards along with three jet fuel samples each containing different levels of FAME contamination. Standards and sample volumes were reduced 10-fold from 1 mL to 100 µL to save resources such as solvents, stock standard solutions and the internal standard solution. The analysis results from the WorkBench prep were compared to results from a manual prep using the precision specifications in the IP585 method.

Designing the Automated Workbench Procedure

Calibration Standards Prepared by Linear Dilution

The IP585 method uses 10 working calibration standards (WCS) to calibrate the GC/MS system. Each WCS contains different concentrations of the six FAMEs shown in Table 1. The linear dilution scheme outlined in Table 2 is described in the method to manually prepare 1 mL quantities of each WCS. For the automated WorkBench preparation, this manual scheme was translated from 1 mL to 100 µL final volumes for each standard as shown in Table 3. To prepare the standards, four resources were defined in the WorkBench software (Table 4). The first resource was 10 empty vials used to contain the final WCS. The next resource was a vial containing 1,000 µL of 99% n-dodecane used as the dilution solvent. The third resource was a vial containing 1,000 µL of the working standard solution (WSS). The last resource was a vial containing 500 µL of the internal standard solution. Figure 1 shows the resource layout used by the WorkBench software for automated preparation of the calibration standards.

Table 1. Compounds used to Quantify Total FAME in Jet Fuel

Chemical name	Common name	Symbol	Molecular formula	Molecular weight
Methyl hexadecanoate	Methyl palmitate	C16:0	$C_{17}H_{34}O_2$	270.45
Methyl heptadecanoate	Methyl margarate	C17:0	$C_{18}H_{36}O_2$	284.45
Methyl octadecanoate	Methyl stearate	C18:0	$C_{19}H_{38}O_2$	298.50
Methyl octadecenoate	Methyl oleate	C18:1	$C_{19}H_{36}O_2$	296.49
Methyl octadecadienaote	Methyl linoleate	C18:2	$C_{19}H_{34}O_2$	294.47
Methyl octadecatrienoate	Methyl linolenate	C18:3	$C_{19}H_{32}O_2$	292.45

These six FAMEs are found in 95% of the common feed stocks used to produce biodiesel.

Table 2. Manual Scheme to Prepare 1-mL of each Working Calibration Standard (WCS) using Linear Volumetric Dilution

Volume (µL) of working standard solution (WSS)	Volume (µL) of n-C12 solvent	Volume (µL) of internal standard (ISTD)	Final concentration (mg/kg) of each FAME
1000	0	10	100
800	200	10	80
600	400	10	60
400	600	10	40
200	800	10	20
100	900	10	10
80	920	10	8
60	940	10	6
40	960	10	4
20	980	10	2
0	1000	10	0

Table 3. Agilent WorkBench Linear Volumetric Dilution Preparation to Make 100 µL of each Working Calibration Standard (WCS)

Volume (µL) of working standard solution (WSS)	Volume (µL) of n-C12 solvent	Volume (µL) of internal standard (ISTD)	Final concentration (mg/kg) of each FAME	Working calibration standards (WCS)
100	0	1	100	High Std 5
80	20	1	80	High Std 4
60	40	1	60	High Std 3
40	60	1	40	High Std 2
20	80	1	20	High Std 1
10	90	1	10	Low Std 5
8	92	1	8	Low Std 4
6	94	1	6	Low Std 3
4	96	1	4	Low Std 2
2	98	1	2	Low Std 1
0	100	1	0	Blank

Table 4. WorkBench Resource Layout for Automated Preparation of IP585 Calibration Standards

Resource	Resource type	Vial range	Usage
Working calibration standards (WCS)	Empty container	51-60	1
n-Dodecane solvent	Chemical resource	61	1000 µL
Working standard solution (WSS)	Chemical resource	71	1000 µL
Internal standard solution (ISTD)	Chemical resource	81	500 µL

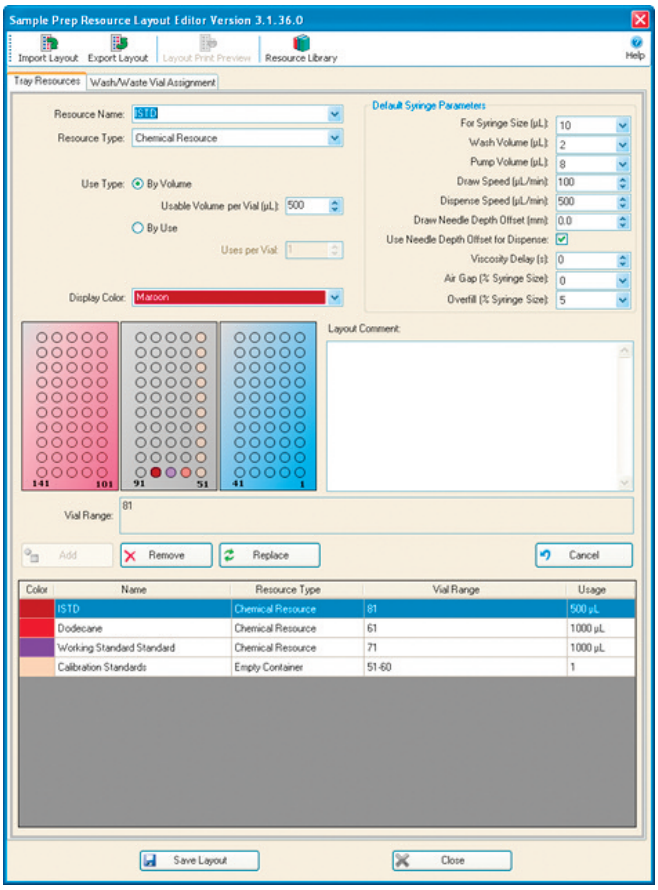

Figure 1. Agilent WorkBench resource layout for the automated preparation of IP585 calibration standards. The empty vials in positions 51 to 60 will contain each of the 10 calibration standards after the automated preparation is complete.

With the resource layout complete, two Agilent WorkBench methods were designed to prepare the standards listed in Table 3. The first method, "IP585_Low.M", was used for the 2 to 10 mg/kg low level standards and the second method, "IP585_High.M", was used for the 20 to 100 mg/kg high level standards. Details of the sample prep steps for each of these methods are listed in Tables 5 and 6. The WorkBench software allows the user to quickly and easily build methods using a graphical "drag-and-drop" interface. The IP585_Low.M method shown in Figure 2 is an example of a typical method.

Table 5. Agilent WorkBench Method to Prepare 100 µL of each Low Level Working Calibration Standard (WCS)

Step	Agilent WorkBench action	Description	Syringe
1	Wash	Solvent wash 250 µL syringe	250 µL
2	Add	100 µL n-C_{12} to Low Blank (Vial 1)	250 µL
3	Add	98 µL n-C_{12} to Low Std 1 (Vial 51)	250 µL
4	Add	96 µL n-C_{12} to Low Std 2 (Vial 52)	250 µL
5	Add	94 µL n-C_{12} to Low Std 3 (Vial 53)	250 µL
6	Add	92 µL n-C_{12} to Low Std 4 (Vial 54)	250 µL
7	Add	90 µL n-C_{12} to Low Std 5 (Vial 55)	250 µL
8	Wash	Solvent wash 25 µL syringe	25 µL
9	Add	2 µL WSS to Low Std 1 (Vial 51)	25 µL
10	Add	4 µL WSS to Low Std 2 (Vial 52)	25 µL
11	Add	6 µL WSS to Low Std 3 (Vial 53)	25 µL
12	Add	8 µL WSS to Low Std 4 (Vial 54)	25 µL
13	Add	10 µL WSS to Low Std 5 (Vial 55)	25 µL
14	Wash	Solvent wash 25 µL syringe	25 µL
15	Add	1 µL ISTD to Low Blank (Vial 1)	25 µL
16	Add	1 µL ISTD to Low Std 1 (Vial 51)	25 µL
17	Add	1 µL ISTD to Low Std 2 (Vial 52)	25 µL
18	Add	1 µL ISTD to Low Std 3 (Vial 53)	25 µL
19	Add	1 µL ISTD to Low Std 4 (Vial 54)	25 µL
20	Add	1 µL ISTD to Low Std 5 (Vial 55)	25 µL
21	Wash	Solvent wash 25 µL syringe	25 µL
22	Mix	Low Blank (Vial 1) for 30 s @ 1500 rpm	
23	Mix	Low Std 1 (Vial 51) for 30 s @ 1500 rpm	
24	Mix	Low Std 2 (Vial 52) for 30 s @ 1500 rpm	
25	Mix	Low Std 3 (Vial 53) for 30 s @ 1500 rpm	
26	Mix	Low Std 4 (Vial 54) for 30 s @ 1500 rpm	
27	Mix	Low Std 5 (Vial 55) for 30 s @ 1500 rpm	

Table 6. Agilent WorkBench Method to Prepare 100 µL of each High Level Working Calibration Standard (WCS)

Step	Agilent WorkBench action	Description	Syringe
1	Wash	Solvent wash 250 µL syringe	250 µL
2	Add	100 µL n-C_{12} to High Blank (Vial 2)	250 µL
3	Add	80 µL n-C_{12} to High Std 1 (Vial 56)	250 µL
4	Add	60 µL n-C_{12} to High Std 2 (Vial 57)	250 µL
5	Add	40 µL n-C_{12} to High Std 3 (Vial 58)	250 µL
6	Add	20 µL n-C_{12} to High Std 4 (Vial 59)	250 µL
7	Wash	Solvent wash 250 µL syringe	250 µL
8	Add	20 µL WSS to High Std 1 (Vial 56)	250 µL
9	Add	40 µL WSS to High Std 2 (Vial 57)	250 µL
10	Add	60 µL WSS to High Std 3 (Vial 58)	250 µL
11	Add	80 µL WSS to High Std 4 (Vial 59)	250 µL
12	Add	100 µL WSS to High Std 5 (Vial 60)	250 µL
13	Wash	Solvent wash 25 µL syringe	25 µL
14	Add	1 µL ISTD to High Blank (Vial 2)	25 µL
15	Add	1 µL ISTD to High Std 1 (Vial 56)	25 µL
16	Add	1 µL ISTD to High Std 2 (Vial 57)	25 µL
17	Add	1 µL ISTD to High Std 3 (Vial 58)	25 µL
18	Add	1 µL ISTD to High Std 4 (Vial 59)	25 µL
19	Add	1 µL ISTD to High Std 5 (Vial 60)	25 µL
20	Wash	Solvent wash 25 µL syringe	25 µL
21	Mix	High Blank (Vial 2) for 30 s @ 1500 rpm	
22	Mix	High Std 1 (Vial 56) for 30 s @ 1500 rpm	
23	Mix	High Std 2 (Vial 57) for 30 s @ 1500 rpm	
24	Mix	High Std 3 (Vial 58) for 30 s @ 1500 rpm	
25	Mix	High Std 4 (Vial 59) for 30 s @ 1500 rpm	
26	Mix	High Std 5 (Vial 60) for 30 s @ 1500 rpm	

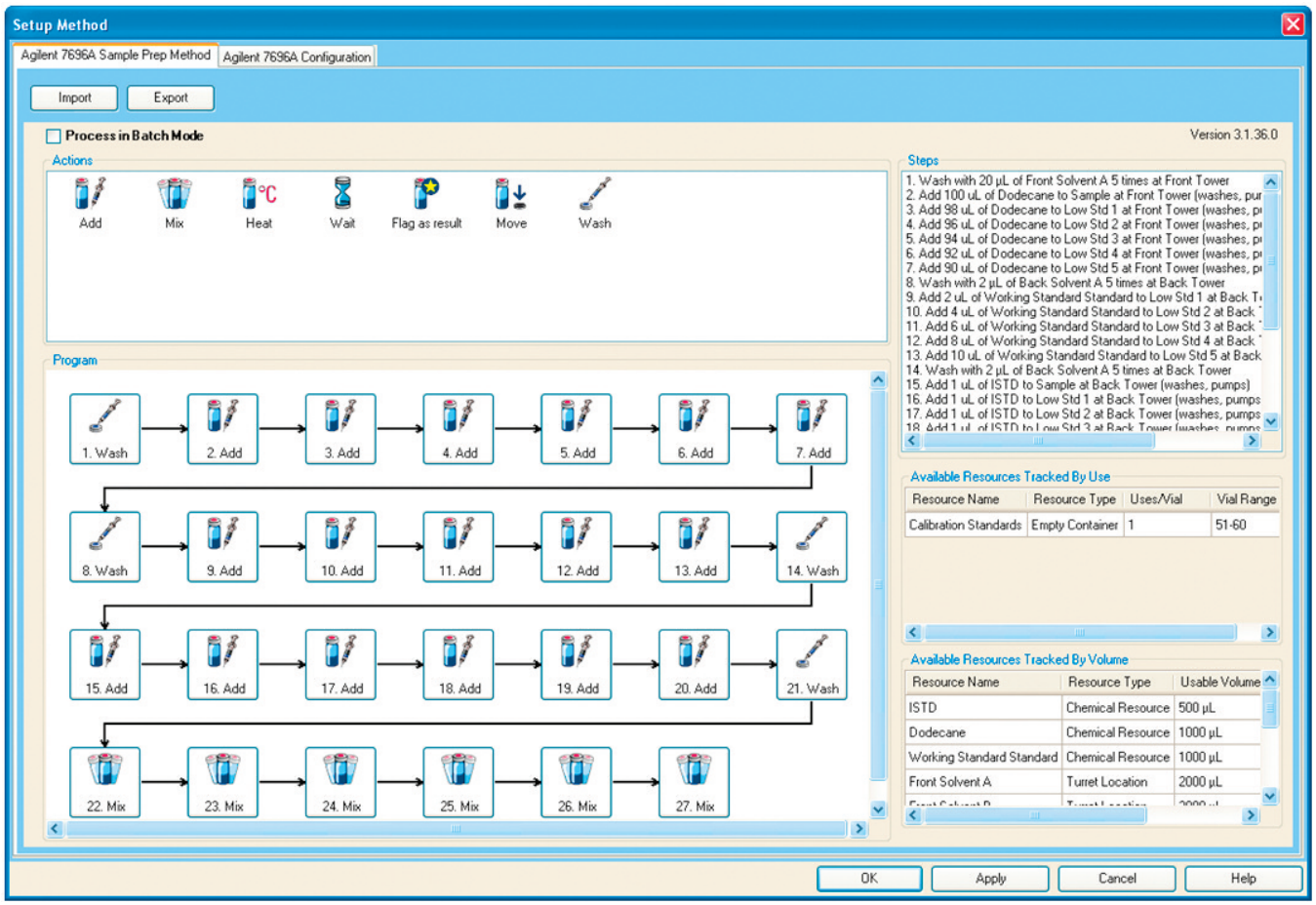

Figure 2. Agilent WorkBench method IP585_Low.M for preparing five low level calibration standards. Each of the method's steps were built using a "drop-and-drag" graphic ser interface.

Jet Fuel Sample Preparation Using Agilent Workbench Batch Mode

For the IP585 method, samples were prepared by pipetting 1 mL of jet fuel into a 2-mL vial followed by the addition of 10 µL of the internal standard solution. A laboratory chemist manually preparing multiple samples performs a workflow by adding each jet fuel sample into individual vials followed by adding the internal standard to each sample. This efficient workflow can be performed by using the Batch Mode feature of the Agilent WorkBench software. In Batch Mode, each sample preparation step was completed for every sample before moving on to the next step so that sample preparation time was minimized. Solvent wash and waste resources are also conserved since syringe solvent washing is only needed between resource changes.

For jet fuel sample preparation, the WorkBench needs only two resources; vials containing each jet fuel sample and a single vial containing the internal standard solution. In this application note, ten separate jet fuel samples were defined as resources for the WorkBench. These vials were placed in tray positions 51 to 60 and usage was set to one use per vial to eliminate any possibility of cross contamination during preparation. The internal standard vial was placed in tray position 81. During the sample preparation runs, 10 empty and capped 2-mL vials were placed in tray positions 1 to 10 (Figure 3). The batch mode WorkBench method, IP585_Samples.M, dispensed 100 uL of each jet fuel sample into separate, empty vials, followed by the addition of 1 µL of internal standard solution and mixing. Figure 4 shows this batch mode method for the jet fuel sample preparation.

Experimental

Manual Preparation of Working Calibration Standards (WCS) and Samples

Following the procedure described in the method (Table 2), the 10 calibration standards and a solvent blank were manually prepared in 2-mL vials using 1,000 µL graduated pipettes and a 25 µL pipetting syringe. Manual sample preparation was done by pipetting 1 mL each of three different jet fuel samples into individual 2-mL vials followed by addition of 10 µL of the internal standard. These samples contained known amounts of total FAME and were prepared in duplicate to determine overall repeatability. Each standard and sample was manually shaken to assure mixing.

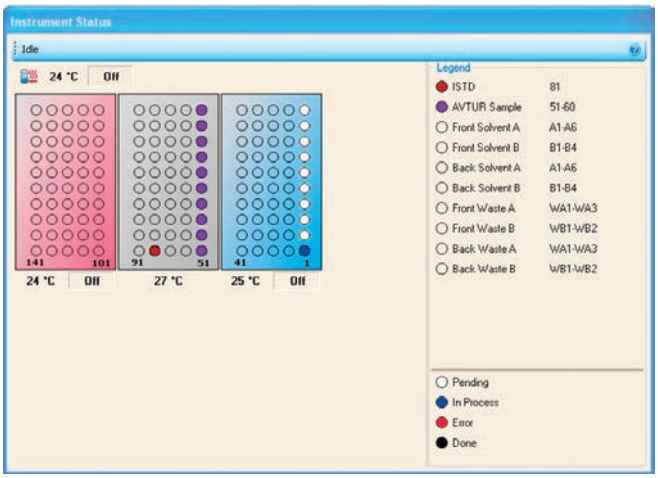

Figure 3. Agilent WorkBench resource layout for the automated preparation of 10 jet fuel samples. The empty vials in positions 1 to 10 will contain the final 100 mL of each jet fuel sample and internal standard after the automated preparation is complete.

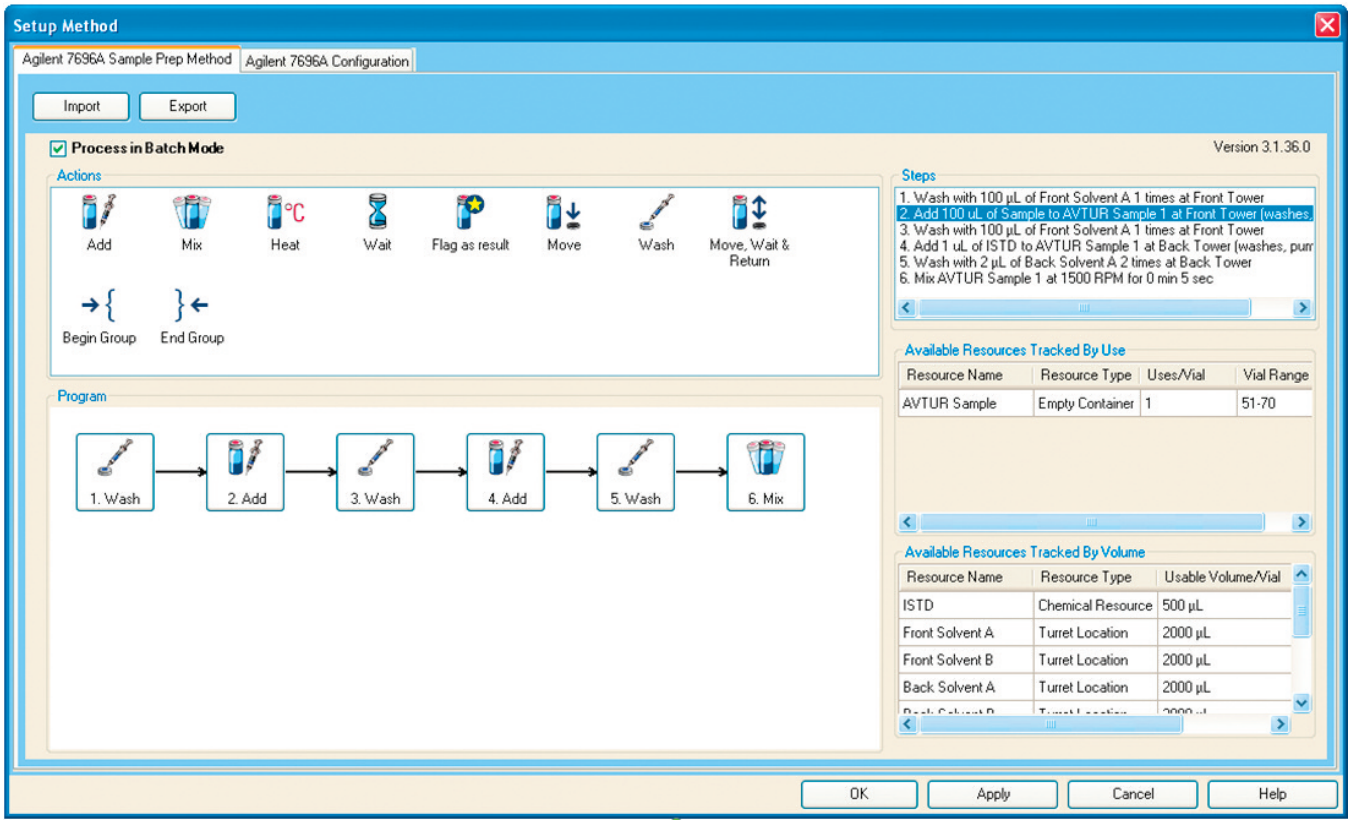

Figure 4. Batch mode Agilent WorkBench method for preparing 10 jet fuel samples. Each step was performed for all 10 samples before moving onto the next step. This efficient workflow minimized time and resource usage.

Automated Preparation of Calibration Standards and Jet Fuel Samples

The Agilent WorkBench was configured with a 250 µL syringe in the front tower and a 25 µL syringe in the rear tower. The 250 µL syringe used a draw speed of 500 µL/min and a dispense speed of 1000 µL/min. A draw speed of 100 µL/min and dispense speed of 500 µL/min was used for the 25 µL syringe. For each syringe, the dispense depth was set to 0 mm so the needle was close to the bottom of the vial when dispensing liquids. This ensured complete transfer of the liquid into the vial resulting in the best possible precision. High recovery vials were used because the internal v-shape allows the GC/MS autosampler to have access to the small 100 µL volumes of standards and samples.

The WorkBench sequence queue was used to prepare 5 low level standards and 5 high level standards using the IP585_Low.M and the IP585_High.M methods. After GC/MS calibration verification, the WorkBench batch mode method, IP585_Samples.M, was used to prepare duplicates of the three jet fuels samples spiked with different amounts of FAME.

GC/MS Analysis of FAME in Jet Fuel

An Agilent 5975C GC/MS system with an Agilent 7693A Automated Liquid Sampler was configured according to the IP585 method. This configuration is described in Table 7 and the instrument operating conditions are shown in Table 8. The mass spectrometer was tuned using the Agilent 5975C Autotune program before running any standards or samples. The calibration standards and the n-dodecane solvent blank were run first and the linear performance of the low level calibration and the high level calibration were evaluated before running the jet fuel samples. Upon successful calibration, a single GC/MS analysis of each jet fuel sample duplicate was made. The individual FAME peaks were quantified and the total FAME content in each sample was calculated by summing the individual FAME results.

Table 7. Instrument Configuration for GC/MS Analysis of FAMEs in Jet Fuel

Component	Description
Agilent 5975C Series MSD	Mass spectrometer with inert electron ionization source
Agilent 7890A GC system	Gas Chromatograph with 100 psi split/splitless inlet and mass spectrometer interface
Agilent 7693A ALS	Automatic liquid injector for Agilent 7890A GC with 150-vial tray
G1701EA	MSD Chemstation Software for data acquisition and analysis

Table 8. GC/MS Instrument Conditions

GC conditions	
Inlet temperature	260 °C
Inlet mode	Splitless
Inlet liner	Splitless liner, single taper glass wool (p/n 5062-3587)
Sample volume	1 uL
Column	HP-INNOWAX, 50 m x 0.2 mm, 0.4 µm film (p/n 19091N-205)
Column flow	Helium at 0.6 mL/min constant flow
Oven program	
Initial temperature	150 °C for 5 min
Oven ramp no 1	12 °C /min to 200 °C for 17 min
Oven ramp no 2	3 °C/min to 252 °C for 6.5 min
Mass spec interface	260 °C
Mass Spec Conditions	
Ionization source	70 eV electron ionization
Source temperature	230 °C
Quadrupole temperature	150 °C
Data acquisition delay	20 min

Results

Comparison of Manual and Agilent WorkBench Calibration Performance

The calibration standards from both the manual and the Agilent WorkBench preparations were run on the Agilent 5975C GC/MS system. The individual FAME calibration curves resulting from the low and high level WorkBench standards are shown in Figures 5 and 6. All of these curves appear to be linear after regression analyses with the origins forced through 0. Comparisons of the manual and WorkBench calibrations are shown in Table 9. For the low level calibrations, the slopes of the manual and WorkBench calibrations are very similar and the correlation coefficients (R^2) all meet the method requirement of greater than 0.985. The high level calibrations show the same performance with the exception of the methyl linoleate (C18:2) and methyl linolenate (C18:3) calibrations. In this case, the WorkBench prepared standards easily met the method requirements, while the manually prepared standards failed the linearity test. Therefore the manually prepared jet fuel samples could not be run until the high level standards were remade and the calibrations correctly verified. This added considerable time in obtaining results for the manually prepared samples. However, since the WorkBench calibrations were initially correct, the WorkBench prepared jet fuel samples could be run immediately.

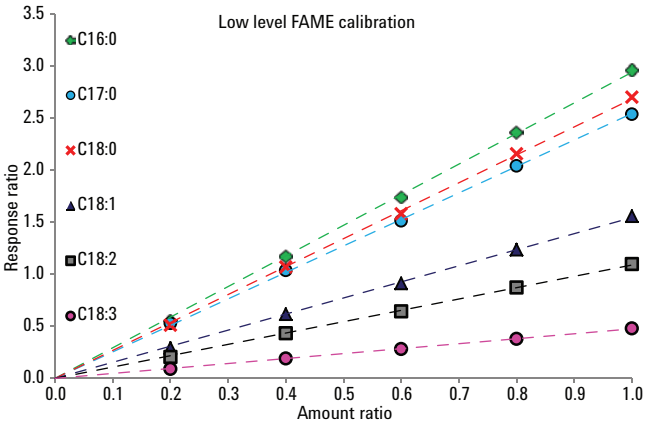

Figure 5. Low level calibration curves for 2, 4, 6, 8, and 10 mg/kg FAME standards prepared using the Agilent WorkBench. The calibration curves were forced through zero according to the method's protocol. Each curves exceeded the method's linearity requirement of $R^2 > 0.985$.

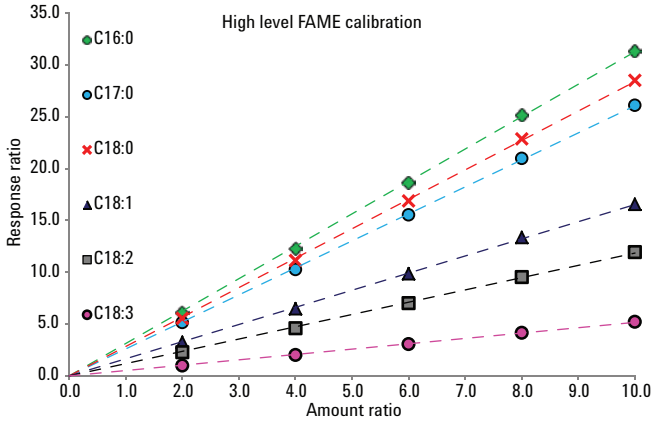

Figure 6. High level calibration curves for 20, 40, 60, 80, and 100 mg/kg FAME standards prepared using the Agilent WorkBench. The calibration curves were forced through zero according to the method's protocol. Each curve exceeded the method's linearity requirement of $R^2 > 0.985$.

Table 9. Comparison of the Slopes and Correlation Coefficients (R^2) Determined for Calibration Curves made using Manual and Agilent WorkBench Prepared Standards

Low Level Calibration (2–10 mg/kg)

	Slope		R^2	
FAME	**Manual**	**WorkBench**	**Manual**	**WorkBench**
C16:0	2.941	2.941	1.000	0.999
C17:0	2.441	2.544	1.000	1.000
C18:0	2.664	2.684	1.000	0.999
C18:1	1.539	1.545	1.000	0.999
C18:2	1.105	1.090	1.000	0.999
C18:3	0.478	0.475	1.000	0.999

High Level Calibration (20–100 mg/kg)

	Slope		R^2	
FAME	**Manual**	**WorkBench**	**Manual**	**WorkBench**
C16:0	4.962	3.127	0.985	1.000
C17:0	4.777	2.606	0.985	1.000
C18:0	4.815	2.840	0.985	1.000
C18:1	2.510	1.653	0.985	1.000
C18:2	1.713	1.184	0.984	0.999
C18:3	0.705	0.516	0.983	0.999

The manual high level calibrations curves for the C18:2 and C18:3 FAMEs failed the minimum R^2 requirement of 0.985.

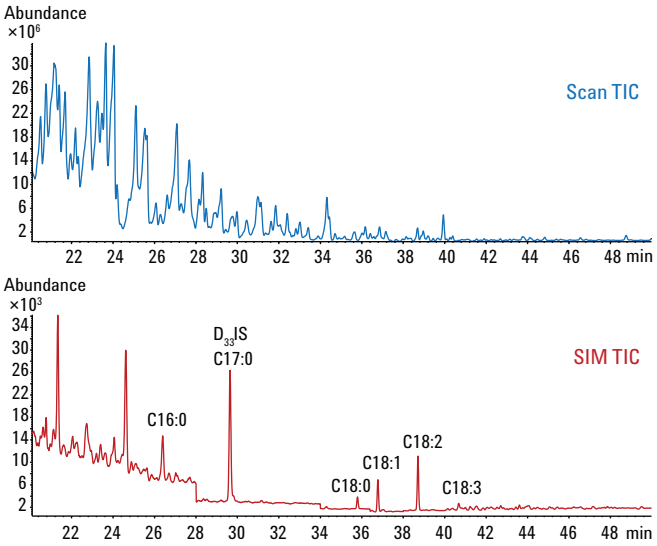

Figure 7. SIM/SCAN GC/MS data obtained from an Agilent WorkBench prepared jet fuel sample containing 5 mg/kg total FAME.

Comparison of Manual and Agilent WorkBench Sample Preparation

A typical GC/MS SIM/SCAN chromatogram for a jet fuel FAME analysis is shown in Figure 7. Comparisons of the analysis results for the manually prepared and the Agilent WorkBench prepared jet fuels are shown in Tables 10, 11, and 12. For each sample duplicate, repeatability (r) was calculated for the total FAME content and compared to the specification published in the IP585 method. Repeatability is a measurement of precision calculated by taking the difference between two duplicate results obtained on the same sample, by the same operator, using the same instrument, on the same day. For the 5 mg/kg FAME spike (Table 11), the repeatability of the manually prepared samples does not meet the IP585 method specification. Therefore, this result is invalid. However, for all WorkBench samples, the repeatabilities were much better than the method's specifications. Additionally, the results obtained with the Workbench samples more closely matched the total FAME content spiked into the jet fuel samples.

Table 10. Comparison of Analysis Results from a Manual and Agilent WorkBench Samples Preps for a 1 mg/kg FAME Jet Fuel Spike

1 mg/kg Jet fuel spike - Manual prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	0.8	0.0	0.1	0.3	0.1	0.0	1.3
Run 2	0.8	0.0	0.1	0.3	0.1	0.0	1.3
						Avg	1.3
						r (calc)	0.0
						r (IP585)	0.7

1 mg/kg Jet fuel spike - Agilent WorkBench prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	0.8	0.0	0.1	0.3	0.1	0.0	1.3
Run 2	0.7	0.0	0.1	0.3	0.1	0.0	1.2
						Avg	1.3
						r (calc)	0.1
						r (IP585)	0.7

Table 11. Comparison of Analysis Results from a Manual and Agilent WorkBench Samples Preps for a 5 mg/kg FAME Jet Fuel Spike

5 mg/kg Jet fuel spike - Manual prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	1.1	0.0	0.3	0.4	3.8	1.2	6.8
Run 2	0.5	0.0	0.2	0.9	2.6	0.7	4.9
						Avg	5.9
						r (calc)	1.9
						r (IP585)	1.4

5 mg/kg Jet fuel spike - Agilent WorkBench prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	0.5	0.0	0.1	0.9	2.7	0.5	4.7
Run 2	0.6	0.0	0.2	0.9	2.7	0.6	5.0
						Avg	4.9
						r (calc)	0.3
						r (IP585)	1.3

Table 12. Comparison of Analysis Results from a Manual and Agilent WorkBench Samples Preps for a 40 mg/kg FAME Jet Fuel Spike

40 mg/kg Jet fuel spike - Manual prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	4.4	0.0	1.7	7.9	24.0	4.1	42.1
Run 2	4.7	0.0	1.8	8.3	25.1	4.3	44.2
						Avg	43.1
						r (calc)	2.1
						r (IP585)	7.5

40 mg/kg Jet fuel spike - Agilent WorkBench prep

	C16:0	C17:0	C18:0	C18:1	C18:2	C18:3	Total
Run 1	4.8	0.0	1.8	8.3	25.4	4.2	41.4
Run 2	4.3	0.0	1.7	7.9	24.0	4.1	39.1
						Avg	40.2
						r (calc)	2.3
						r (IP585)	7.1

Conclusion

The Agilent WorkBench was shown to successfully automate the preparation of the calibration standards and samples when measuring FAME in jet fuel using the IP585 GC/MS method. By comparison, it was also shown that good analysis results can be difficult to obtain when using manual preparation techniques that require precise handling very small amounts of samples and reagents. This application note has demonstrated that the WorkBench can achieve better overall method performance compared to manual preparation. Considerable time was saved in avoiding rework and 10 times less reagents used with the WorkBench.

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Agilent 7696A Sample Prep WorkBench Automated Sample Preparation for the GC Analysis of Biodiesel Using Method EN14105:2011

Application Note

Fuels

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Abstract

The recently revised European Union method EN14105 describes complex, multistep procedures to manually prepare standards and samples for the GC analysis of glycerol contaminants in B100 biodiesel. The Agilent 7696A Sample Prep WorkBench was successfully used to automate the standard and sample prep of this method while reducing the reagent use and chemical wastes by a factor of 10. Calibration performance of the WorkBench prepared standards exceeded the method requirements. Using a commercial biodiesel sample, the WorkBench was shown to prepare the samples with an extremely high degree of precision that surpassed the method's specifications.

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Introduction

In countries adhering to European Union norms, B100 biodiesel quality is assured by measuring the amount of free and total glycerol and the mono-, di-, and triglycerides contained in the fuel. A gas chromatography (GC) method, EN14105, was developed to separate and quantify these compounds. Since glycerol, mono-, and diglycerides are not volatile, the method outlines a complex procedure to derivatize these compounds and create volatile silanized species prior to GC analysis. In 2011, the European Committee for Standardization (CEN) updated this method to improve GC performance, glyceride quantification, and overall precision [1]. This application note describes using the Agilent 7696A Sample Prep WorkBench to automate the preparation of calibration standards and samples for analysis with the Agilent 7890A Series GC.

The WorkBench is a standalone instrument specifically designed to perform automated sample preparation. It uses two Agilent 7693A injection towers to volumetrically transfer liquids between 2-mL vials. Vials containing various chemical resources, standards, and samples are housed in three 50-positions trays. The sample tray compartment contains a robotic arm, a vortex mixing station, and a sample heating station. For biodiesel analysis, the WorkBench was used to successfully prepare samples for ASTM method D6584, which is similar to the EN14105 method [2]. In that application note, the analysis results from WorkBench prepared samples were identical to results obtained with manually prepared samples.

The Agilent WorkBench Easy SamplePrep (ESP) software was recently updated to provide more efficient use of chemical resources and time. At its core, ESP provides a simple software platform allowing users to quickly build sample preparation methods using drag-and-drop icons representing each WorkBench action. A new mode of ESP operation called **Batch Mode** allows the WorkBench to repeat common actions for all samples before moving on to the next action. For methods where Batch Mode can be used, significant increases in solvent wash and waste capacity can be realized along with faster sample preparation times [3,4].

Experimental

WorkBench Preparation of EN14105 Calibration Standards

The WorkBench was configured with a Blue Line 25 µL gas tight syringe (p/n G4513-80241) in the rear tower and a Blue Line 500 µL gas tight syringe (p/n G4513-60561) in the front tower. The chemical resources used to prepare standards and samples are listed in Table 1. The three reference glycerides used to prepare the Standard Glycerides Solution were purchased as pure compounds from Nu-Chek Prep (www.nu-chekprep.com). Each chemical resource was placed into separate 2-mL high recovery vials (p/n 5183-2030) and sealed using screw caps with PTFE lined septa (p/n 5040-4682).

Table 1. Chemical Resources and Standards used for Method EN14105:2011

Resource	Description	Supplier
Heptane	Capillary GC grade	Sigma Aldrich p/n H9629
Glycerol stock	0.5 mg/mL in pyridine	Sigma Aldrich p/n 44892-U
Butanetriol solution	1 mg/mL in pyridine	p/n 5982-0024
MSTFA	Silanizing reagent	p/n 5190-1407
Std glycerides solution	2.5 mg/mL in THF	Nu-Chek Prep
Monoglycerides RT std	10 mg/mL in pryridine	p/n 5190-1410
Pyridine	Anhydrous grade	Sigma Aldrich p/n 270970

Using the Agilent ESP software, the chemical resources were arranged in the WorkBench and assigned initial properties. This resource layout is described in Table 2 and graphically shown in Figure 1.

Table 2. Agilent WorkBench Chemical Resources used to Prepare Standards and Samples as Shown in Figure 1

Resource name	Resource type	Use type	Capacity (µL)	Vial range
Heptane	Chemical resource	By volume	1,000	81–95
Glycerol stock	Chemical resource	By volume	1,000	61
Butanetriol solution	Chemical resource	By volume	1,000	62
MSTFA	Chemical resource	By volume	1,000	63
Std glycerides solution	Chemical resource	By volume	1,000	64
Monoglycerides RT std	Chemical resource	By volume	1,000	65
Pyridine	Chemical resource	By volume	500	71
Empty vials				51–55

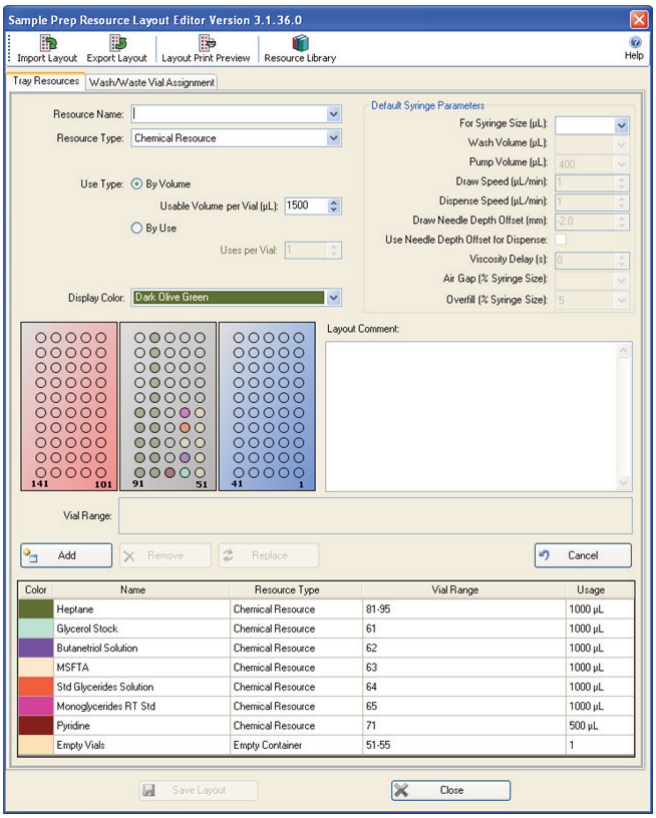

Figure 1. Easy Sample Prep (ESP) software layout for preparing standards and samples using method EN14105.

The EN14105 method requires the preparation of five calibration standards using a linear dilution technique. Four standards contain different amounts of glycerol and the same amount of the internal standard 1,2,3-butanetriol. The fifth calibration standard contains three monoglycerides used to identify these compounds in biodiesel by retention time comparison. The EN14105 method outlines the steps used to prepare approximately 10 mL of each calibration standard. Since the WorkBench uses 2-mL vials, automating the method required a volume reduction by a factor of 10 [2]. Table 3 describes the 37 individual steps used to prepare these five calibration standards. Since this is a linear dilution technique, the ESP Batch Mode was not used for standard preparation (Figure 2). It is important to note that a Needle Depth Offset of 0 was used in combination with the high recovery vials to assure complete mixing of the small volumes needed to prepare these standards. Additionally a 5% Overfill was used when dispensing each resource to eliminate any potential errors causes by bubble formation in the syringe.

WorkBench Preparation of B100 Biodiesel Samples for EN14105

The EN14105 method calls for weighing 100 mg of biodiesel sample into a reaction vial for silation. Since the WorkBench sample prep scale was reduced by a factor of 10, only 10 mg of sample was weighed into 2-mL high recovery vials. Automatic sample weighing cannot be performed using the WorkBench because there is no analytical balance. Since weighing 10 mg of biodiesel can be very challenging, an Eppendorf Reference Adjustable-Volume Pipettor (10–100 µL) was used to transfer the sample. Weighing 10 mg of biodiesel was done by manually pipetting 11.5 µL of biodiesel into tared 2-mL high recovery vials and recording the weight to the nearest 0.01 mg.

Table 3. WorkBench Method used to Prepare Calibration Standards for Method EN14105

Step	WorkBench action	Description	Syringe	Draw speed (µL/min)	Dispense speed (µL/min)	Needle depth offset (mm)	Viscosity delay (sec)	Overfill %
1	Wash	Syringe three times with 5 µL of butanetriol	25 µL	250	1,000		0	
2–6	Add	8 µL butanetriol to empty vials 1, 2, 3, 4, 5	25 µL	250	1,000	0	2	5
7	Wash	Syringe with wash solvent A	25 µL	250	1,000		0	
8	Wash	Syringe with 5 µL of glycerol stock	25 µL	250	1,000		0	
9	Add	1 µL glycerol stock to empty vial 1	25 µL	250	1,000	0	2	5
10	Add	4 µL glycerol stock to empty vial 2	25 µL	250	1,000	0	2	5
11	Add	7 µL glycerol stock to empty vial 3	25 µL	250	1,000	0	2	5
12	Add	10 µL glycerol stock to empty vial 4	25 µL	250	1,000	0	2	5
13	Add	5 µL monoglyceride RT std to empty vial 5	25 µL	250	1,000	0	2	5
14	Add	20 µL std glycerides to empty vial 5	25 µL	250	1,000	0	2	5
15	Add	20 µL of pyridine to empty vial 5	25 µL	250	1,000	0	2	5
16	Wash	Syringe three times with wash solvent A	25 µL	250	1,000		0	
17–21	Add	15 µL of MSTFA to empty vials 1, 2, 3, 4, 5	25 µL	250	1,000	0	2	5
22–26	Mix	Empty vials 1, 2, 3, 4, 5 at 2,500 RPM for 15 sec						
27	Wait	15 minutes						
28–32	Add	800 µL heptane to empty vials 1, 2, 3, 4, 5	500 µL	1,250	5,000	0	2	5
33–37	Mix	Empty vials 1, 2, 3, 4, 5 at 2,500 RPM for 15 sec						

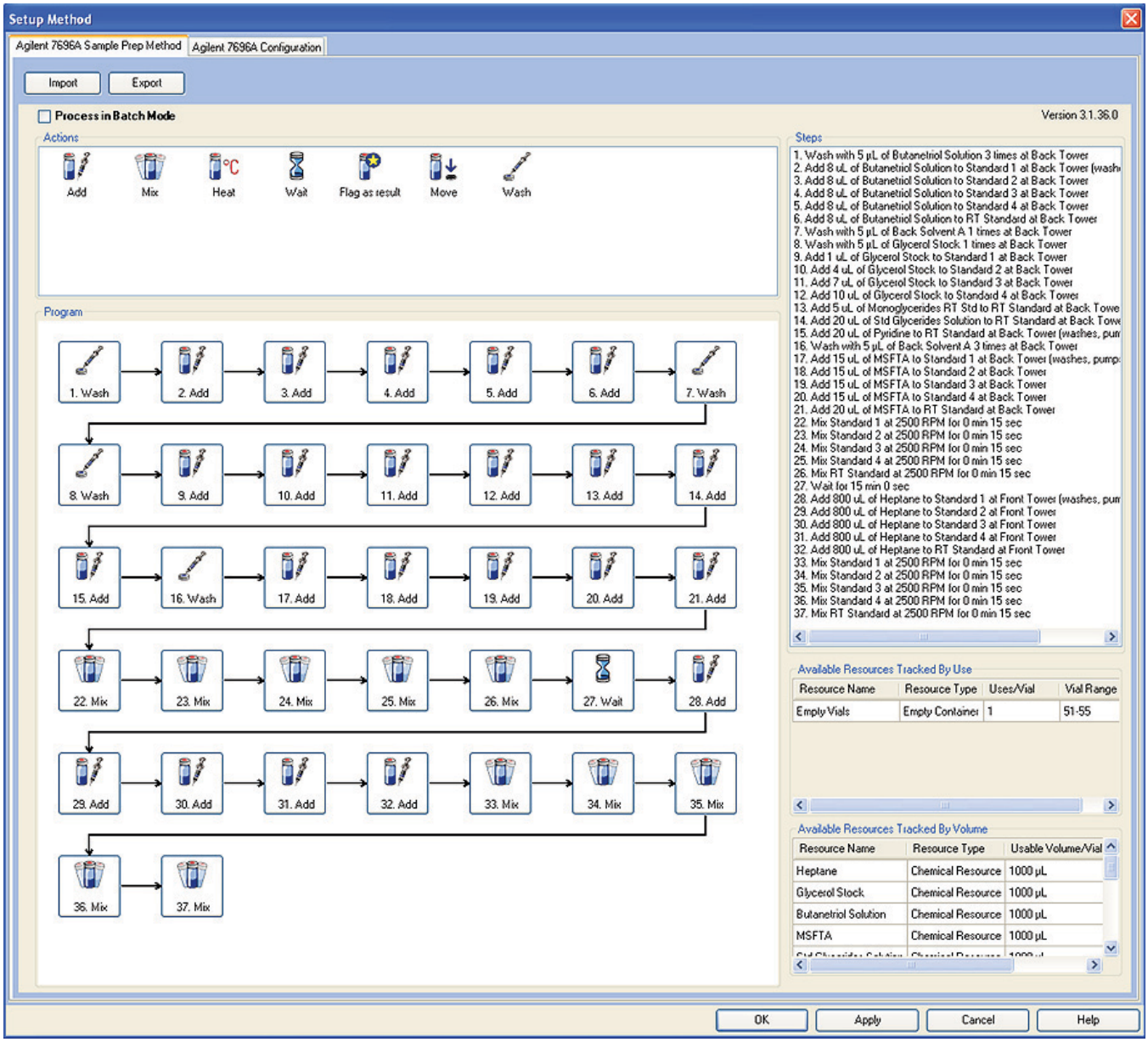

Figure 2. Easy Sample Prep (ESP) software method used to prepare calibration standards for method EN14105.

Sample preparation for the EN14105 method is performed by adding fixed volumes of the butanetriol stock, the standard glycerides stock, pyridine, and MSTFA to the sample to derivatize the non-volatile components. After the 15 minutes, heptane is added to the mix to quench the reaction. Since 2-mL vials were used for the WorkBench, the volumes of each added reagent was reduced by a factor of 10. The individual steps for this sample preparation are listed in Table 4. The ESP software was used to create a Batch Mode method for the sample prep while saving time and resources. This Batch Mode method is shown in Figure 3.

Since both the standards preparation and sample preparation use the same resource layout, the WorkBench can run both methods together using an ESP software Sequence Queue. For this application note, 10 duplicates of a soybean oil derived B100 biodiesel were prepared to evaluate the precision of the WorkBench sample prep.

Table 4. Ten Individual Steps used by the WorkBench to Prepare Biodiesel Samples for Method EN14105

Step	WorkBench action	Description	Syringe	Draw speed (µL/min)	Dispense speed (µL/min)	Needle depth offset (mm)	Viscosity delay (sec)	Overfill %
1	Wash	Syringe three times with 5 µL of butanetriol	25 µL	250	1,000		0	
2	Add	20 µL of pyridine to each sample	25 µL	250	1,000	0	2	5
3	Add	8 µL butanetriol to each sample	25 µL	250	1,000	0	2	5
4	Add	20 µL std glycerides to each sample	25 µL	250	1,000	0	2	5
5	Add	20 µL of MSTFA to each sample	25 µL	250	1,000	0	2	5
6	Mix	Each sample at 2,500 PRPM for 15 sec						
7	Wait	15 minutes						
8	Wash	Syringe one time with 200 µL of wash solvent A	25 µL	250	1,000		0	
9	Add	800 µL heptane to each sample	500 µL	1,250	5,000	0	2	5
10	Mix	Each sample at 2,500 RPM for 15 sec						

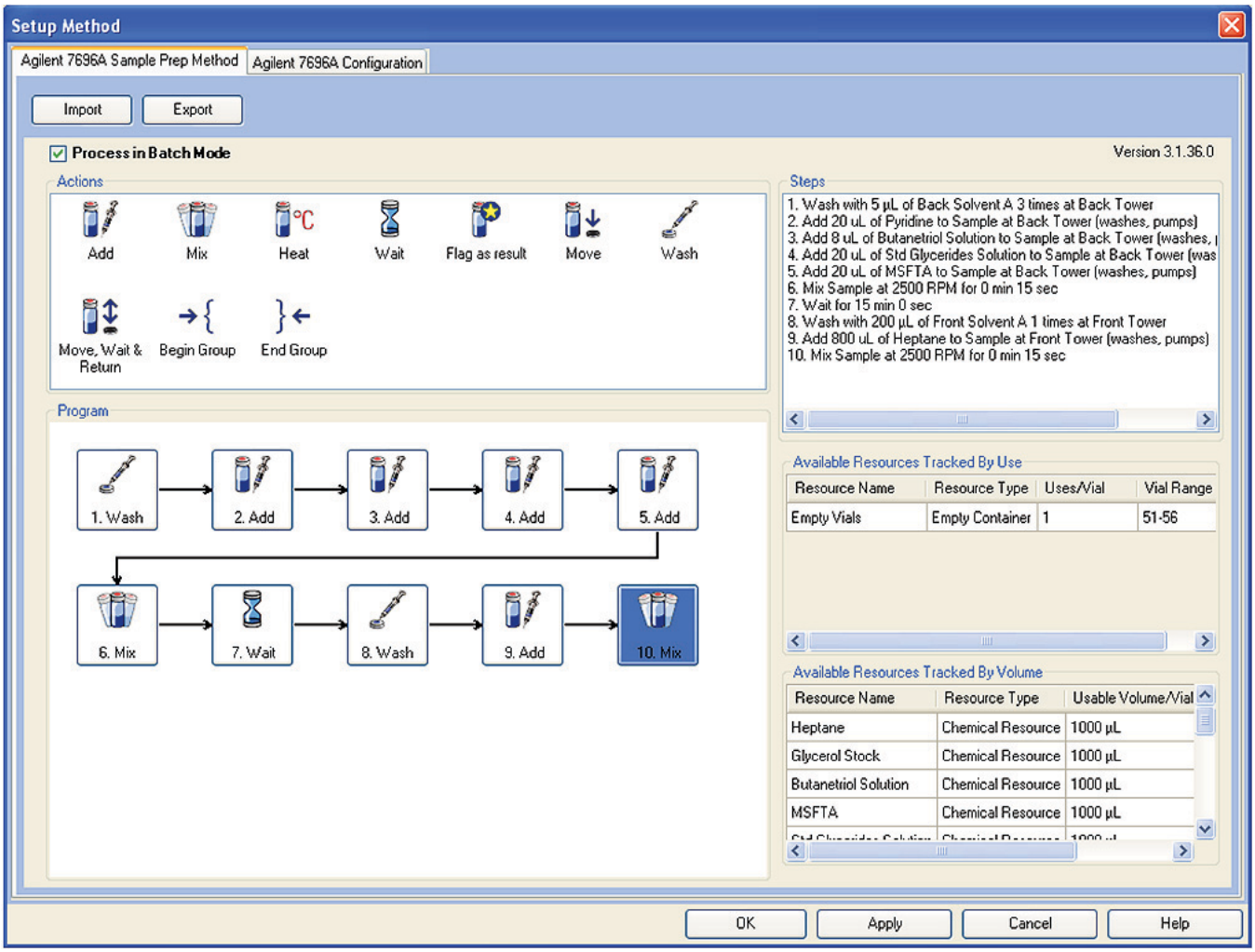

*Figure 3. Easy Sample Prep (ESP) software **Batch Mode** method used to prepare biodiesel samples for EN14105.*

GC Analysis of WorkBench Prepared Standards and Samples

An Agilent 7890A Gas Chromatograph (GC) was configured to comply with the EN14105:2011 requirements. Table 5 lists the instrument configuration and the instrument operating conditions. A single, 1-µL injection of each standard and each sample was made on this system. The Agilent OpenLab CDS Chemstation was used to control the 7890A GC, collect the data, and perform data analysis.

Table 5. Agilent 7890A GC Configuration and Operating Conditions for the Analysis of WorkBench Prepared Standards and Samples using Method EN14105:2011

Instrument configuration	
G3440A	Agilent 7890A Series GC
Option 122	Cool-on-column Inlet with EPC control
Option 211	Capillary FID with EPC control
G4513A	Agilent 7693A ALS
Column	Select Biodiesel for Glycerides
	15 m × 0.32 mm, 0.1 µm film (p/n cp9078)
Data system	Agilent OpenLab CDS Chemstation C.01.03

GC operating conditions	
Cool-on-column inlet	
Initial pressure	Helium at 11.353 psi
Initial temperature	50 °C
Temperature program	Oven track mode
Column flow	Helium at 5 mL/min constant flow
Column temperature	
Initial	50 °C for 1 min
Rate 1	15 °C/min to 180 °C, hold 0 min
Rate 2	7 °C/min to 230 °C, hold 0 min
Rate 3	10 °C/min to 370 °C, hold 10 min
Flame ionization detector	380 °C

Results and Discussion

WorkBench Prepared EN14105 Standards

The retention times of the three monoglycerides and the standard glycerides were determined using the data obtained from the retention time standard. This chromatogram is shown in Figure 4. A glycerol calibration curve was prepared using the data obtained from the four glycerol calibration standards. This curve is shown in Figure 5. The correlation coefficient for this curve was 1.000 which meets the EN14105 method requirement of 0.9.

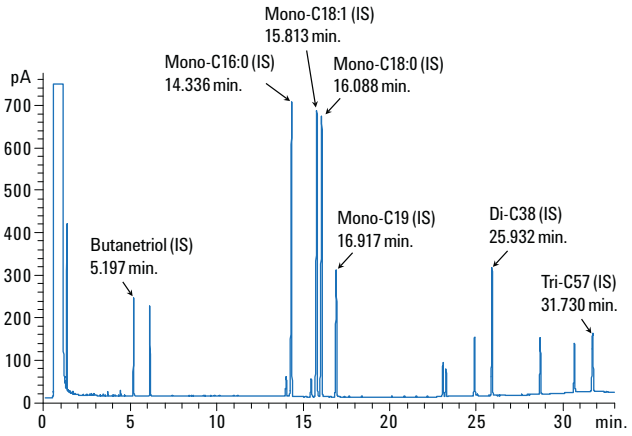

Figure 4. Retention time identification standard prepared using the WorkBench. In addition to the three monoglycerides, the four internal standards (Butanetriol, Mono-C19, Di-C38 and Tri-C57) were also added to this mix.

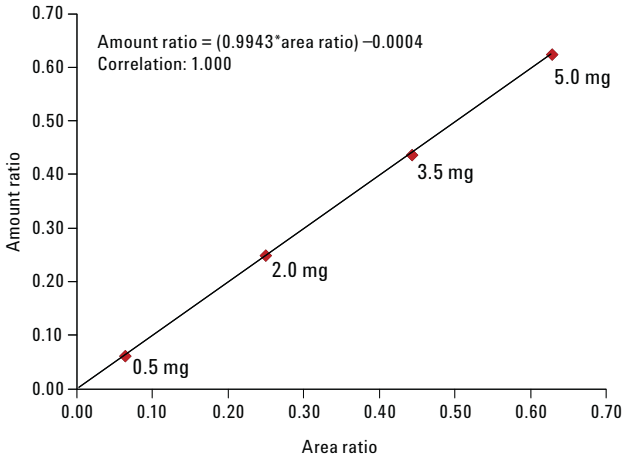

Figure 5. Glycerol calibration curve made using the data from four WorkBench prepared calibration standards. The correlation coefficient exceeds a value of 0.9 as required by the EN14105 method.

WorkBench Prepared B100 Biodiesel Samples

Figure 6 shows a chromatogram of a single sample compared to an overlay of the 10 WorkBench prepared samples. The 10 overlaid chromatograms are nearly identical to the single chromatogram in both retention time and peak response. This result graphically illustrates the WorkBench ability to prepare each sample with precision. Figure 7 shows the four quantification zones in greater detail. Again, these chromatograms are overlays of the 10 WorkBench prepared biodiesel samples and show nearly identical results. In the glycerol and the monoglyceride zones, only the identified peaks are quantified and reported. In the di- and triglyceride zones, any peaks eluting in the respective zone is quantified and reported as a diglyceride or triglyceride.

Before one can determine the final results, a column performance control must be calculated for the analysis. This control is measured by calculating the relative response factors (RRF) of the Di-C38 internal standard versus the Tri-C57 internal standard. The RRF must be lower than 1.8 to be certain of good triglyceride detection. This column performance control was passed for each WorkBench prepared sample as shown in Table 6.

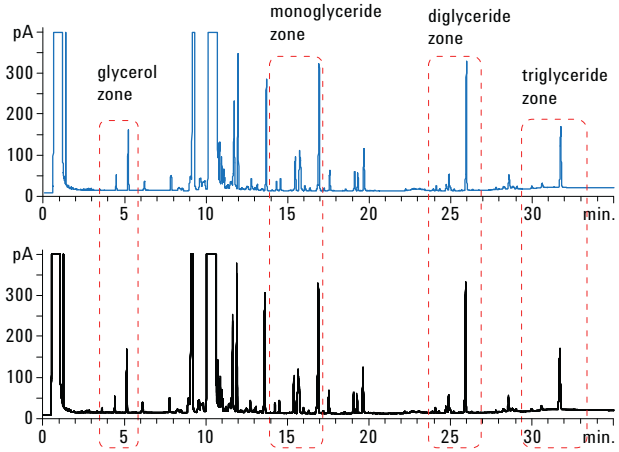

Figure 6. The upper chromatogram is a single run of a B100 sample prepared using the Agilent WorkBench. Each zone for quantification of glycerol and glycerides is outlined in red. The lower chromatogram is an overlay of 10 separate samples prepared using the WorkBench.

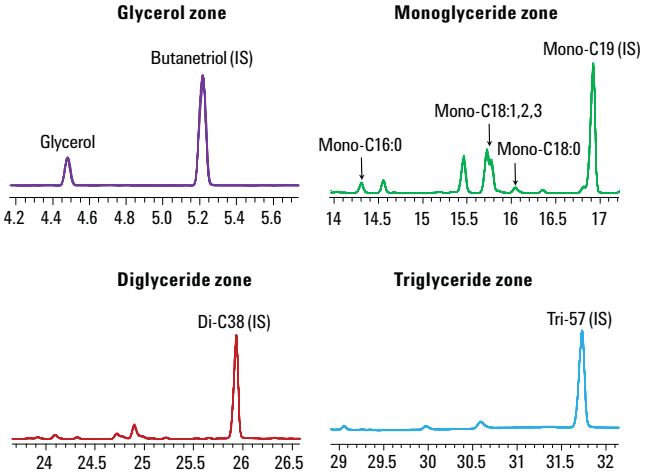

Figure 7. Expanded views of the four quantification zones identified in Figure 5. Note that these chromatograms are overlays of 10 separate samples prepared using the Agilent WorkBench.

Table 6. Column Performance Control Parameters

Sample	A_{DiC38}/M_{DiC38}	A_{TriC57}/M_{TriC57}	RRF
SRM01	24.4	16.5	1.5
SRM02	24.4	16.4	1.5
SRM03	24.4	16.4	1.5
SRM04	24.4	16.4	1.5
SRM05	24.5	16.5	1.5
SRM06	24.6	16.5	1.5
SRM07	24.5	16.0	1.5
SRM08	24.9	16.0	1.6
SRM09	24.9	16.0	1.6
SRM10	25.0	16.2	1.5

As a column performance control, the relative response factor (RRF) for the Di-C38 versus Tri-C57 internal standards must be less than 1.8. All 10 WorkBench prepared biodiesel samples meet this requirement. (A = peak area, M = compound mass)

With the glycerol calibration and column performance control criteria met, the contents of free glycerol, mono-, di-, triglycerides and total glycerol were determined for the 10 WorkBench prepared biodiesel samples. These results are shown in Table 7. The precision for these 10 results was excellent as measured by the low RSDs calculated for each component. However, the EN14105:2011 method does provide a complete statement for both single user and multiple lab precision. For this application note, single user precision can be determined from the results and compared to the method's criteria.

Single user precision is also known as repeatability (r). Repeatability is the difference between two test results obtained by the same operator using the same equipment on identical test material. The EN14105 method provides repeatability statements for each component measured in the sample. To use this statement, the two results with the largest difference, SRM01 and SRM10, were used. The absolute value of the difference for each sample's results was taken and compared to the minimum difference required by the method. As shown in Table 8, samples prepared using the WorkBech comfortably meet the method's repeatability specifications for all quantified components in biodiesel.

Table 7. Results for the Analysis of Ten B100 Biodiesel Prepared using the Agilent WorkBench

		Weight %				
Sample	**Sample weight (mg)**	**Free glycerol**	**Monoglycerides**	**Diglycerides**	**Triglycerides**	**Total glycerol**
SRM01	10.90	0.016	0.39	0.14	0.19	0.156
SRM02	10.40	0.017	0.39	0.14	0.19	0.157
SRM03	10.63	0.017	0.39	0.14	0.19	0.157
SRM04	9.59	0.017	0.39	0.14	0.19	0.157
SRM05	11.12	0.017	0.39	0.14	0.19	0.157
SRM06	9.93	0.017	0.39	0.14	0.19	0.157
SRM07	10.46	0.017	0.39	0.14	0.19	0.157
SRM08	9.66	0.017	0.39	0.14	0.19	0.157
SRM09	9.74	0.017	0.39	0.14	0.19	0.157
SRM10	10.01	0.017	0.39	0.14	0.19	0.157
	Avg	**0.017**	**0.39**	**0.14**	**0.19**	**0.157**
	Std Dev	**0.000**	**0.00**	**0.00**	**0.00**	**0.000**
	RSD	**1.871%**	**0.00%**	**0.00%**	**0.00%**	**0.202%**

Table 8. Analysis Precision as Expressed by Repeatability (r) for two B100 Biodiesel Samples Prepared using the Agilent WorkBench. The Repeatability for Each Component (r calc) Meets the Specification of the EN14105:2011 Method (r spec)

	Weight %				
Sample	**Free glycerol**	**Monoglycerides**	**Diglycerides**	**Triglycerides**	**Total glycerol**
SRM01	0.016	0.39	0.14	0.19	0.156
SRM10	0.017	0.39	0.14	0.19	0.157
r calc	**0.001**	**0.00**	**0.00**	**0.00**	**0.001**
r spec	**0.003**	**0.04**	**0.02**	**0.02**	**0.020**

Conclusion

The Agilent 7696A WorkBench is shown to have successfully performed an automated preparation of standards and samples for the GC analysis of glycerol contaminants in biodiesel according to the revised European Union method EN14105:2011. Since the WorkBench uses 2-mL vials, the scale of the EN14105 preparation was reduced by a factor of 10. This served to lower reagent costs and reduced the generation of waste chemicals when performing this analysis. Calibration standards prepared with the WorkBench met all performance criteria set forth by the method. Ten duplicates of a biodiesel sample were prepared using the WorkBench and the resulting GC analysis showed extremely high precision that exceeded the requirement of the EN14105 method.

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ENVIRONMENTAL

Reproducible dilutions and automated final clean-up to exceed method QC requirements

The number of tasks and samples to report in an environmental laboratory can make it easy to miss something during a simple procedure of sample preparation. Automation can eliminate simple errors like dilution calibration curves, adding internal standards, or adding surrogates. In some cases final sample clean-up of oils can also be automated. When these mundane tasks are unloaded from the analysts workload, that analyst can become more productive, focusing in the more important tasks in the lab.

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ENVIRONMENTAL

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Automated Clean-up for Mineral Oil (Hydrocarbon Oil Index) Analysis using the Agilent 7696A Sample Prep WorkBench

Application Note

Automated Sample Preparation

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Abstract

Mineral oil (or Hydrocarbon oil) in water samples is determined by liquid-liquid extraction, followed by a clean-up step on Florisil, and GC-FID analysis. Using the Agilent 7696A Sample Prep WorkBench system, drying and clean-up of samples obtained after liquid-liquid extraction can be automated. High recoveries and excellent reproducibility are obtained for the mineral oil fraction, while the clean-up step is very efficient. The extracts are analysed by GC-FID. In combination with low thermal mass (LTM) – GC, an automated high throughput method is obtained.

Agilent Technologies

Introduction

Environmental contamination by hydrocarbon fractions, such as diesel or motor oil, is measured using GC-FID. This method, also called hydrocarbon oil index (HOI), mineral oil or total petroleum hydrocarbon (TPH) determination is one of the most important applications in environmental analysis. For water samples, liquid-liquid extraction (LLE) with an apolar (hydrocarbon) solvent with a boiling point between 36 °C and 69 °C (for example, hexane) is the first step in sample preparation. Next, the extract is dried over sodium sulphate and cleaned by passing over Florisil to remove more polar, co-extracted solutes such as lipids. The extract is then concentrated by N_2 blowdown (or Kuderna-Danish) and analyzed by GC-FID [1].

The sample clean-up procedure can be minimized using dispersive SPE instead of classical column chromatography or solid phase extraction. In d-SPE, a small amount of adsorbent is added to the sample. The matrix solutes (in this case: more polar solutes that are co-extracted in LLE) bind to the adsorbent and are removed from the solution. The purified extract is analyzed by GC-FID. For efficient clean-up, the extract needs to be dried on Florisil before d-SPE.

The mineral oil extract is then analyzed by GC-FID. Normally, a 10 – 30 m column with low film thickness is used. Typically the analysis time is about 30 min. Recently, it was demonstrated that sample throughput can be significantly increased by applying low thermal mass (LTM) technology [2].

In this application note, the automation of the drying and dispersive SPE steps of the hydrocarbon oil index method on the Agilent 7696 Sample Prep WorkBench is described. Prior to the WorkBench method, the extract obtained by LLE is concentrated (for example, by rotavapor or nitrogen blow-down) to about 1–1.5 mL. This fraction is then dried on sodium sulphate and cleaned on Florisil using autosampler vials, preloaded with the adsorbents. The final extract is analysed by fast GC-FID using LTM technology

Experimental

Chemicals and test solutions

A 1:1 mixture of diesel and motor oil (5,000 µg/mL each in cyclohexane) was used as mineral oil test sample. An alkane standard containing even numbered n-alkanes from C10 to C40 (50 µg/mL each in hexane) was used for repeatability testing and GC-FID calibration.

Stearyl stearate was used for checking the performance of the clean-up procedure, as described in ISO 9377 method. A stock solution of 2,000 µg/mL in acetone was prepared.

From these stock solutions, a calibration solution containing 5 ng/µL of each n-alkanes and and 80 ng/µL stearyl stearate was prepared in hexane. Also, a calibration solution containing 400 ng/µL mineral oil and 80 ng/µL stearyl stearate was prepared in hexane. These solutions were used for direct injection.

Two spiking solutions were prepared in acetone. These solutions were then spiked into 900-mL water samples and extraction was performed with 50 mL hexane. The hexane fraction was concentrated under nitrogen using a Turbovap system to 10 mL. This extract was divided over several vials for repeatability testing of the 7696 WorkBench method. The first spiking solution contained 50 µg per n-alkane and 800 µg stearyl stearate. The second spiking solution contained 4,000 µg mineral oil and 800 µg stearyl stearate. Assuming 100% recovery in liquid-liquid extraction, the concentrations in the 10-mL hexane extract are identical to the calibration solutions.

Drying of the extract (in hexane) is done on anhydrous sodium sulphate. The clean-up adsorbent was Florisil. Both Na_2SO_4 and Florisil were first heated overnight at 140 °C.

First, 20 mg (± 2 mg) sodium sulphate was added to a series of 1.5-mL high recovery vials. These vials are labeled Dry. To a second series of 1.5-mL high recovery vials, 30 mg (± 2 mg) Florisil was added. These vials are labeled Clean-up. All vials were closed with screw caps to protect them from humidity.

Sample Preparation

The hexane extracts of water samples are placed in 1.5-mL high recovery vials (samples). A series of 1.5-mL high recovery vials containing 20 mg dried sodium sulphate (Dry) and 1.5-mL high recovery vials containing 30 mg Florisil (Clean-up) are also positioned in the racks, together with empty vials with a 200-µL insert (for final extract).

The front tower was configured with a 500 µL syringe, the back tower with a 250 µL syringe. Syringe rinsing is done with hexane.

An example of the resource lay-out of the 7696 WorkBench is shown in Figure 1.

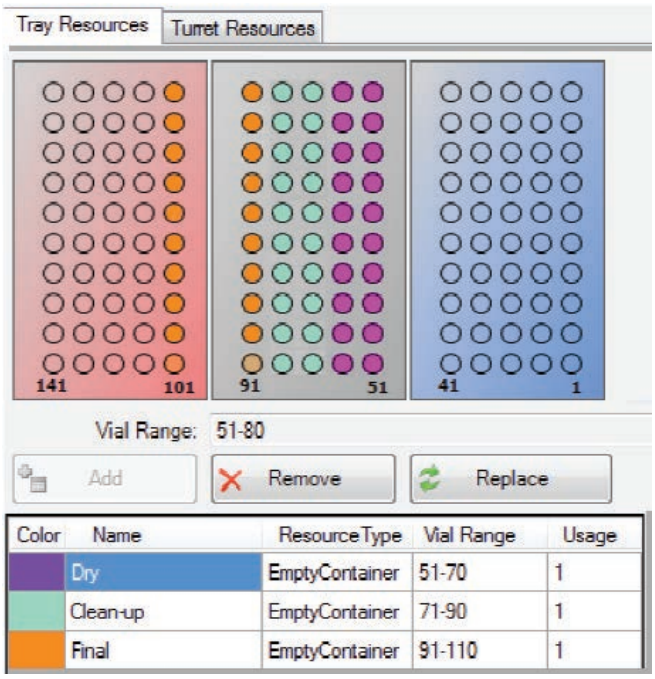

Figure 1. Resource lay-out for an Agilent 7696 Sample Prep WorkBench.

The Workbench method can be summarized as follows (command in bold, comment in italic):

1. **Add 500 µL of Sample to Dry at Front Tower**
   (Aliquot of extract is added to sodium sulphate)
2. **Mix Dry for 1 min (2,000 rpm, bidirectional, 4 s on, 1 s off)**
   (Mixing allows good contact between sample and adsorbent, removal of water)
3. **Add 350 µL of Dry to Clean-up at Back Tower**
   (Aliquot of dried extract is added to Florisil)
4. **Mix Clean-up for 1 min (2,000 rpm, bidirectional, 4 s on, 1 s off)**
   (Mixing allows good contact between sample and adsorbent, removal of polars)
5. **Add 150 µL of Clean-up to Final at Back Tower**
   (Transfer of cleaned extract to vial for further analysis by GC-FID)
6. **Flag Final as Result**

Instrumental Configuration

The analyses were performed on an Agilent 7890 GC system. The GC was equipped with a SSI inlet, an LTM II oven door and FID detection. Separation was done on a 0.32 mm × 10 m, 0.10 µm DB-5HT column (p/n 123-5701LTM).

The analytical conditions are summarized in Table 1 (see also [2]).

Table 1. Analytical Conditions

Injection	1 µL, splitless (0.4 min purge delay) 350 °C split/splitless liner (p/n 5183-4647)
Carrier	Helium, 9 mL/min constant flow
GC oven temp	340 °C isothermal
LTM	40 °C (0.5 min) – 200 °C/min –240 °C –100 °C/min –340 °C (0.5 min) Analysis time: 3 min
FID	340 °C, 40 mL/min H_2, 400 mL/min air

Results and Discussion

The first step in the determination of mineral oil in water samples is liquid-liquid extraction. Typically, a large sample (900 mL) is extracted with 50 mL solvent (hexane). This step cannot be automated and is still performed in a classical way. The obtained extract is concentrated (for example, under nitrogen using a Turbovap system, Zymark). The concentrated sample (1–2 mL) is transferred to a 2-mL autosampler vial and the remaining of the procedure is handled by the 7696 Sample Prep WorkBench.

The sample preparation procedure performed on the Agilent 7696 Sample Prep WorkBench is illustrated in Figure 2. In the first step, an aliquot of the hexane extract is dried over sodium sulphate. This step is important because traces of water reduce the performance of the next clean-up. An aliquot of the dried extract is then transferred to a vial containing Florisil. It was observed that the Florisil adsorbent needs to be activated at 140 °C to remove residual water. Also, tests were performed to verify the minimum quantity of Florisil needed. Clean-up performance was not sufficient when less than 20 mg material was used. An amount of 30 mg (± 2 mg) was considered as a safe margin.

After agitation of the extract with the Florisil, an aliquot can be transferred to an empty vial (with 200-µL insert). This extract can be analysed by fast GC-FID. Since the vial contains 150 µL extract, the same method could also be combined by large volume injection if needed.

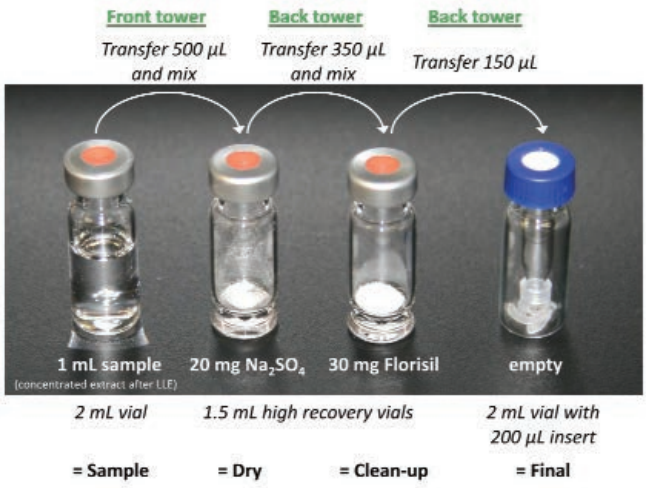

Figure 2. Picture of 2-mL vials during different clean-up steps on Work Bench
1. Original water extract in hexane;
2. After transfer of extract to Na_2SO_4 (drying);
3. After transfer of extract to Florisil;
4. Final extract.

A typical profile obtained for a mineral oil sample is shown in Figure 3. The upper trace shows the chromatogram obtained for a sample (400 µg/mL in extract) without clean-up. To the mineral oil sample, an amount of stearyl stearate (80 µg/mL in extract) was added. This compound (used as simulant for more polar interferences), elutes around C38.

The chromatogram obtained for the purified sample (theoretically same concentration as above) is shown in Figure 3, lower trace. It is clear that a similar profile is obtained for the mineral oil. The stearyl stearate is nearly quantitatively removed (peak area should be < 5% of peak area in non-purified sample.

To test the reproducibility, the Agilent 7696 WorkBench method was applied to a series of 6 samples in hexane, containing alkanes (5 µg/mL) and stearyl stearate (80 µg/mL). In Table 2, the peak areas for 4 n-alkanes and stearyl stearate are given. Typically, the RSD % are around 1% (2.5 % for C40). The recovery (calculated as ratio of peak area in purified sample versus original sample) is higher than 80%. The recovery of stearyl stearate was 1.9% (average of n=6), so well below 5%.

Table 2. Repeatability (n = 6) of Peak Areas for n-alkanes and Stearyl Stearate and Recovery Versus Direct Liquid Injection of Nonpurified Sample

Compound	Average response	SD	RSD (%)	Recovery (%)
C10	58.1	0.542	0.93	99.6
C20	62.7	0.612	0.97	99.8
C30	60.4	0.656	1.09	101.1
Stearyl stearate	**16.0**	**2.792**	(17.5)	**1.9**
C40	50.5	1.293	2.56	86.4

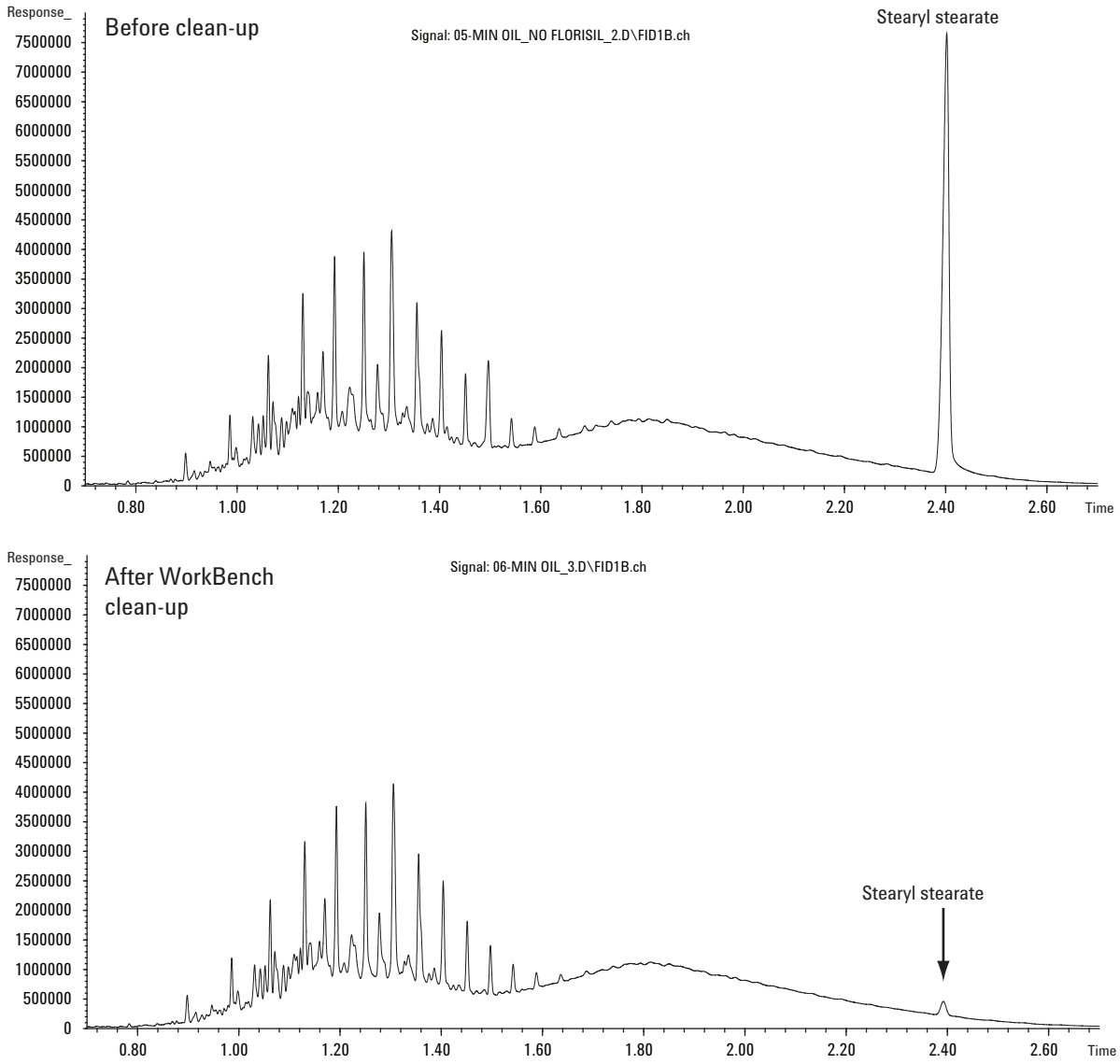

Figure 3. GC-FID chromatograms from water extract containing 400 ng/µL of a petroleum based oil and 80 ng/µL stearyl stearate in hexane. Upper trace: direct injection without clean-up; Lower trace: after Agilent 7696 Sample Prep WorkBench system drying and clean-up procedure.

The same test was performed on a water sample extract containing hydrocarbon oil (400 µg/mL) and stearyl stearate (80 µg/mL). The results are summarized in Table 3.

These data clearly demonstrate that repeatability is excellent (RSD < 1%) on total area for mineral oil and that removal of stearyl stearate was also efficient in this test (recovery = 2.3% on average, so < 5%). The recovery of the mineral oil fraction was higher than 95% (criteria: 80%< recovery < 110%).

Table 3. Repeatability (n = 10) of Peak Areas for Mineral Oil and Stearyl Stearate and Recovery Versus Direct Liquid Injection of Nonpurified Sample

	Peak area		Recovery (%)	
	Mineral oil	**Stearyl stearate**	**Mineral oil**	**Stearyl stearate**
No clean-up	9342.0	895.4		
1	9760.9	20.6	104.5	2.3
2	9745.4	20.6	104.3	2.3
3	9602.1	19.7	102.8	2.2
4	9839.3	16.4	105.3	1.8
5	9841.8	23.0	105.3	2.6
6	9704.5	18.1	103.9	2.0
7	9800.4	16.6	104.9	1.8
8	9745.4	23.0	104.3	2.6
9	9735.6	19.6	104.2	2.2
10	9658.4	31.6	103.4	3.5
Average	**9743.4**	**20.9**	**104.3**	**2.3**
sd	**75.4**		**0.8**	
RSD (%)	**0.77**		**0.77**	

Conclusion

A miniaturized dispersive SPE clean-up method for the determination of mineral oil in water samples was automated on the Agilent 7696A Sample Prep WorkBench system. Hexane extracts from liquid-liquid extraction are dried and purified. The obtained extracts are analysed by GC-FID.

High recoveries and excellent reproducibility are obtained for the mineral oil fraction, while the clean-up step is very efficient. In combination with low thermal mass (LTM) –GC, a high throughput method is obtained.

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Automated Clean-up of PCB extracts from Waste Oil using the Agilent 7696A Sample Prep WorkBench

Application Note

Automated Sample Preparation

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Abstract

Solid waste, including petroleum based waste oils, transformer oil or mineral oil are screened for the presence of polychlorinated biphenyls (PCBs). The analysis is typically performed by GC-ECD or GC-MS after clean-up using column chromatography, solid phase extraction (SPE), or dispersive solid phase extraction (d-SPE). Clean-up on silica or acidified silica removes polar constituents that can interfere with the PCB analytes and/or contaminate the analytical system.

A miniaturized dispersive SPE method using $SiOH/H_2SO_4$ adsorbent was automated on the Agilent 7696A Sample Prep WorkBench system. The extracts could be purified efficiently and reproducible. In combination with GC-ECD, GC-MS or GC-MS/MS, also applying back-flushing, an automated, accurate and robust determination of PCBs in waste oils is possible.

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Introduction

The determination of polychlorinated biphenyls (PCBs) in mineral oils, including transformer oil, waste oil, or solid waste in general is a routine application in environmental laboratories. After dilution/dissolvation of the oil, sample clean-up is applied to remove most of the matrix. Clean-up methods are typically based on classical column chromatography or solid phase extraction (SPE). Several SPE methods are applied and dedicated cartridges for PCB in waste (mineral) oil determination are available. According to EN 12766, for instance, a combination of an acidified silica/anion exchange (SiOH-H_2SO_4/SAX) and a silica (SiOH) is used. The oil samples are applied to the cartridge in hexane solution and the PCB fraction is immediately eluted with hexane. The polar matrix compounds remain on the SPE cartridges [1].

The solid phase extraction method can also be miniaturized and simplified using dispersive solid phase extraction [2]. In comparison to SPE, the adsorbent is added to the extract and the sample is mixed. The polar interferences bind to the active adsorbent, while the apolar solutes remain in solution. A similar approach is used in the well-know QuEChERS method used for pesticide analysis [3].

In this application note, it is demonstrated that the dispersive-SPE method can be miniaturized and automated on an Agilent 7696 Sample Prep WorkBench. From an extract/solution containing oil and (possibly) PCBs, an aliquot is transferred to a vial containing a pre-weighed amount of adsorbent. The vial is vortexed, an aliquot of the supernatant is transferred to another vial containing silica for additional clean-up, and finally, an aliquot of the PCB-fraction is transferred to an empty vial ready to be analysed by GC-ECD, GC-MS or GC-MS/MS.

As this clean-up procedure removes polar interferences, the PCB extract still contains apolar matrix compounds. These compounds will interfere less with the PCB determination, but especially high molecular weight material can build up in the analytical system and contaminate column and ion source. For this reason, it is still recommended to apply back-flushing as described in Application Note 5989-7601 EN [4].

Experimental

Chemicals

A BCR reference sample was used to demonstrated the performance of the clean-up method. BCR-449 (IRMM, Geel, Belgium) is a waste mineral oil sample, containing a high (mg/kg) level of PCBs. From the oil, a 100 mg/mL solution in hexane was prepared.

Octachloronaphthalene (Sigma-Aldrich, Beerse, Belgium) was used as internal standard. A stock solution of 10 ppm in iso-octane was used.

Three adsorbents were used: 44% H_2SO_4 coated silica gel (BONDESIL-SAX, 40UM, p/n 12213041, Agilent Technologies) and washed silica gel (BONDESIL-SI, 40UM, p/n 12213001, Agilent Technologies). To a first series of 2-mL vials, 100 mg H_2SO_4 coated silica and 100 mg SAX materials was added. These vials are labeled SiH. To a second series of 2-mL vials, 100 mg washed silica was added. These vials are labeled SiOH. All vial were closed with screw caps to protect them from humidity. All weighed amounts were with a precision: 100 mg ± 5 mg.

Sample Preparation

Aliquots of the waste oil solution are pipetted in 2-mL vials (samples). Eventually, a vial with a 100-µL insert can be used. A series of 2-mL vials containing the acidified silica/SAX material (SiH) and 2-mL vials containing silica (SiOH) are also positioned in the racks, together with empty vials with a 100-µL insert (for final extract), vials with hexane solvent (chemical resource) and vials containing the internal standard solution (chemical resource). An example of the resource lay-out of the 7696 WorkBench is shown in Figure 1.

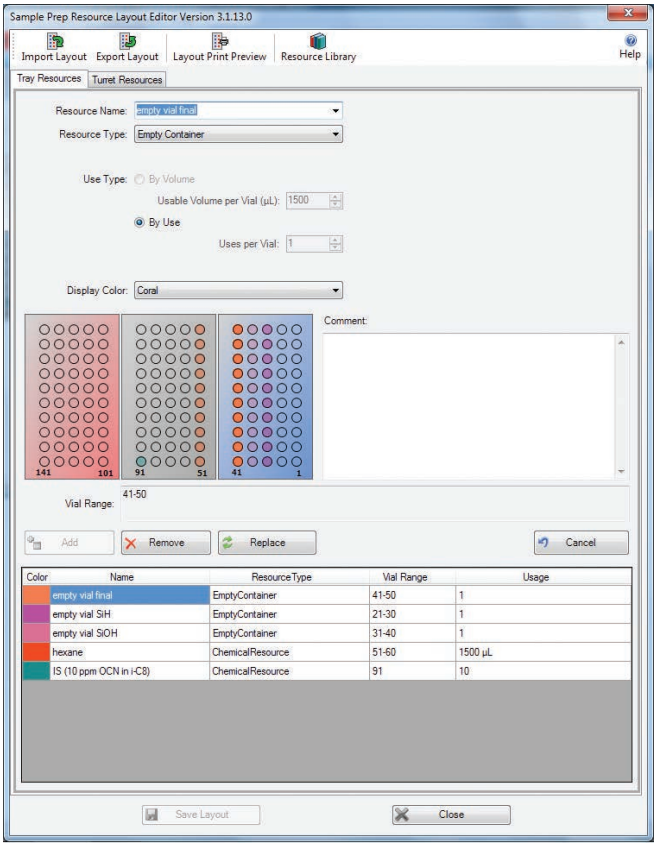

figure 1. Resource lay-out for an Agilent 7696 Sample Prep WorkBench

The WorkBench method can be summarized as follows:

1 **Add 50 uL of Sample (10% waste oil in hexane) to SiH at Front Tower.**
(A fraction of the sample is added to the vial containing the acidified silica and SAX material.)

2 **Add 1,350 uL of hexane to SiH at Front Tower.**
(Additional hexane solvent is added.)

3 **Add 150 uL of IS to SiH at Front Tower.**
(Internal standard is added, final volume is 1.5 mL, corresponds to 5 mg oil and 1,500 ng IS.)

4 **Mix SiH at 4,000 RPM for 5 min 0 sec.**
(The vortex allows good mixing of the adsorbents with the sample.)

5 **Wait for 2 min 0 sec.**
(This allows the polar fraction to bind with the adsorbents.)

6 **Add 1,000 uL of SiH to SiOH at Front Tower.**
(Transfers supernatant to a second clean-up vial.)

7 **Mix SiOH at 4,000 RPM for 5 min 0 sec.**
(The vortex allows good mixing of the adsorbents with the sample.)

8 **Wait for 2 min 0 sec.**
(This allows the polar fraction to bind with the adsorbent.)

9 **Add 200 uL of SiOH to Vial final at Front Tower.**
(Transfer of cleaned extract to a vial with an insert.)

10 **Flag Vial final as Result.**

Instrumental Configuration

The analyses were performed on an Agilent 7000 Triple Quad GC/MS system. The GC was equipped with a MMI inlet. Separation was done on a 0.25 mm × 30 m, 0.25 µm DB-5MS column (p/n 122-5532). The outlet of the column was connected to a Quick-Swap connector at 28 kPa constant pressure. The fused silica restrictor in the MS transfer line was 0.17 m × 110 µm.

The analytical conditions are summarized in Table 1.

Table 1. Analytical Conditions

Injection	1 µL, pulsed splitless
Inlet temp	85 °C (0 min) – 720 °C/min – 325 °C (5 min)
Carrier	Helium, 1 mL/min constant flow
	During backflush: –2 mL/min
Oven temp	80 °C (1 min) – 10 °C/min – 305 °C – 7.5 min hold
MS	MRM mode
	CE 25 V, dwell time 100 ms per transition
	Trichloro-biphenyls: 256.0 > 186.0; 258.0 > 186.0
	Tetrachloro-biphenyls: 293.8 > 222.0; 291.8 > 222.0
	Pentachloro-biphenyls: 325.8 > 256.0; 327.8 > 256.0
	Hexachloro-biphenyls: 359.9 > 289.9; 361.9 > 289.9
	Heptachloro-biphenyls: 393.8 > 323.8; 395.8 > 323.8
	Octachloronaphthalene (IS): 404.0 > 404.0 (CE 0V)
Backflush	Start at 23.5 min

Results and Discussion

The sample preparation procedure performed on the Agilent 7696 Sample Prep WorkBench is illustrated in Figure 2. The solution of the waste mineral oil is dark-brown, due to the presence of oil components. After transfer of an aliquot of this sample, corresponding to 5 mg oil, to a vial containing 100 mg $SiOH/H_2SO_4$ + 100 mg SAX adsorbent, the solution becomes much clearer, while the adsorbent turns black. This step is the most important, removing the bulk of the matrix. In a second clean-up step, some remaining contaminants are removed on silica material. Finally, the fraction is transferred in an insert vial. This solution is clear, indicating that purification was efficient.

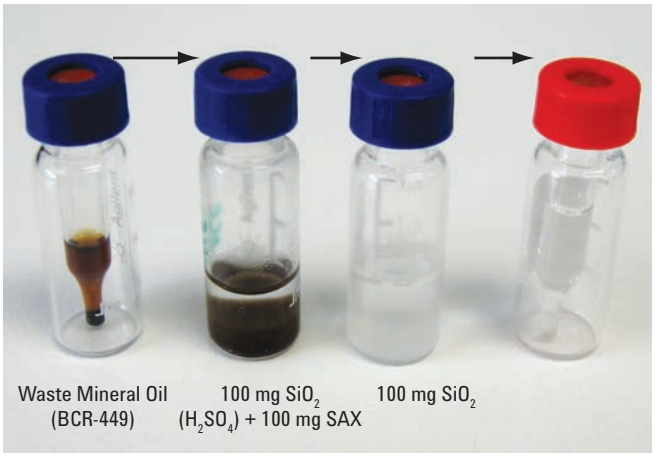

Figure 2. Picture of 2-mL vials during different clean-up steps on an Agilent 7696 Sampe Prep WorkBench.

In Figure 2, from left to right:

1. Original solution of waste oil in hexane
2. After transfer of waste oil to SiH (binding of contaminants to acidified silica/SAX material)
3. After transfer of extract to SiOH vial
4. Final extract

The obtained extracts were analyzed by GC-MS/MS. The obtained chromatograms (TIC from MRM acquisition) of 5 aliquots from the reference sample BCR-449, prepared according to the above described sample preparation method, are shown in Figure 3. The internal standard elutes at 22.8 min. The PCBs can easily be detected. From these chromatograms, it is clear that reproducibility is quite good.

In Table 2, the repeatabilities of the relative peak areas for six target compounds are given. Typically, the RSD % are around 5%.

Table 2. Repeatabilities of the Relative Peak Areas

Solute	RT (min)	Rel Area (solute/IS)	RSD (%)
PCB52	15,463	0,027	4,51
PCB101	17,151	0,037	6,10
PCB118	18,326	0,105	9,76
PCB153	18,731	0,086	4,54
PCB138	19,246	0,095	5,58
PCB180	20,448	0,026	4,03
OCN (IS)	22,849		

The same results are expected using GC-ECD or GC-MSD analysis.

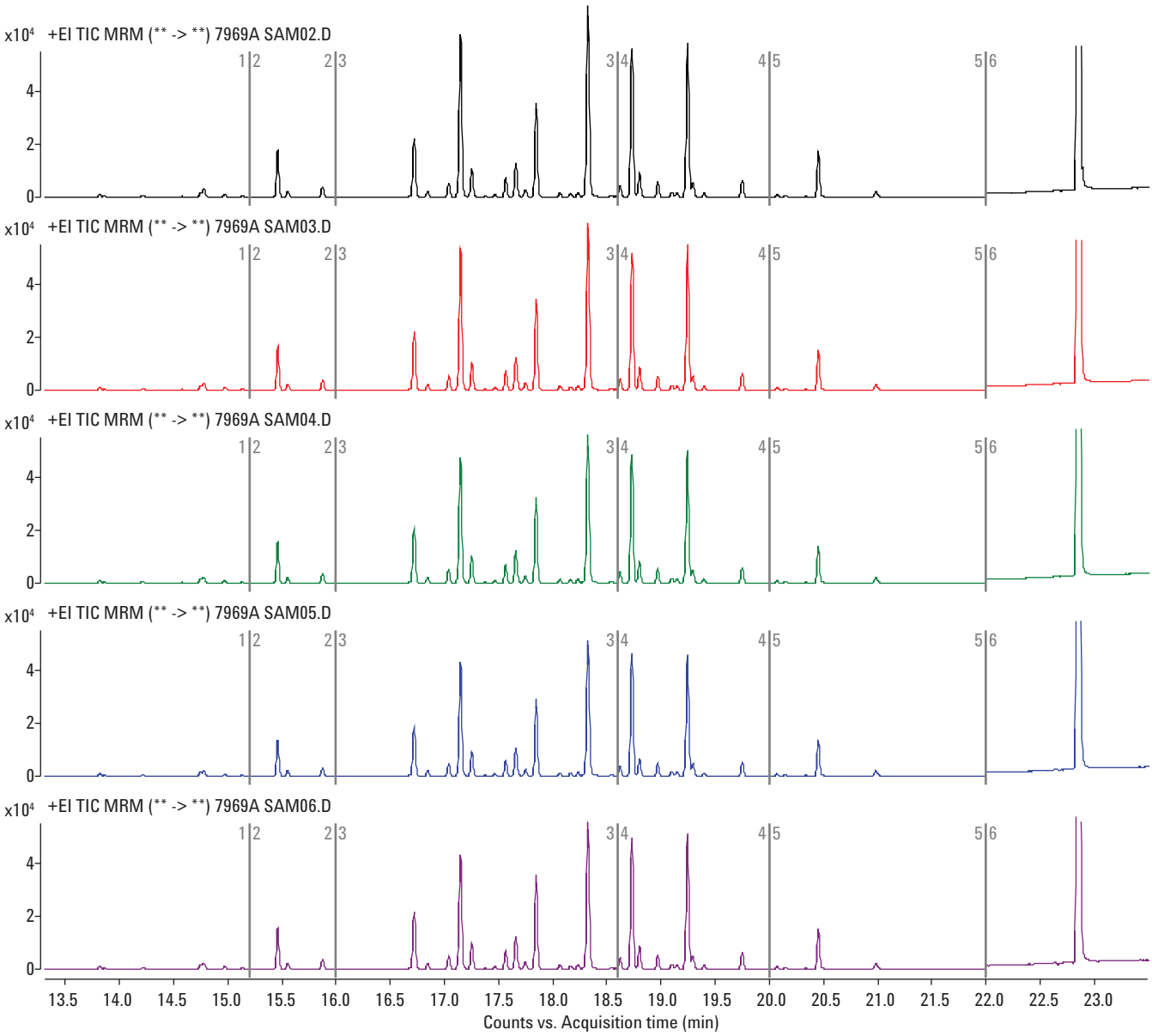

Figure 3. TIC Chromatograms from GC-MS/MS analysis in MRM mode obtained for the extract of PCBs from waste oil.

It should however be kept in mind that in the PCB fraction, apolar matrix compounds are still present. These compounds are not removed on the different adsorbents. In the analysis by GC-ECD, GC-MS operated in SIM mode, or GC-MS/MS in MRM mode, the co-extracted solutes are not detected. Their presence can however contaminate the inlet, column and finally also the detector. For this reason, the use of back-flushing, as described in reference [4], is still recommended.

Conclusion

A miniaturized dispersive SPE method for the determination of PCBS in waste mineral oils was automated on the Agilent 7696A Sample Prep WorkBench system. A two-step d-SPE using $SiOH/H_2SO_4$ + SAX adsorbent, followed by silica adsorbent was used. The extracts were efficiently and reproducibly purified. In combination with GC-MS or GC-MS/MS, also applying backflushing, an automated, accurate and robust determination of PCBs in waste oils is possible.

References

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Sub µg/L Level Analysis of Chlorinated Pesticide and Herbicide Analysis in Water by GC/µECD using Agilent J&W DB-35ms Ultra Inert and DB-XLB columns

Application Note

Environmental

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Abstract

Chlorinated pesticides and herbicides in water samples are successfully extracted with Agilent SPEC C18AR liquid-solid extraction (LSE) disks. A dual column GC/µECD approach was used employing Agilent J&W DB-35ms Ultra Inert (UI) primary analysis and DB-XLB confirmatory analysis columns. This approach provided consistent and sensitive analysis for the chlorinated compounds at and below established maximum contaminant level concentrations. The method was calibrated over a range of 1 to 100 ng/mL, which corresponds to the expected analyte extraction concentration levels. A water sample fortified at a level of 0.01 µg/L and a tap water sample were extracted and analyzed to demonstrate the effectiveness of this application.

Introduction

Pesticides and herbicides are commonly used in agricultural and residential environments. Pesticide residues are found in many environmental ground and surface waters. These residues enter the water supplies through runoff from pesticide applications and leaching through the soil into groundwater. Human exposure through contaminated drinking water is of concern as pesticides have been linked to serious health and environmental effects. Potential health effects from long term exposure include liver problems and an increased risk of cancer, while recent studies have raised concerns over endocrine disruption [1,2]. The European Union (EU) and United States Environmental Protection Agency (EPA) have established regulations for maximum pesticide levels in drinking water [2,3,4].

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Column and liner inertness are critical to achieving consistently reliable analytical results, especially for challenging pesticides such as endrin and DDT, which are particularly susceptible to interaction with active sites in the inlet or on the column [5,6]. Minimizing flow path activity is essential to accurate detection at the trace levels required by the current regulations. This application uses both an Agilent Ultra Inert column and liner to help insure an inert sample flow path.

Quantitative determination of the chlorinated pesticides was achieved by GC/µECD using a dual-column approach. An Agilent J&W DB-35ms Ultra Inert GC column was chosen for primary analysis, while an Agilent J&W DB-XLB column provided confirmatory analysis with a less polar stationary phase than the primary column to help verify the analyte's identity.

The DB-35ms UI offers excellent selectivity for chlorinated pesticides, effectively resolving all thirty-seven of the pesticides and herbicides targeted by the EPA 508.1 method [7]. The EPA 508.1 method recommends pentachloronitrobenzene as an internal standard and 4,4'-dibromobiphenyl as the surrogate standard. Because these two compounds coeleute with analytes of interest, this application was modified by shifting to two surrogate standards commonly used in CLP pesticides analysis, tetra-chloro-m-xylene (TCMX) and decachlorobiphenyl, which are well resolved from the pesticides.

Calibration curve standard preparation can be time consuming and resource intensive. Manual sample preparation can also be prone to errors, resulting in poor reproducibility and precision. The Agilent 7696A Sample Prep WorkBench allows automation of many sample preparation tasks, while significantly reducing solvent use and analysis time. The Agilent 7696A WorkBench has demonstrated high precision and reproducibility, while decreasing variability errors in several sample preparation applications [8,9,10].

The chlorinated pesticides and herbicides are extracted from water using liquid-solid extraction. Because the targeted analytes can be present at trace levels, a large sample volume is needed to extract detectable levels of the pesticides. Current method procedures use a 1 L sample size, which can be time consuming to extract using typical cartridge extractions. Agilent SPEC C18AR 47 mm LSE disks allow faster sample extraction while effectively retaining targeted analytes.

Experimental

An Agilent 7890A Series GC equipped with dual µECD detection and an Agilent 7683B autosampler was used for this study. An inert tee split the effluent 1:1 to the primary and confirmation columns. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies and Table 3 lists the sample preparation supplies.

Table 1. Chromatographic Conditions

Column 1	Agilent DB-35ms UI 30 m × 0.32 mm, 0.25 µm (p/n 123-3832UI)
Column 2	Agilent DB-XLB 30 m × 0.32 mm, 0.5 µm (p/n 123-1236)
GC/µECD	Agilent 7890 Series GC
Sampler	Agilent 7683 automatic liquid sampler, 5.0 µL tapered syringe (p/n 5181-1273)
CFT device	Inert tee (p/n G3184-60065)
Split ratio	1:1
Retention gap	5 m × 0.32 mm id deactivated fused silica tubing
Inlet	2 µL splitless; 250 °C,
Purge flow	60 mL/min at 0.5 min
Carrier	Helium, average velocity 35 cm/s at 80 °C
Oven	80 °C (0.5 min), 26 °C/min to 175 °C, 6.5 °C/min to 235 °C, 15 °C/min to 300 °C (6 min)
µECD	340 °C, constant column + makeup (N_2) = 30 mL/min

Table 2. Flow Path Supplies

Vials and caps	MS certified amber crimp top glass vials and caps kit (p/n 5190-2283)
Vial inserts	250 µL glass/polymer feet (p/n 5181-8872)
Syringe	5 µL tapered (p/n 5181-1273)
Septum	Advanced Green (p/n 5183-4759)
Inlet liner	Ultra Inert single tapered liner (p/n 5190-2292)
Ferrules	0.5 mm id short; 85/15 Vespel/graphite (p/n 5062-3514)
CFT fittings	Internal nut (p/n G2855-20530)
CFT ferrules	SilTite ferrules, 0.32 mm id (p/n 5188-5362)
20x magnifier	20× magnifier loop (p/n 430-1020)

Table 3. Sample Prep Supplies

SPEC disks	Agilent SPEC C18AR 47 mm (p/n A74819)
SPEC manifold system	SPEC 6-position manifold (p/n A712) SPEC disk holders (p/n A713) SPEC 1 L flasks (p/n A714)

Reagents and chemicals

All reagents and solvents were ACS or Ultra Resi grade. Ethyl acetate (EtOAc), methanol (MeOH), and methylene chloride ($MeCl_2$) from JT Baker was purchased through VWR International (West Chester, PA). Hydrochloric acid (HCl) and sodium sulfite (Na_2SO_3) were purchased from Sigma-Aldrich (St. Louis, MO). The EPA 508.1 analyte and surrogate standards were purchased from Ultra Scientific (North Kingstown, RI, USA).

Solutions and standards

An aqueous sodium sulfite solution was prepared at a 50 mg/mL concentration. This solution was added to the sample during collection to reduce any residual chlorine. A 1:1 EtOAc:$MeCl_2$ solution was prepared by mixing equal parts of each solvent.

A 6 N HCl solution was prepared by adding 25 mL hydrochloric acid dropwise to a 50 mL volumetric flask containing approximately 22 mL water in a cooling bath. The solution was allowed to reach room temperature, then diluted to volume with water and mixed thoroughly.

The analyte primary dilution standard was prepared by diluting the commercially prepared pesticide stock solutions with ethyl acetate to yield the analytes at a concentration of 1 µg/mL. This solution was used to fortify a reagent water sample for method analysis. A surrogate standard was prepared at concentrations of 1 µg/mL in ethyl acetate and added to water samples prior to extraction.

The Agilent 7696A Sample Prep WorkBench was used to prepare the calibration standards in ethyl acetate from the neat analyte and surrogate standards over a concentration range of 1 to 100 ng/mL.

Sample preparation

A 1-L water sample was extracted using Agilent SPEC C18AR 47 mm solid-liquid extraction disks, and the extract dried and concentrated prior to GC analysis. Figure 1 illustrates the LSE sample extraction procedure.

A 1-L aliquot of water was collected and 1 mL of 50 mg/mL aqueous Na_2SO_3 was added to convert any residual free chlorine. The pH of the sample was adjusted to pH $\leq$ 2 with 6 N HCl. A quality control sample was spiked with an appropriate amount of spiking solutions to yield a QC sample with an analyte concentration of 0.01 µg/L.

After assembling the vacuum manifold system, the SPEC disk was placed wrinkle side up on the filter. A 5-mL aliquot of 1:1 EtOAc:$MeCl_2$ was added and allowed to soak the disk for 1 minute, then drawn through slowly under vacuum. Next, 5 mL of MeOH was added to the disk and again drawn through slowly, leaving a layer on the disk surface, ensuring the disk did not go dry. The disk was then rinsed with 5 mL of reagent water, which was drawn through under vacuum, again leaving a layer on the disk surface.

A 5-mL aliquot of MeOH was added to the 1-L water sample and mixed well. The appropriate amount of surrogate standard spiking solution was added and the sample shaken. The water sample was drawn through the extraction disk at a rate of about 75 to 100 mL/min. The disk was then dried by drawing air through the disk for about 10 minutes.

The filtration glassware was removed and replaced with a flask containing a collection tube, ensuring the tube fit around the drip tip of the fritted base, and the filtration apparatus reassembled. The sample bottle was rinsed with 5 mL of EtOAc which was transferred to the disk using a disposable pipet. The solvent was drawn through very slowly under vacuum. This bottle rinse step was repeated with 5 mL $MeCl_2$. A glass disposable pipet was used to rinse the filtration reservoir with two 3-mL portions of 1:1 EtOAc:$MeCl_2$.

The eluent was passed through a glass drying tube containing 5 to 7 g anhydrous sodium sulfate. The drying tube was rinsed with two 3-mL portions of 1:1 EtOAc:$MeCl_2$. The extract and washings were collected in a concentrator tube and concentrated to approximately 0.8 mL using a Labconco CentriVap centrifugal concentrator (78100 Series). The inside walls of the tube were rinsed two to three times with EtOAc during concentration. The final extract volume was adjusted to 1.0 mL with EtOAc and transferred to autosampler vials for GC analysis.

Sample extraction procedure

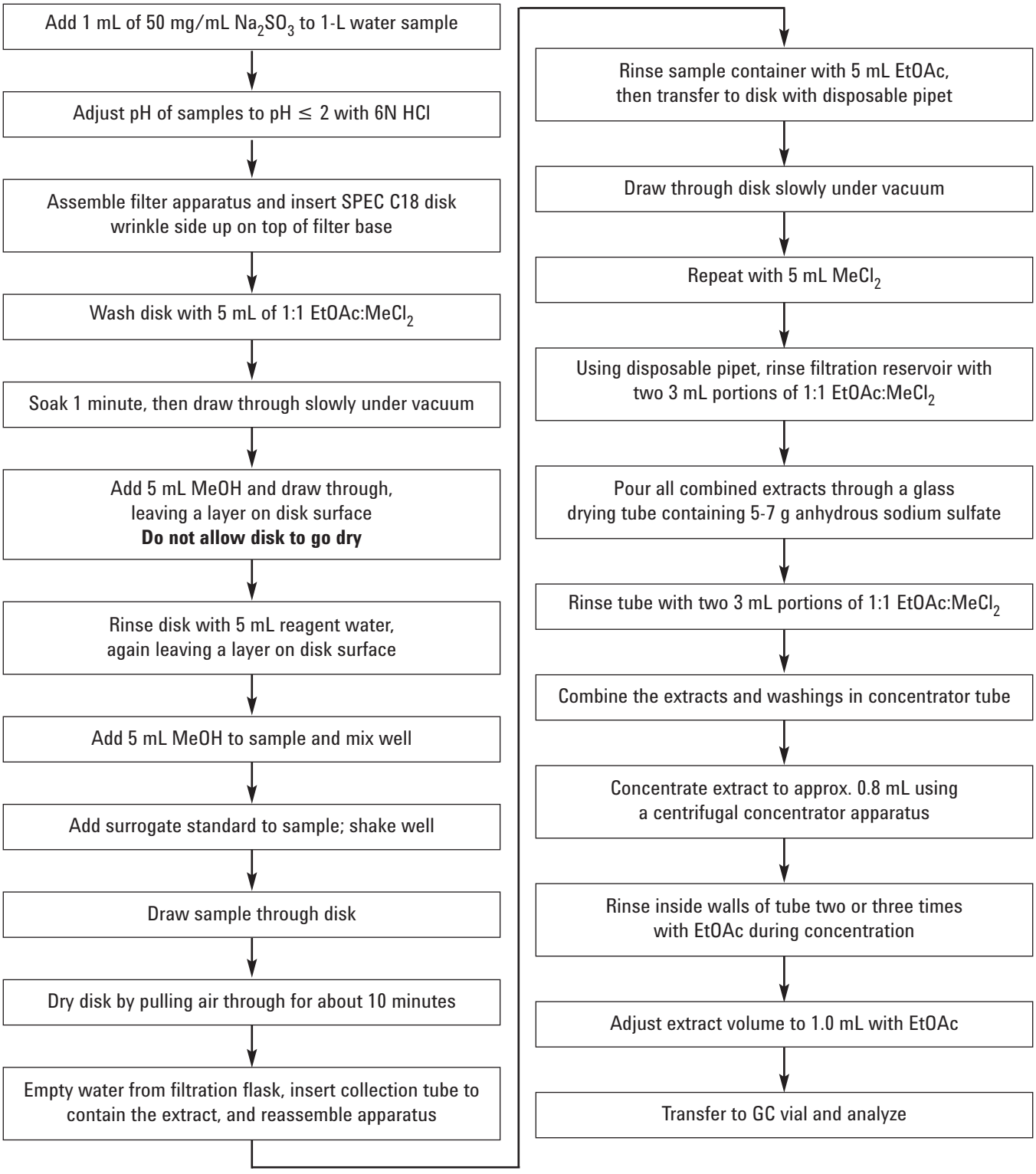

Figure 1. Flow chart for the extraction of chlorinated pesticides in water.

Results and Discussion

The thirty seven targeted chlorinated pesticides and herbicides were resolved on the Agilent DB-35ms UI primary analysis column and the Agilent DB-XLB confirmation column in less than 23 minutes. Figure 2 depicts the dual column GC/μECD chromatograms of a 50 ng/mL standard prepared in ethyl acetate. The enlarged section of the chromatograph in Figure 3 shows the excellent peak response and resolution of a 10 ng/mL EPA 508.1 standard analyzed on the DB-35ms UI column. Figure 4 illustrates the separation and differences in selectivity of the DB-XLB column, demonstrating its benefits as a confirmation column.

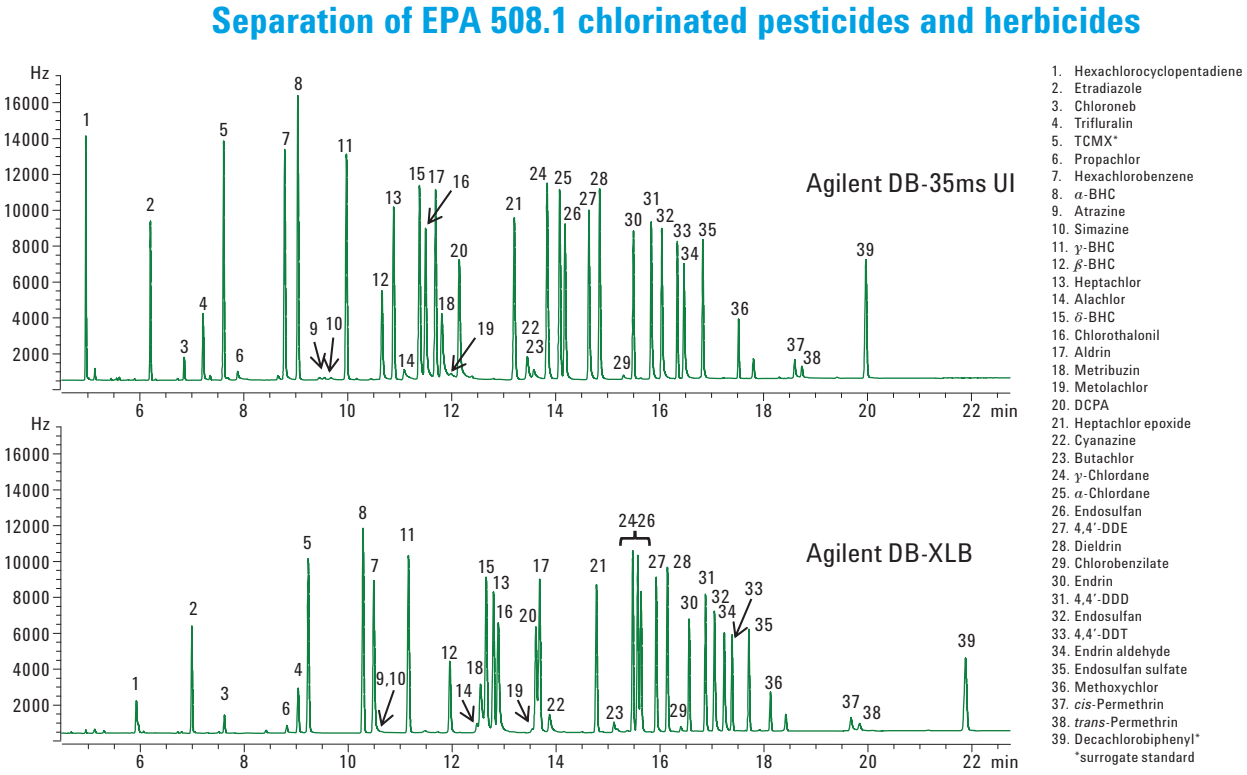

Figure 2. GC/μECD chromatogram of a 50 ng/mL pesticide standard analyzed on an Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 μm column (p/n 123-3832UI) and DB-XLB 30 m × 0.32 mm, 0.5 μm column (p/n 123-1236). This standard was prepared in ethyl acetate using an Agilent 7696A Sample Prep WorkBench. Chromatographic conditions are listed in Table 1.

EPA 508.1 low level pesticides peak shape and resolution with an Agilent DB-35ms UI

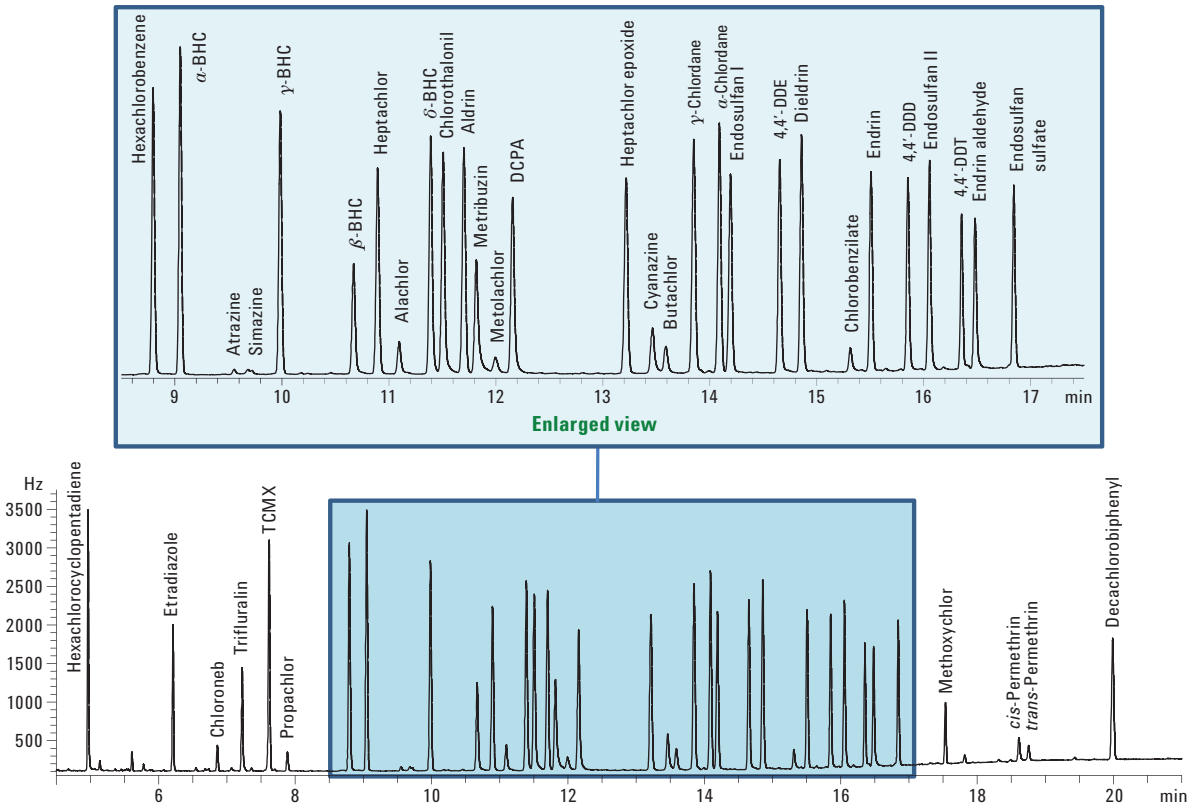

Figure 3. Enlarged section of the GC/μECD chromatogram of a 10 ng/mL chlorinated pesticide standard analyzed on an Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 μm column. The chromatographic conditions are listed in Table 1.

EPA 508.1 low level pesticides peak shape and resolution with an Agilent DB-XLB

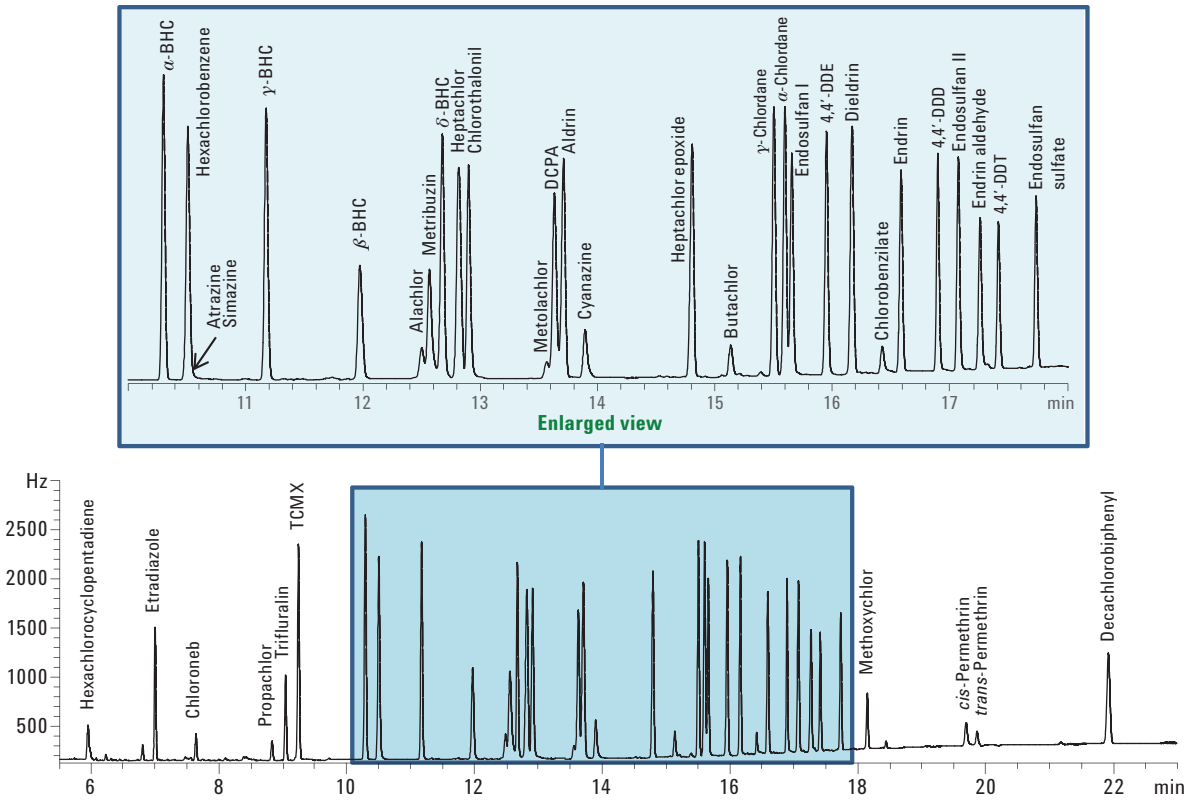

Figure 4. Enlarged section of the GC/μECD chromatogram of a 10 ng/mL chlorinated pesticide standard analyzed on an Agilent J&W DB-XLB 30 m × 0.32 mm, 0.5 μm column. The chromatographic conditions are listed in Table 1.

A seven-point calibration curve was generated to test the linearity of the method. Linearity as defined by the correlation coefficient (R^2) of the calibration curve can be used to evaluate the performance of a gas chromatographic column. The seven-level calibration solutions were prepared by appropriate dilution of commercially prepared standards in ethyl acetate. The Agilent 7696A Sample Prep WorkBench was used to prepare the calibration curve standards at 1, 2.5, 5, 10, 25, 50, and 100 ng/mL.

A nonlinear response can be indicative of breakdown or adsorption of the compound in the inlet or column. The performance of the Agilent DB-35ms UI and Agilent DB-XLB columns yielded correlation coefficient (R^2) values ≥ 0.993 over the calibration range of this study. The individual pesticide analyte values are shown in Table 4.

Table 4. Correlation Coefficients (R^2) for the EPA 508.1 Chlorinated Pesticides Calibration Standards Analyzed by GC/μECD

Linearity results

	R^2 values	
Analyte	**Agilent DB-35ms UI**	**DB-XLB**
Hexachlorocyclopentadiene	0.9996	0.9930
Etradiazole	0.9982	1.0000
Chloroneb	0.9982	0.9981
Trifluralin	0.9976	0.9976
TCMX (ss)	0.9997	0.9997
Propachlor	0.9996	0.9986
Hexachlorobenzene	0.9996	0.9991*
α-BHC	0.9998	1.0000
Atrazine	0.9941	*
Simazine	0.9971	*
γ-BHC	0.9999	0.9998
β-BHC	0.9998	0.9999
Heptachlor	0.9999	0.9998
Alachlor	0.9986	0.9989
δ-BHC	0.9999	0.9996
Chlorothalonil	1.0000	1.0000
Aldrin	0.9998	0.9994
Metribuzin	0.9997	0.9985
Metolachlor	0.9973	0.9987
DCPA	0.9996	0.9998

	R^2 values	
Analyte	**Agilent DB-35ms UI**	**DB-XLB**
Heptachlor epoxide	0.9998	0.9998
Cyanazine	0.9994	0.9998
Butachlor	0.9990	0.9992
γ-Chlordane	0.9998	0.9999
α-Chlordane	0.9998	0.9998
Endosulfan I	0.9998	0.9997
4,4′-DDE	0.9998	0.9998
Dieldrin	0.9998	0.9999
Chlorobenzilate	0.9940	0.9985
Endrin	0.9998	0.9996
4,4′-DDD	1.0000	0.9999
Endosulfan II	0.9999	0.9999
4,4′-DDT	0.9993	0.9996
Endrin aldehyde	1.0000	0.9999
Endosulfan sulfate	0.9997	0.9997
Methoxychlor	0.9993	0.9982
cis-Permethrin	0.9992	0.9992
trans-Permethrin	0.9988	0.9995
Decachlorobiphenyl (ss)	0.9998	0.9997

(ss)-surrogate std *Coelution

The method was able to detect chlorinated pesticides with a high level of sensitivity at trace levels. The European Union Directive sets the content limit of individual pesticides in drinking water at 0.1 μg/L [3]. To reliably achieve this detection level, the method should be capable of a limit of detection (LOD) well below the established threshold. Figure 5 shows an extracted 0.01 μg/L fortified reagent water sample on the Agilent DB-35ms UI and Agilent DB-XLB columns. This sample is fortified at an order of magnitude below the target limit, and is also at or below the maximum contaminant levels (MCLs) established by the EPA for pesticides in drinking water [1].

Sample preparation using Agilent SPEC C18AR liquid-solid extraction disks was effective in retaining and preconcentrating the chlorinated pesticides in the spiked water sample. To determine the trace amounts of pesticides in water at the regulated MCLs, a large sample volume is needed to concentrate the pesticides at a detectable level. The use of the large 47 mm C18 disks enabled extraction of a 1 L water sample at a rate of 75 to 100 mL/min. This allowed samples to be processed in about 10 minutes, reducing sample preparation time and increasing sample throughput.

GC/µECD chromatogram of extracted water samples blank relative to fortified sample

1. Hexachlorocyclopentadiene
2. Etradiazole
3. Chloroneb
4. Trifluralin
5. TCMX*
6. Propachlor
7. Hexachlorobenzene
8. α-BHC
9. Atrazine
10. Simazine
11. γ-BHC
12. β-BHC
13. Heptachlor
14. Alachlor
15. δ-BHC
16. Chlorothalonil
17. Aldrin
18. Metribuzin
19. Metolachlor
20. DCPA
21. Heptachlor epoxide
22. Cyanazine
23. Butachlor
24. γ-Chlordane
25. α-Chlordane
26. Endosulfan
27. 4,4′-DDE
28. Dieldrin
29. Chlorobenzilate
30. Endrin
31. 4,4′-DDD
32. Endosulfan
33. 4,4′-DDT
34. Endrin aldehyde
35. Endosulfan sulfate
36. Methoxychlor
37. *cis*-Permethrin
38. *trans*-Permethrin
39. Decachlorobiphenyl*

*surrogate standard

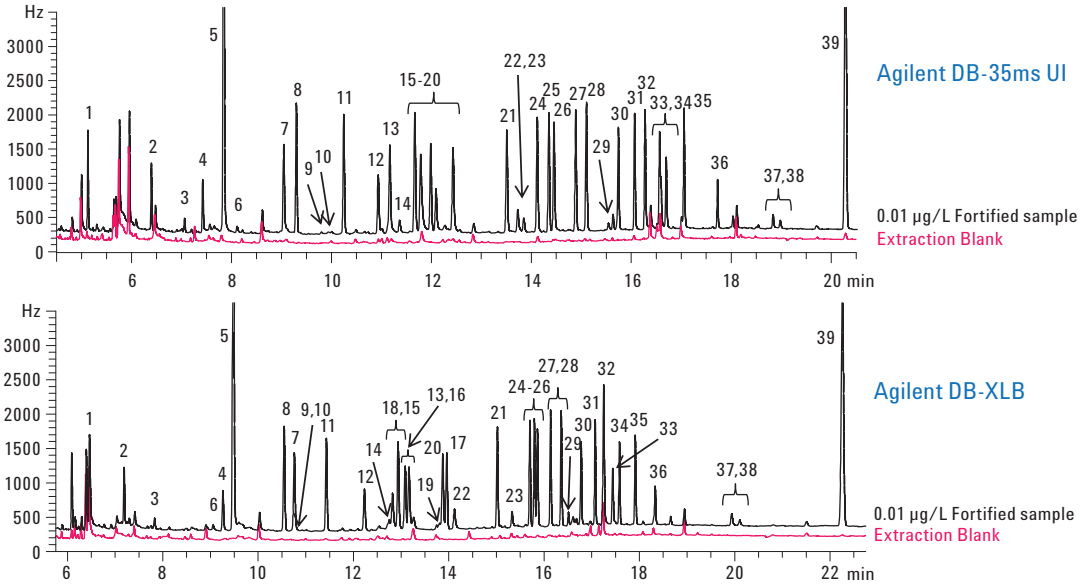

Figure 5. GC/µECD chromatogram for a 0.01 µg/L fortified water sample and extraction blank analyzed using Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 µm column (p/n 123-3832UI) and an Agilent DB-XLB 30 m × 0.32 mm, 0.5 µm column (p/n 123-1236). These samples were prepared and extracted according to the sample preparation procedure detailed in Figure 1. Chromatographic conditions are listed in Table 1.

A drinking water sample was also analyzed for chlorinated pesticides using this method. The tap water sample was collected and prepared according to the sample preparation steps shown in Figure 1 and evaluated under the chromatographic conditions listed in Table 1. The targeted chlorinated compounds were not detected in the tap water sample at the calibrated range of this study. The GC/µECD chromatograms of the sample are shown in Figure 6.

GC/µECD chromatogram of extracted water samples blank relative to drinking water sample

1. Hexachlorocyclopentadiene
2. Etradiazole
3. Chloroneb
4. Trifluralin
5. TCMX*
6. Propachlor
7. Hexachlorobenzene
8. α-BHC
9. Atrazine
10. Simazine
11. γ-BHC
12. β-BHC
13. Heptachlor
14. Alachlor
15. δ-BHC
16. Chlorothalonil
17. Aldrin
18. Metribuzin
19. Metolachlor
20. DCPA
21. Heptachlor epoxide
22. Cyanazine
23. Butachlor
24. γ-Chlordane
25. α-Chlordane
26. Endosulfan
27. 4,4′-DDE
28. Dieldrin
29. Chlorobenzilate
30. Endrin
31. 4,4′-DDD
32. Endosulfan
33. 4,4′-DDT
34. Endrin aldehyde
35. Endosulfan sulfate
36. Methoxychlor
37. *cis*-Permethrin
38. *trans*-Permethrin
39. Decachlorobiphenyl*

*surrogate standard

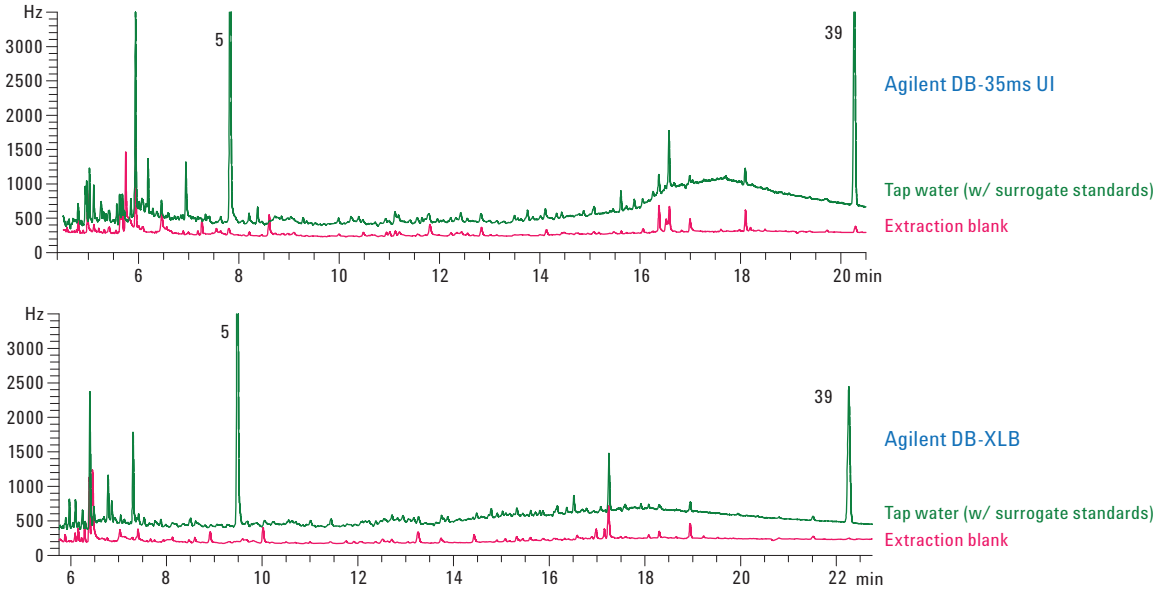

Figure 6. GC/µECD chromatogram for a tap water sample and extraction blank analyzed using Agilent J&W DB-35ms UI 30 m × 0.32 mm, 0.25 µm column (p/n 123-3832UI) and an Agilent DB-XLB 30 m × 0.32 mm, 0.5 µm column (p/n 123-1236). These samples were prepared and extracted according to the sample preparation procedure detailed in Figure 1. Chromatographic conditions are listed in Table 1.

Conclusions

This application note demonstrates an effective analytical method to extract and detect sub-µg/L level chlorinated pesticides and herbicides in water samples. The Agilent J&W DB-35ms UI capillary column adequately resolves all thirty-seven targeted analytes, while providing excellent sensitivity and reliable quantitation at low levels. The separation of the chlorinated pesticides with the Agilent DB-XLB column provides consistent analyte confirmation.

The Agilent SPEC C18AR 47 mm liquid-solid extraction disks successfully extracted and preconcentrated pesticides from water samples, delivering improved trace analyte detection, while reducing sample preparation time. Calibration standards prepared with the Agilent 7696A Sample Prep WorkBench yielded regression coefficients $R^2 \geq 0.993$ for both columns over the range studied.

Pesticide levels were detectable ten fold below the EU and EPA maximum contaminant levels for pesticides in water. A water sample fortified at 0.01 µg/L was successfully prepared and analyzed by this application demonstrating the effectiveness of using Agilent J&W DB-35ms UI and Agilent DB-XLB columns for low level chlorinated pesticide determination. Analysis of a tap water sample did not detect any pesticides at the calibrated levels of this method.

Acknowledgements

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Agilent Technologies

Generation of AQA Standards for EPA Method 8270 Using the Agilent 7696A Sample Prep WorkBench

Application Note

Environmental

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Abstract

Using the Agilent 7696A Sample Prep WorkBench to prepare calibration standards enables relative response factor reproducibility and recoveries that are well within the analytical quality assurance standards set by this laboratory for EPA Method 8270.

Introduction

Analytical quality assurance (AQA) is essential for the proper operation of any analytical laboratory, be it commercial, government, or academic. Reliability of data, particularly for analyses regulated by the Environmental Protection Agency (EPA), depends on strict adherence to a wide range of operating procedures for analysis. Two of the most common procedures are the use of calibration curves and the measurement of recovery from spiked samples.

A calibration curve is constructed by preparing a series of standards across a range of concentrations near the expected concentration of analyte in the unknown sample. The deviation of individual calibration points from the line of best fit is used to assess the precision of the calibration. This precision is directly dependent on the quality of the source standard material used as well as the accuracy and reproducibility of the preparation of the calibration standards. Measurement of recovery of analyte from a sample spiked with a standard is another assessment of the efficiency and precision of the analysis, and is also dependent on the quality of the calibration standards used.

Calibration standards are most commonly prepared manually, involving tedious and time-consuming pipetting steps whose accuracy is dependent on the skill of the operator and the possibility of human error. In addition, the operator may be exposed to hazardous chemicals. Automated dispensing systems remove human error from the process, and assure the accuracy and precision of the preparation of the calibration standards.

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This application note demonstrates the ability of the Agilent 7696A Sample Prep WorkBench to automatically prepare calibration standards that meet AQA requirements for calibration curve precision and recovery levels. EPA Method 8270 for the detection of semi-volatile organic compounds (SVOCs) was used as a model for the demonstration. This method uses gas chromatography/mass spectrometry (GC/MS) to analyze solid, liquid, and gaseous samples for a list of 90 SVOCs regulated by the EPA.

The automated method for preparing the calibration standards resulted in standards that met the laboratory's AQA requirements for relative standard deviation of the calibration curve average relative response factors (RRFs) for 13 calibration check compounds (CCCs). Recoveries were also within allowed limits for these 13 compounds. Additionally, all other requirements outlined by the method for system performance compounds (SPCCs) and non-CCC compounds were met.

Experimental

Standards and Reagents

Methylene chloride of pesticide grade or higher was used to prepare the calibration standards. SVOC standards were obtained from Sigma-Aldrich and Restek, at a concentration of 1,000 µg/mL. Deuterated internal and surrogate standards were also obtained from Restek.

Instruments

The calibration standards were prepared on an Agilent 7696A Sample Prep WorkBench using the settings shown in Table 1. The analysis was performed by GC/MS.

Table 1. Agilent 7696A Sample Prep WorkBench Settings

Front syringe	500 µL
Back syringe	100 µL
Heater setpoint	Ambient
Heater offset	0 °C
Number of pumps	2
Wash volume	400 µL
Draw speed	800 (µL/min)
Dispense speed	2,500 (µL/min)
Draw needle depth offset	0 mm
Viscosity delay	4 seconds
Overfill	5% of syringe size
Air gap	0% of syringe size
Sample processing scheme	Sequential

Preparation of Calibration Standards on the Sample Prep WorkBench

Calibration standards were prepared sequentially on the WorkBench. Varying amounts of methylene chloride were first dispensed into vials, then varying amounts of the SVOC standard working solution were dispensed into the same vials to provide a total volume of 1 mL in each vial, and eight calibration standards ranging in concentration from 0.5 to 80 µg/mL. The syringe was rinsed with 400 µL of methylene chloride between each dispensing step.

Results

Calibration Curves

The GC/MS analysis of SVOC calibration standards results in separate peaks whose area can be integrated to relate peak area to concentration (Figure 1). Response factors are calculated at each concentration on the calibration curve by dividing the area of the peak by the concentration of the calibration standard. An RRF is then calculated by dividing the response factor of the calibration standard by the response factor of the internal standard.

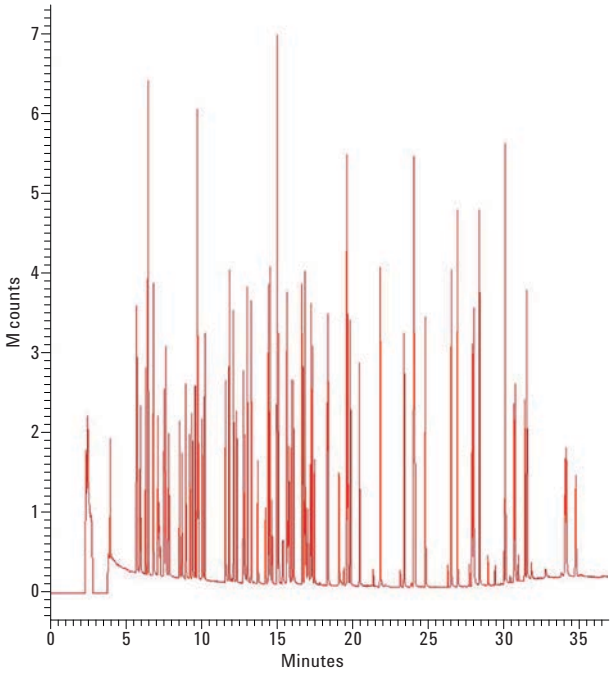

Figure1. Typical total ion current (TIC) chromatogram obtained for an EPA Method 8270 GC/MS analysis, spiked at 10 µg/mL with calibration standard mix.

An average RRF is calculated across all concentrations of the calibration standard used to generate the calibration curve. Each average RRF is determined in replicate and the relative standard deviation (RSD) is calculated across the replicates. The AQA standard in this laboratory requires that the RSDs be calculated for 13 CCCs, and that they must be less than 30%. Table 2 illustrates that all of the RRFs for the CCCs generated using the WorkBench met this quality criterion. In fact, 8 of the 13 RSDs were ≤10%.

Recoveries

Recoveries of spiked calibration standards were determined using the calibration curves prepared using the WorkBench. The AQA standard in this laboratory requires that all recoveries fall in the range of 80 to 120%. Table 3 shows that all recoveries met this criterion, and 7 of the 13 measured recovery values were in the 95 to 100% range. These high recoveries attest to the accuracy of quantitation using the calibration curves derived from standards prepared by the WorkBench automated system.

Table 3. Recoveries for Spiked Calibration Standards

Calibration check compound	% Recovery
Phenol	114.21
1,4-Dichlorobenzene	97.28
2-Nitrophenol	99.69
1,2,4-Dichlorophenol	108.1
Hexachlorobutadiene	98.53
4-Chloro-3-methylphenol	96.33
2,4,6-Trichlorophenol	95.71
Acenapthylene	92.92
N-Nitrosodiphenylamine	97.70
Pentachlorophenol	115.06
Fluoranthene	89.91
Di-*n*-octyl phthalate	108.55
Benzo[a]pyrene	99.05

Table 2. Relative Response Factors (RRFs) Across the Calibration Curve, Average RRFs, and % RSDs

	RRF for each concentration (µg/mL) on the calibration curve									
Calibration check compound	**0.5**	**1.0**	**2.0**	**5.0**	**10**	**20**	**50**	**80**	**Average RRF**	**%RSD**
Phenol	1.581	1.502	1.597	1.722	1.413	1.342	1.053	1.082	1.412	17.1
1,4-Dichlorobenzene	1.600	1.618	1.848	1.725	1.710	2.074	1.722	2.030	1.791	10.0
2-Nitrophenol	0.119	0.113	0.106	0.114	0.116	0.111	0.126	0.109	0.114	5.3
2,4-Dichlorophenol	0.330	0.306	0.332	0.330	0.338	0.293	0.299	0.280	0.314	6.8
Hexachlorobutadiene	0.227	0.230	0.241	0.265	0.283	0.261	0.283	0.312	0.263	11.2
4-Chloro-3-methylphenol	0.417	0.416	0.420	0.422	0.442	0.379	0.438	0.464	0.425	5.8
2,4,6-Trichlorophenol	0.529	0.495	0.476	0.460	0.477	0.451	0.410	0.403	0.463	9.1
Acenapthylene	2.195	2.137	1.960	2.012	2.062	1.914	1.962	2.389	2.079	7.6
N-Nitrosodiphenylamine	0.868	0.795	0.721	0.673	0.650	0.646	0.658	0.678	0.711	11.3
Pentachlorophenol	0.157	0.140	0.129	0.136	0.128	0.118	0.094	0.088	0.124	18.8
Fluoranthene	1.006	0.961	0.948	0.920	0.956	0.990	0.972	1.147	0.988	7.1
Di-*n*-octyl phthalate	2.038	2.557	2.825	3.159	3.281	3.471	4.018	--	3.050	21.2
Benzo[a]pyrene	1.092	1.080	1.069	0.984	0.951	0.914	0.987	1.060	1.017	6.6

Conclusion

Preparation of accurate and precise calibration standards is an absolute necessity for every analytical laboratory. This is particularly true for EPA methods in environmental laboratories. Method 8270 is challenging due to the large number of chemically diverse compounds analyzed. The Agilent Sample Prep WorkBench provides the precision and accuracy required for an analytical laboratory to obtain reportable EPA Method 8270 results, without the tedium, time, and human error associated with manual preparation of calibration standards.

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Agilent Technologies

Using the Agilent 7696A Sample Prep WorkBench for the analysis of estrone by GC Triple Quadrupole Mass Spectrometry

Application Note

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Abstract

Analysis of endocrine disruptors is increasingly becoming a high volume analysis in many labs and crossing disciplines such as clinical chemistry, industrial exposure, drug discovery and development and environmental analyses including emerging contaminate and persistent organic pollutants. The demand placed on laboratories for these high volume tests places a burden on not only the analytical measurement tools but most importantly accurate and reproducible sample preparation. This application note briefly outlines how the Agilent 7696A Sample Prep WorkBench can be used to prepare samples for analysis through GC/MS/MS using an automated workflow.

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Introduction

The need for accurate analysis of endocrine disruptors (EDCs) such as estrogens, androgens, progestins, corticosteroids, and glucocorticoids in ground, surface, and potable water sources is growing in demand. The major source of these compounds in the environment is an iatrogenic artifact of hormonal therapies for agricultural livestock and humans. The excretions of the nonmetabolized parent drug and its metabolites are often not fully degraded through conventional wastewater treatment processes. Thus, these compounds are found in freshwater bodies such as rivers and transported to aquifers. Adverse effects even at ppt levels include, but are not limited to, abnormal population ratios of male to female in fish and amphibian communities, reversible feminization of fish species, inhibition of reproduction pathways, morphological changes such as an increased occurrence of hermaphroditism, and disruption of normal pheromone responses. Due to decades of extensive use, these compounds have become ubiquitous, persistent, organic pollutants, and could pose a risk to human health. The need to study their transport and fate in the environment is of paramount importance. This application note illustrates automated sample preparation including preparation of calibrators and derivatization protocol using the 7696A Sample Prep WorkBench for the analysis of a group of known endocrine disruptors by GC/MS/MS.

Experimental

Standards and Reagents

Estrone (E1), BSFTA/TCMS (99%/1%), anhydrous acetonitrile, and anhydrous pyridine were purchased from Sigma-Aldrich (USA). A stock solution of E1 was prepared in anhydrous acetonitrile and used to create a working mixture required for calibrator preparation.

Instruments

The Agilent 7696A Sample Prep WorkBench was used to prepare calibration standards and perform automated derivatization of the analytes. The measurement experiments were performed on an Agilent 7890A Series GC equipped with a multimode inlet (MMI) in cold split-less injection mode and an Agilent 7693A 150 position auto-sampler coupled to an Agilent 7000B Triple Quadrupole GC/MS in EI mode. The instrument conditions are listed in Tables 1 and 2.

Table 1. GC/MS Conditions

GC run conditions	
Analytical columns	Two 15 m HP-5MS UI, (p/n 19091S431UI) connected sequentially using the Agilent Purged Ultimate Union (p/n G1472A)
Injection volume	2 µL
Injection mode	Cold, split-less using Multi-Mode Inlet (MMI)
Inlet temperature	70 °C for 0.01 minutes 450 °C/min to 280 °C for 3 minutes
Gas saver	On 20 mL/min after 3 minutes
Purge flow	30 mL/min at 1.5 minutes
Cryo	On
Cryo use temperature	72 °C
Fault detection	30 minutes
Timeout detection	On 10 minutes
Oven temperature	120 °C for 0.5 minutes 40 °C/min to 240°C, hold for 0 minutes 5 °C/min to 280°C, hold for 3.75 minutes
Carrier gas	Helium in constant flow mode Column 1: 0.8 mL/min Column 2: 1.0 mL/min
Average velocity	23.498 cm/sec
Transfer line temperature	280 °C
Run time	15.25 minutes
MS conditions	
Tune	atunes.eiex.tune.xml
Gain factor	50
Acquisition parameters	Multiple reaction monitoring (MRM)
Collision gas	1.5 mL/min
Quench gas	2.25 mL/min
Solvent delay	6.0 minutes
MS temperatures	Source 300 °C Quadrupoles 150 °C

Table 2. MRM Parameters

Time segment	Start time	Compound name	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell (ms)	Collision energy (V)
1	10.5	E1	342.0	257.0	150	15
1	10.5	E1	342.0	244.0	150	15

Sample Preparation using the Agilent 7696A Sample Prep WorkBench

Trinh *et al* (2011) have demonstrated an E1 MDL near 1.0 ng L-1, taking into consideration a 1,000-fold concentration (1.0 L sample volume concentrated to 1.0 mL). For this evaluation, five calibrators were prepared at 1.0, 2.5, 5.0, 10.0, and 50.0 ng/mL using the 7696A Sample Prep WorkBench. For the derivatization, a stock reagent of 10/10/80 (% v/v) BSTFA+TCMS/anhydrous pyridine/anhydrous acetonitrile was prepared and added to the dried calibrators and heated to 60 °C for 30 minutes also by the 7696A Sample Prep WorkBench.

Results and Discussion

7696A Sample Prep WorkBench sample preparation

Automation using the 7696A Sample Prep WorkBench significantly reduces analyst time spent on sample preparation, removes the potential for sampling errors while maintaining the recovery, and precision achieved through manual work up. In this application note, a recovery of 133.37% was determined at the 1.0 ng/mL (1 pg on column) level with three replicate injections and an average precision of 5.162% RSD (range 3.32–6.89) over the five levels. Tables 3 and 4 illustrate these results. Figure 1 illustrates the quantitative and qualitative SRMs for E1 at 1.0 ng/mL or 1 pg mass on column.

Table 3. Low Calibrator S/N and % Recovery at 1.0 ng/mL (1 pg on Column)

Name	Sample type	Level	E1 method Exp. conc.	Area	E1 Final conc.	S/N
Std_1_1	Cal	1	1.0 ng/mL	48.18	1.29	11.20
Std_1_2	Cal	1	1.0 ng/mL	42.01	0.94	9.00
Std_1_3	Cal	1	1.0 ng/mL	45.97	1.17	12.40
		% Recovery			113.37	

Table 4. Calibrator %RSD (5 Levels, n = 3 Replicates)

Name	Sample type	Level	Exp. conc.	E1 area
Std_1_1	Cal	1	1	48.18
Std_1_2	Cal	1	1	42.01
Std_1_3	Cal	1	1	45.97
		% RSD		6.89
Std_2_1	Cal	2	2.5	65.86
Std_2_2	Cal	2	2.5	65.75
Std_2_3	Cal	2	2.5	59.74
		% RSD		5.49
Std_3_1	Cal	3	5	134.20
Std_3_2	Cal	3	5	147.65
Std_3_3	Cal	3	5	137.09
		% RSD		5.07
Std_4_1	Cal	4	10	184.80
Std_4_2	Cal	4	10	167.32
Std_4_3	Cal	4	10	173.81
		% RSD		5.04
Std_6_1	Cal	5	50	931.48
Std_6_2	Cal	5	50	874.49
Std_6_3	Cal	5	50	887.74
		% RSD		3.32

GC/MS/MS analysis

For this study, three replicate injections were made at five concentration levels ranging from 1.0 ng/mL to 50.0 ng/mL. Figure 2 illustrates the resulting calibration curve with a correlation coefficient of $R^2 = 0.996$ for the 15 total injections.

Instrument Detection Limit

Wells *et al* (2011) state that, when the sample set is less than 30, the one-tail Students-t distribution can be used to estimate the instrument detection limit (IDL). For 99% confidence and n–1 degrees of freedom, the Students-t Table value for this study is 6.965. Substitution of 6.965 and 6.89% RSD for the low calibrator into the IDL equation (Equation 1) results in an estimated IDL of 0.48 pg E1 on column. This value is in fair agreement with Trinh *et al* (2011) who determined MDLs of 0.7 ng L-1 with 99% confidence and n = 7 replicates.

$$IDL_{\%RSD} = \frac{(6.965 \times 6.89\% \times 1.0\ pg)}{100} = 0.48\ pg$$

Equation 1. Estimated IDL based on area % RSD for 1.0 ng/mL calibrators (n = 3).

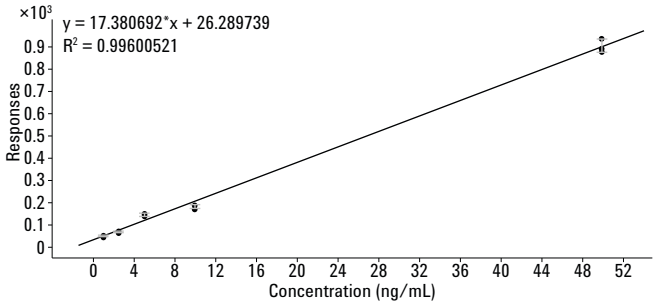

Figure 2. E1 Calibration curve: three replicate injections at five levels.

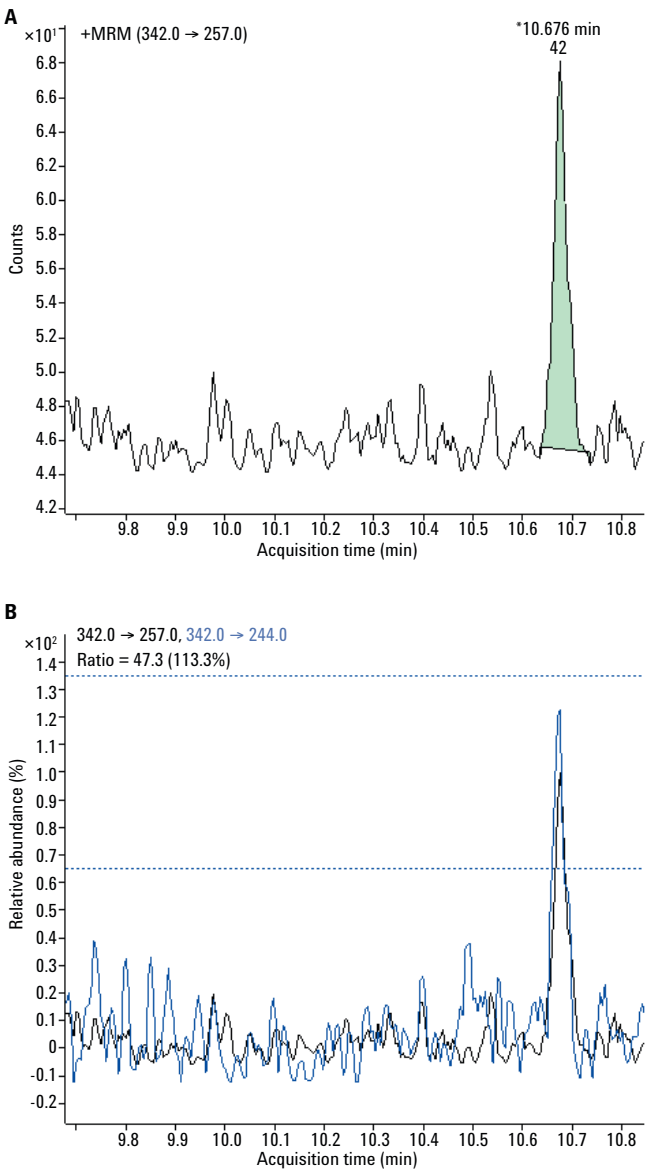

*Figure 1. 1.0 ng/mL EI. **A** shows the quantitative SRM 342 → 257. **B** shows the qualitative transition 342 → 244. The dotted lines in **B** represent the allowable uncertainty for qualifier ratio. Noise region for S/N calculation is 10.4 to 10.6 minutes.*

Conclusions

The Agilent 7696A Sample Prep WorkBench can be used to accurately prepare samples, calibrators, and QC's for the analysis of estrogenic and other endocrine disruptors in an automated workflow that includes on board derivatization. This application note illustrates the effectiveness of automating sample derivatization followed by analysis using GC triple quadrupole mass spectrometry. Excellent recoveries and precision were obtained over the calibration levels and an IDL was determined in good agreement with MDLs reported in the literature.

References

1. Trinh T., *et al.* "Simultaneous determination of estrogenic and androgenic hormones in water by isotope dilution gas chromatography–tandem mass spectrometry". *J. Chrom A*, 1218 (2011) 1668–1676.

2. Wells G, Prest H, Russ CW. "Why use signal-to-noise as a measurement of MS performance when it is often meaningless". Agilent Application Note 5990-8341EN. (2011) Agilent Technologies, Inc.

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Agilent Application Solution

Analysis of bisphenol A leaching from baby feeding bottles

Application Note

Consumer Products

Author

Syed Salman Lateef
Agilent Technologies, Inc.
Bangalore, India

Abstract

Bisphenol A can leach out from the plastic surfaces of food containers and has been detected in various matrices such as plasma, urine and groundwater. Bipshenol A is an endocrine disruptor, which can mimic the body's own hormones such as estrogen and may lead to negative health effects. In this Application Note, we describe a method to quantify bisphenol A and a structurally similar analog bisphenol F extracted from baby feeding bottles. The method was developed on an Agilent 1260 Infinity LC system using an Agilent ZORBAX Eclipse Plus C18 column. Partial method validation was performed to demonstrate linearity, robustness and precision of area and retention time. The dilution series was generated automatically using an Agilent 7696A Sample Prep WorkBench, saving analyst time. The limit of quantitation (LOQ) for bisphenol A was found to be 1.06 ng/mL. During sample recovery studies, 80% recovery was obtained for bisphenol A. The method was transferred to an ultrahigh performance liquid chromatography (UHPLC) method using an Agilent 1290 Infinity LC System. The UHPLC method has the same experimental conditions but showed narrower and higher peaks, better resolution, and improved signal-to-noise response. Both methods can be applied for bisphenol A quantification in quality control of food containers such as baby feeding bottles.

Agilent Technologies

Introduction

Bisphenol A (BPA) is a monomer used to make polycarbonate plastic and epoxy resins. Traces of BPA can leach out of these polycarbonate plastic surfaces under various environmental conditions such as heat or pH changes and eventually are consumed by humans. BPA was detected in various matrices such as urine, groundwater and plasma. Based on the assumption that a threshold exists above which toxic effects are seen, the U.S. Environmental Protection Agency (US EPA) has established 50 µg/kg body weight/day as the reference dose (RfD) for BPA[1]. Baby bottles made of polycarbonate plastic are a potential risk to children. In this Application Note, we used a structurally similar commercially available compound bisphenol F, (BPF), (Figure 1) along with BPA to determine separation efficiency.

Ballesteros-Gomez *et.al.*, reviewed various analytical methods used to separate, identify and quantify BPA[2]. Also, the ASTM standard test method, D 7574-09, describes a SPE based offline method for extracting bisphenol A from environmental waters[3]. BPA is a fluorescent compound, and fluorescence detector (FLD) is sensitive to detect BPA concentration in baby bottles. In this Application Note, a method is described to simultaneously quantify BPA and BPF using a SPE based offline extraction procedure with (U)HPLC/FLD detection.

Experimental

Instruments and software

An Agilent 1260 Infinity Binary LC system consisting of the following modules was used:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Autosampler and Thermostat (G1367E, G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (TCC) (G1316A)
- Agilent 1260 Infinity Fluorescence Detector (G1312B) with 8 µL flow cell

The UHPLC analysis was developed and performed using an Agilent 1290 Infinity LC System consisting of the following modules:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Autosampler and Thermostat (G4226A, G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Fluorescence Detector (G1312B) with 8 µL flow cell

Software:

- Agilent ChemStation B.04.02

Sample preparation:

- Agilent 7696A Sample Prep WorkBench

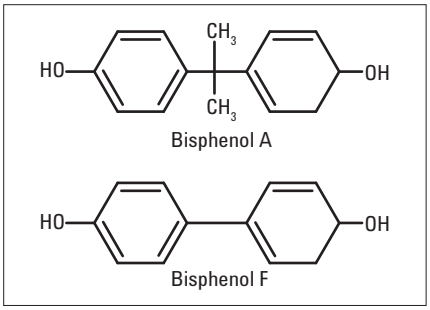

Figure 1
Molecular structures of bisphenol A and bisphenol F

Reagents and materials

All chemicals and solvents used were HPLC grade. Purified water was used from a Milli Q water purification system (Millipore Q-POD Element, USA). Acetonitrile and methanol super gradient were purchased from Lab-Scan (Bangkok, Thailand) and potassium phosphate monobasic was obtained from Fluka (Germany). Standards of bisphenol A and bisphenol F were purchased from Sigma-Aldrich (India). BPA-free baby bottles manufactured in USA and three different brands of polycarbonate baby bottles manufactured locally were purchased.

Chromatographic parameters

The chromatographic parameters for reverse phase liquid chromatography using Agilent 1260 and Agilent 1290 Infinity LC systems are shown in Table 1.

Parameters	Agilent 1260 Infinity LC system	Agilent 1290 Infinity LC system
Column:	Agilent ZORBAX Eclipse Plus C18 4.6×100 mm 5 µm (p/n 959996 902)	Agilent ZORBAX Eclipse Plus C18 4.6×100 mm 1.8 µm (p/n 959964 902)
TCC temperature:	40 °C	
FLD:	Ex: 230. Em: 316	
FLD acquisition rate, gain:	9.26 Hz, 15	
Sample thermostat:	4 °C	
Mobile phase A:	10 mM monobasic potassium phosphate in water	
Mobile phase B:	100% Acetonitrile	
Gradient:	Time (min) %B	
	0 5	
	2 5	
	2.1 35	
	12.5 35	
	12.6 70	
	17 70	
	18.1 5	
	23 5	
Flow:	0.9 mL/min	
Injection volume:	20 µL. 5 sec needle wash at flush port for 5 sec, using mobile phase A	

Table 1
Chromatographic parameters used in the Agilent 1260 Infinity LC and Agilent 1290 Infinity LC systems.

Preparation of standards

BPA and BPF were accurately weighed out and dissolved in 100% methanol separately to obtain stock solutions of about 300 µg/mL each, which were stored at 4 °C when not in use.
A 400 ng/mL solution of BPA and BPF was prepared freshly by diluting the stock solutions using the dilution buffer of 5% acetonitrile and 95% 10 mM monobasic potassium phosphate in water. Linearity levels shown in Table 2, were prepared by subsequent dilution of a 400 ng/mL solution. The Agilent 7696A Sample Prep WorkBench was used to make linearity levels, using serial dilutions. In the first sequence, 400 µL of dilution solution was added to all the vials. In the second sequence, 300 µL of 400 ng/mL solution was added to a Level 7 vial from the first sequence and vortexed for 15 seconds. Serial dilutions were carried out by taking 300 µL from the previous level and added to the next level vial. Note that instead of running two sequences, the steps can also be programmed into one method and running one sequence. The syringe parameters used in the setup of the Agilent 7696A Sample Prep WorkBench are given in Table 3. An Agilent Application Note[5], describes in detail the set up of 7696A Sample Prep WorkBench[4].

Sample preparation

BPA from polycarbonate baby bottles was extracted following the schematic shown in Figure 2. An SPE adapter (p/n 12131001) and 3 mm OD tubing (p/n 5062-2483) were used to load the sample onto an Agilent Bond Elut Plexa SPE column, 200 mg, 6 mL (p/n 12109206). An Agilent 20 port vacuum extraction manifold (p/n 12234104) was used for the setup of SPE. We followed the sample handling precautions as described in the ASTM method[3]. The reconstituted solution from the final step (Figure 2) was used directly for sample analysis.

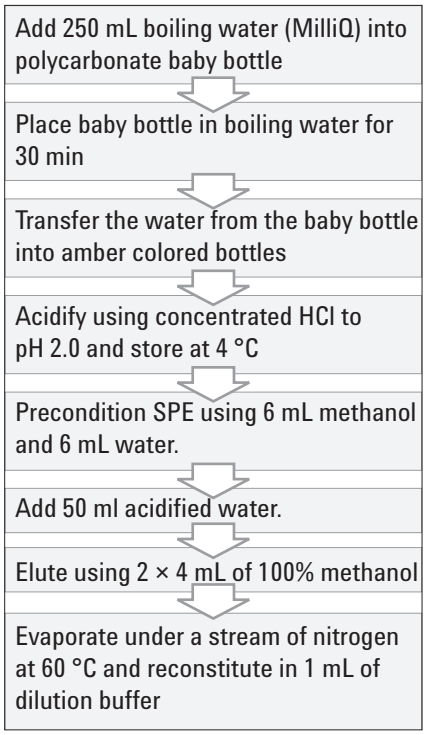

Figure 2
Extraction of BPA from baby bottles and sample preparation using SPE.

Linearity levels	Bisphenol A (ng/mL)	Bisphenol F (ng/mL)
LOD	0.195105	0.195105
1	1.06224	1.06224
2	2.478559	2.478559
3	5.783305	5.783305
4	13.49438	13.49438
5	31.48688	31.48688
6	73.46939	73.46939
7	171.4286	171.4286

Table 2
Dilution table for bisphenol A and bisphenol F.

	Solvent prewash 1	Dispense wash	Dispense pumps	Dispense settings
Number of pumps or washes	1	1	3	
Wash volume (µL)	50	50	20	
Draw speed (µL/min)	1250	1250	1250	1250
Dispense speed (µL/min)	2500	2500	2500	2500
Needle dept off set (mm)	-2.0	-2.0	-2.0	-2.0
Viscosity delay (s)	0	0	0	0
Turret solvent	A			
Air gap (% syr. vol.)	0			0

Table 3
500 µL syringe parameters used for the Agilent 7696A Sample Prep WorkBench.

Procedure

The reconstituted extracts from the baby bottles were injected to measure the approximate concentration of BPA before establishing the linearity range. A 20 µL solution of mobile phase A was injected as blank, followed by each linearity level in six replicates. Area and retention time (RT) information for each level was used to calculate the relative standard deviation (RSD) values. The average area of each linearity level in the linearity range was plotted against the concentration to obtain a calibration curve. The limit of detection (LOD) and limit of quantitation (LOQ) for BPA and BPF were established from the lower linearity level injections.

To evaluate the robustness of the method, six critical method parameters were evaluated:

- Flow rate ±2%
- Column temperature ±2.5%
- Injector volume ±5%
- Excitation and emission wavelength ±3%
- Step gradient ±10%
- Buffer concentration ±10%

For each robustness parameter, a standard concentration of 30 ng/mL solution of BPA and BPF was injected in seven replicates.

To perform the recovery studies, we extracted samples from BPA-free baby bottles as described in Figure 2. To 50 mL of this sample, either a low or a higher quantity of BPA and BPF was spiked. Both spiked samples were subjected to SPE. The resulting concentrations of the samples were determined using the calibration curves. The theoretical concentrations were compared against the experimental values to obtain the recovery values.

Finally, three different brands of baby bottles were analyzed to determine the leaching concentrations of the two bisphenols using the standard HPLC method.

The method was then transferred to an Agilent 1290 Infinity LC system and run on a 1.8 µm column using the same experimental conditions to test resolution and sensitivity of the method. For this method, we also evaluated LOD, LOQ, linearity of each standard and precision of the method by area and RT RSD.

Results and Discussion

Separation and detection

The separation of BPA and BPF was tested on C18 columns using acidic and basic mobile phases during method development. Extracted water samples from baby bottles were also tested before finalizing the method.

An Agilent ZORBAX Eclipse Plus C18 column was used for further experiments. A low temperature (35 °C) of the TCC provided optimal separation of BPA from a closely eluting impurity, however 40 °C was found to be better when analyzing matrix samples. A linear gradient separated the two bisphenols, however a preliminary method robustness study showed large variation when the gradient was modified. A step gradient method was therefore adapted, which gave comparatively robust results. The ASTM method recommended storing bisphenols at low temperature, therefore the autosampler was maintained at 4 °C during the analysis. Figure 3 shows a chromatogram separating the two bisphenols using the final method.

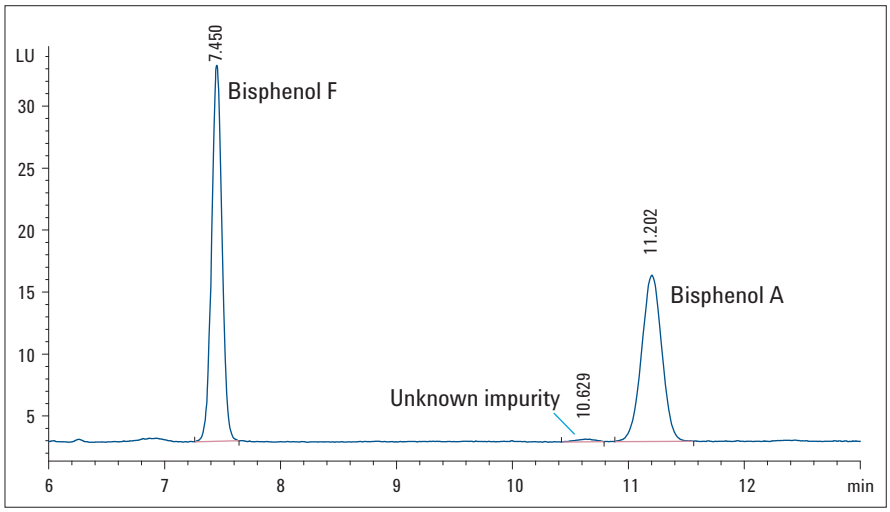

Figure 3
Separation of 30 ng/mL solution of bisphenol F and bisphenol A using an Agilent ZORBAX Eclipse Plus C18 column. The chromatogram was collected using FLD settings of excitation at 230 and emission at 316 nm.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The analyte concentration that provides a signal-to-noise ratio (S/N) of > 3 was considered as LOD and the analyte concentration with S/N ratio > 10 was considered as LOQ. A peak-to-peak method was used to calculate noise. Figure 4 shows a chromatogram of BPA at the LOQ level overlaid with a blank (mobile phase) injection. For BPA, the LOD was 0.19 ng/mL with S/N = 4.3 and LOQ was 1.06 ng/mL with S/N = 15.1.

Linearity

Calibration curves with linearity range (see Table 2) were prepared using an Agilent 7696A Sample Prep WorkBench. The WorkBench automates the sample handling, providing consistent results. Different sets of linearity ranges can be prepared by simply rerunning the program. The linearity levels were established starting from the LOQ level of BPA. LOD and LOQ values, along with the linearity results are included in Table 4. LOD and LOQ values can be further decreased by increasing the injection volume but it was not necessary for this application since the values obtained from baby bottles were found to be within the linearity range.

Precision of retention time (RT) and area

The area precision was measured as RSD(%) across the linearity levels. The maximum RSD value of 5.6% and 7.2% for level 1 (L1) were obtained for BPA and BPF respectively. Similarly, RT precision calculations showed a maximum RSD value of only 0.14% and 0.11% for the BPA and BPF. Graphical representation of area RSD values are shown in Figure 5.

Sl no.	Name	LOD ng/mL	S/N	LOQ ng/mL	S/N	Linearity range	R^2 value	No. of levels
1	Bisphenol F	0.19	5.1	0.46	12.4	1.06 –171.43	0.99999	7
2	Bisphenol A	0.19	4.3	1.06	15.1	1.06–171.43	0.99998	7

Table 4
LOD, LOQ and linearity for BPA and BPF. Samples were prepared using an Agilent 7696A Sample Prep WorkBench. BPA levels found in polycarbonate baby bottles were within the linearity range.

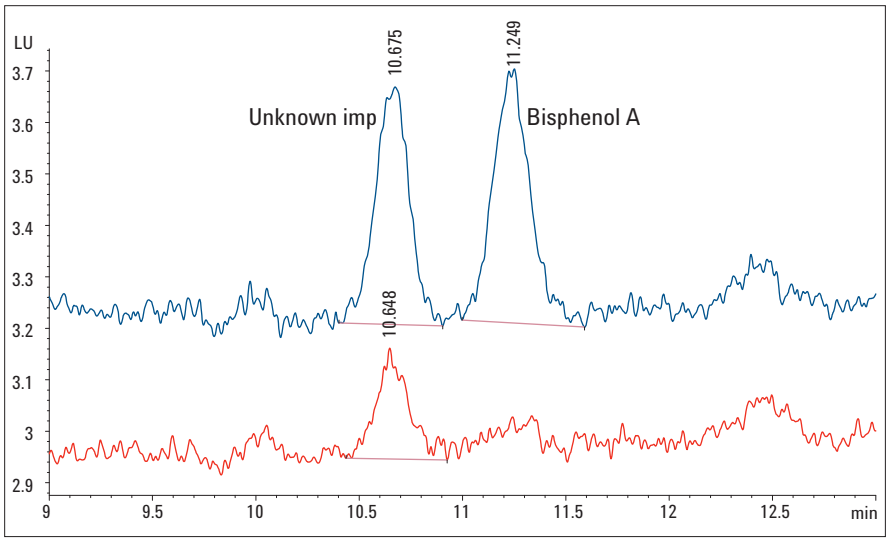

Figure 4
A 20 µL injection of LOQ level, 1.06 ng/mL (21 pg on column) solution, of bisphenol A overlaid with blank injection. S/N ratio obtained at this concentration was 15.

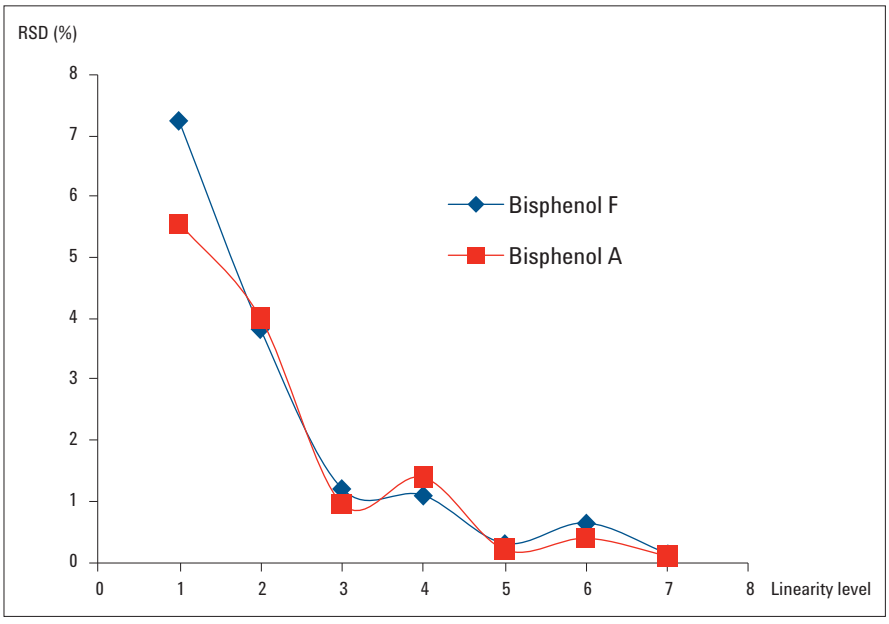

Figure 5
Area precision measured as RSD(%) for six replicates at each concentration level for BPA and BPF.

Robustness

To test the robustness of the method, a standard solution containing 30 ng/mL of BPA and BPF was used. Six critical method parameters (flow rate, column temperature, injector volume, excitation and emission wavelength, step gradient and buffer concentration) were varied separately and data were collected for seven replicate injections. The compound peak areas from the last six replicates were compared for analysis. The allowed deviation for the area and retention time was set to ± 5% and ± 3% respectively.

The results of the robustness tests are summarized in Table 5. The red numbers indicate where the result exceeded the allowed deviation. A flow rate change of +2% resulted in a decreased area of the two bisphenols. Specifically for bisphenol A, the peak area was found to have the negative deviation caused by 2.5% change in column temperature. The resolution of BPA compared to the impurity (see RT 10.6, Figure 3) showed poor results when the temperature was increased and improved results when the temperature was decreased to 35 °C. A temperature of 40 °C was found to be better for sample analysis. Robustness results show the importance of maintaining the column temperature during the analysis. Setting the FLD excitation at 230 nm and emission at 316 nm was found to be ideal, as it displays the maximum peak area. The emission setting of 316 nm is robust since a variation of 3 nm does not deviate the area percentage from the allowed limit. The excitation wavelength however, needs to be controlled. A change in buffer concentration is also critical as a deviation of 10% decreases the peak area for both BPA and BPF. Robustness results indicate that the method is reliable for normal usage and to a great extent the performance remains unaffected by deliberately changing method parameters. However, some parameters are critical and must be carefully controlled.

Recovery from sample matrix

BPA-free baby bottles were used as blank matrix. The recoveries of the BPA and BPF were tested by spiking experiments in duplicates. A low standard spike contained BPA (30 ng) and BPF (30 ng) each spiked into 50 mL water extracts of BPA-free bottles. Another high standard spike consisted of BPA (50 ng), BPF (50 ng) each spiked into a 50 mL water extracts from BPA-free bottles. The analytes were extracted from the water sample as described above. Using the aqueous linearity curve (see section Linearity), the area was converted to concentration values. The low and high concentration values were compared against the theoretical value. The recovery experiment results are shown in Table 6. BPA shows a recovery value of 80% at the high concentration value. The value of 80% is higher than the value reported in the ASTM method where an average single laboratory results shows a recovery of 70%.

Compound name	Recovery low conc. (%)	Recovery high conc. (%)
Bisphenol F	70.2	75.9
	70.1	74.1
Bisphenol A	76.9	79.6
	75.1	81.1

Table 6
Recovery results from spiking experiments performed in duplicates.

Parameters	Changes	BPF % area	BPF % RT	Resolution of BPA with unknown	BPA % area	BPA % RT
Flow: 0.9 mL/min ± 2%	High: 0.92 mL/min	-4.6	-1.2	1.9	-5.1	-1.2
	Low: 0.88 mL/min	0.1	1.9	1.9	-1.9	2.2
TCC: 40 °C ± 2.5%	High: 41 °C	-4.2	-0.4	1.7	-5.0	-0.6
	Low: 39 °C	-3.1	0.9	2.1	-10.0	1.3
Injector: 20 µL ± 5%	High: 21 µL	2.6	0.2	1.9	0.0	0.2
	Low: 19 µL	-7.6	0.1	1.9	-9.8	0.1
Wavelength: 230–316 ±3 nm	233–316	-2.2	0.0	1.9	-5.7	0.0
	227–316	-7.0	0.2	1.9	-4.6	0.2
	230–319	-3.2	0.1	1.9	-4.5	0.1
	230–313	-3.5	0.1	1.9	-3.0	0.1
Step gradient starting point: 2 min ±10%	High: 2.2 min	-3.8	2.9	1.9	-4.1	2.0
	Low: 1.8 min	-3.3	-2.4	1.9	-3.8	-1.5
Buffer concentration: 10 mM ±10%	High: 11 mM	-4.2	0.2	1.9	-6.0	0.2
	Low: 9 mM	-5.9	0.1	1.8	-9.8	0.1

Table 5
Robustness test method results compared to the standard method at concentration of 30 ng/mL. The red values in the table indicate that the deviations exceeding the allowed limits of 5% for area and 3% for retention time.

Sample analysis

The content of BPA and BPF in baby bottles was determined using the extraction procedure and the developed chromatographic method. Baby bottles labeled as brand 1, brand 2, and brand 3, were analyzed in duplicates. The results of the analysis were compared against the calibration curve prepared prior to sample analysis. Blank water samples subjected to SPE did not show BPA, suggesting that no BPA leached out of plastics used in the experiment[3]. Different amounts of BPA were detected in the three brands of baby bottles (see Figure 6A). The BPA emission spectra from the standard was overlaid with the spectra from the sample. A good overlap was observed confirming the presence of BPA (see Figure 6B). Different brand analysis showed a high concentration of 4 ng/mL while a low value of 0.5 ng/mL (see Table 7). These values are consistent with those observed earlier by Sun *et al.*, who showed a value of 0.6 ng/mL[5]. If a baby of 10 kg were to drink 250 mL from brand 2 baby bottle, the baby would consume 0.1 µg/kg/day of BPA. This value is less than the reference dose of 50 µg/kg/day established by the EPA but is of concern according to some other studies[6]. The results also show that BPF was not detected in any bottle.

Compound name	BPF (ng/mL)	BPA (ng/mL)
Brand 1	0	0.76
	0	0.52
Brand 2	0	4.26
	0	4.46
Brand 3	0	2.08
	0	2.58

Table 7
Concentration of BPA and BPF extracted in 250 mL water from different brands of baby bottles.

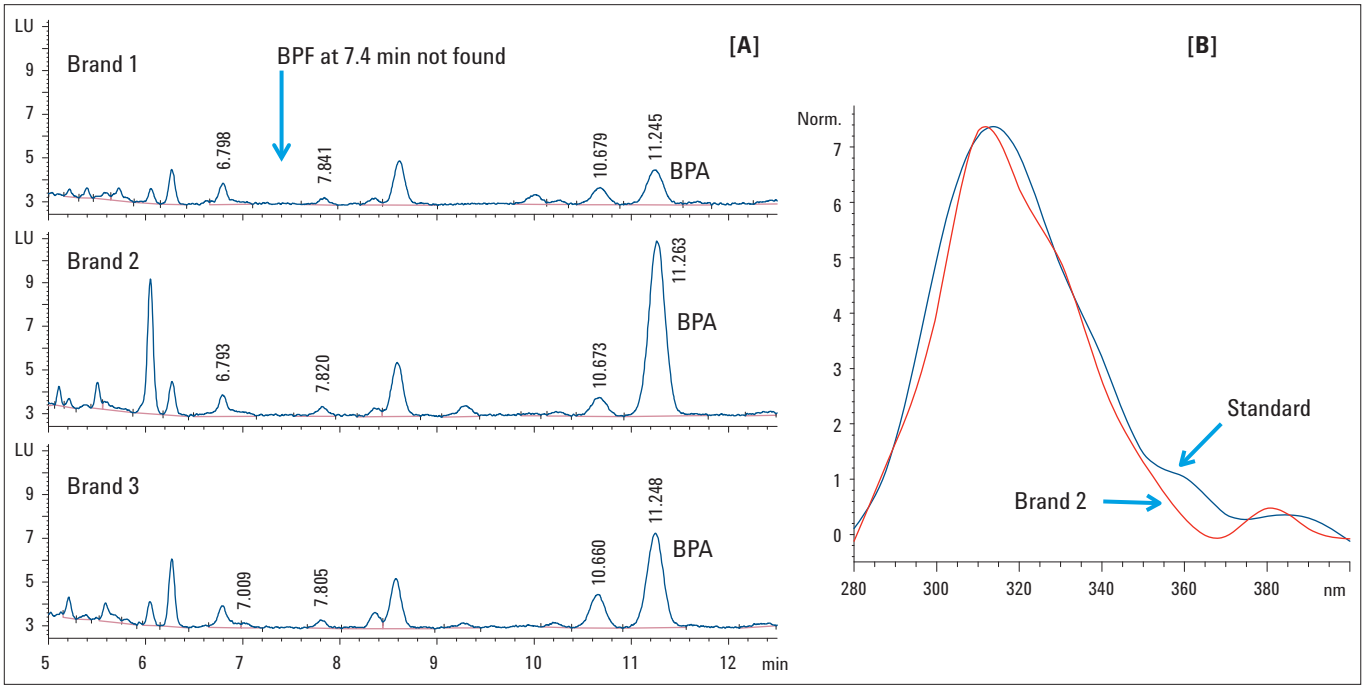

Figure 6
[A] The overlay of chromatogram from three different baby bottles analyzed for BPA and BPF. [B] The overlay of the emission spectrum of BPA from standard and that obtained from brand 2 sample.

UHPLC method

The HPLC method was transferred to an UHPLC method on an Agilent 1290 Infinity LC system keeping the same run time as shown in Figure 7. The transfer was performed to study the effect on resolution and sensitivity. The UHPLC method used the same mobile phase, gradient, and detector settings. The column dimensions were kept the same but the particle size was reduced from 5 µm to 1.8 µm for the UHPLC method. The peaks in the UHPLC method elute about 1.2 minutes earlier, which is due to the lower delay volume in an Agilent 1290 Infinity LC system. The UHPLC method also showed narrower peaks and better resolution compared to the HPLC method. The peak properties such as peak area, peak height, peak width, resolution and S/N at the lowest linearity level L1 and the highest linearity level L7 are compared in Table 8. The results show that resolution of BPA increased from 1.9 in the HPLC method to 2.5 in the UHPLC method. The S/N ratio almost doubled thereby adding sensitivity and allowing the possibility to redefine the LOQ and LOD levels.

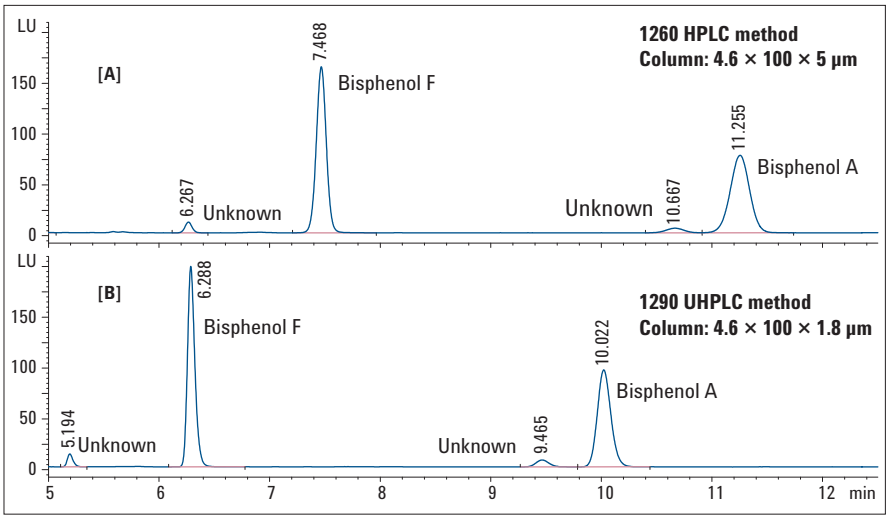

Figure 7

An overlaid chromatogram of the HPLC method [A] and UHPLC method [B] separating the level 7 standards for BPA and BPF on an Agilent ZORBAX Eclipse Plus C18 4.6×100 column. A 5 µm particle size was used for HPLC method while 1.8 µm was used for UHPLC method.

	HPLC method					UHPLC method				
Compound name (Level)	Peak area	Peak width at half height	Peak height	Resolution	S/N	Peak area	Peak width at half height	Peak height	Resolution	S/N
BPF (L7)	1037.0	0.10	163.7	-	3683.0	930.5	0.07	199.6	-	6784.8
BPA (L7)	934.1	0.19	76.3	1.9	1715.7	825.8	0.13	96.4	2.5	3276.7
BPF (L1)	7.5	0.10	1.1	-	27.4	7.2	0.07	1.4	-	42.6
BPA (L1)	8.1	0.20	0.6	1.8	15.1	9.4	0.14	1.0	2.5	31.6

Table 8

Comparison of peak area, peak width at half height, peak height, resolution and S/N ratio between HPLC and UHPLC method from first level and last linearity level. The UHPLC method provides better sensitivity and resolution compared to the HPLC method.

The calibration for BPA and BPF, when using the same calibration levels (see Table 2) was found to be linear - R^2: 0.99991 for BPF and R^2: 0.99993 for BPA. RSD(%) deviation on area and RT was calculated for all concentration levels. The results show that RSD(%) on area deviation was comparatively lower in the UHPLC method. As shown in Figure 8, a value of 3.0% was found for level 1 for BPA. The maximum RSD of RT for both BPA and BPF was less than 0.1%.

Conclusion

Bisphenol A and bisphenol F were separated and quantified using an Agilent 1260 Infinity LC system and an Agilent ZORBAX Eclipse Plus C18 column. Calibration standards were prepared using the Agilent 7696A Sample Prep WorkBench. A method was developed and partially validated. This method quantifies bisphenol A and bisphenol F from various baby bottles with 80% recovery values. The method can be applied to determine BPA and BPF levels for quality control of baby bottles. A method transfer to an Agilent 1290 Infinity LC system was effectively carried out by keeping the same detector and method conditions. Both HPLC and UHPLC methods were linear and give precise results. The UHPLC method however showed better resolution, S/N ratio, narrower peak width and increased peak height compared to the HPLC method.

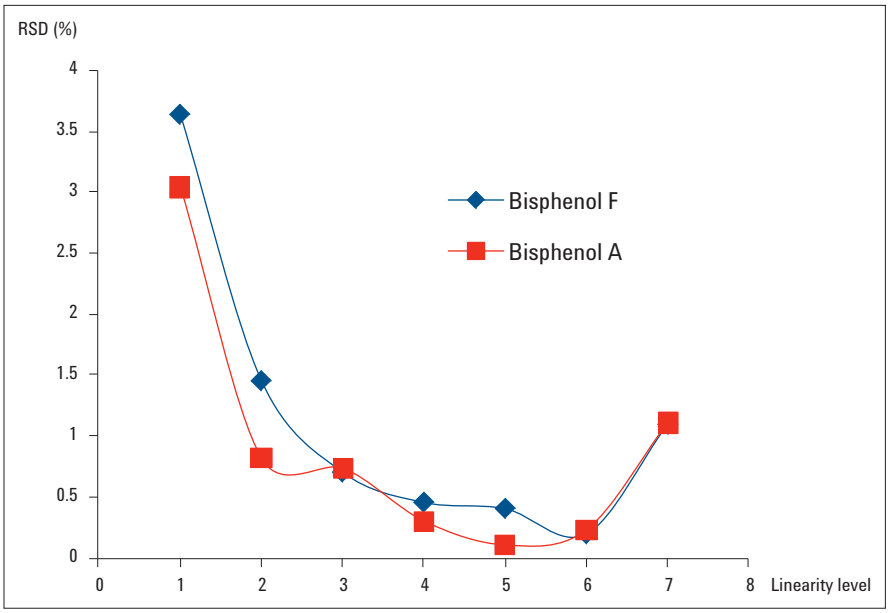

Figure 8
Area precision measured as RSD (%) for BPF and BPA with UHPLC. Six replicates at each concentration level were measured.

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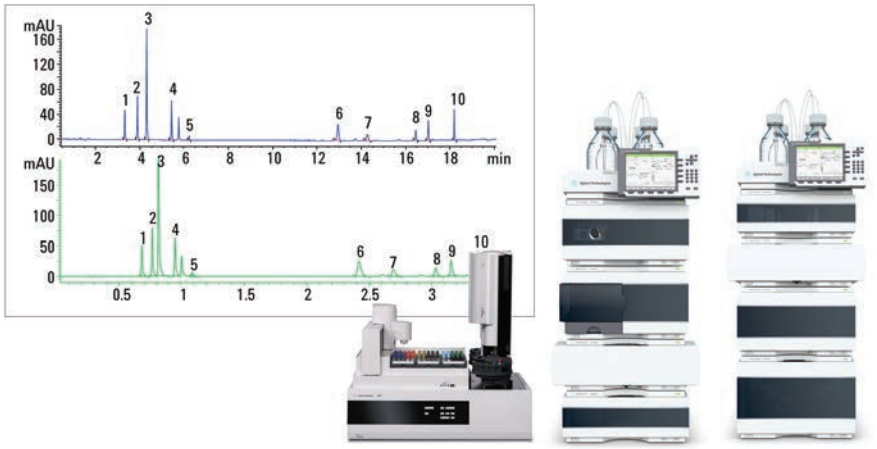
Agilent Application Solution

Analysis of color additives in sweets

Application Note

Food Testing



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Abstract

Synthetic or artificial colors are used as additives in food and drinks to improve the appearance of the product. In this study, a robust reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous determination of 10 synthetic colorants was developed. Separation and quantification was achieved by an Agilent 1260 Infinity LC System using an Agilent Poroshell EC-C18 column. Robustness of the method was established by partial validation. Suitability of this method to quantify artificial colorants from food matrix is demonstrated by analyzing color additives from sweets. Finally, this HPLC method was effectively transferred to a short Ultra High Pressure Liquid Chromatographic (UHPLC) method using an Agilent 1290 Infinity LC System for faster analysis without compromising resolution. With the Agilent 1290 Infinity Diode Array Detector (DAD), various wavelengths were selected to quantify different colorants at their absorbance maxima. The limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and linearity of each colorant were established using both methods. Sample preparation for LOD, LOQ and linearity studies was facilitated by incorporating the Agilent 7696A Sample Prep WorkBench into the analytical workflow.

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Introduction

A color additive is defined as any dye, pigment, or substance which, when added to food, is capable of imparting color[1]. There are natural and synthetic color additives which mainly originate from plants or animals. Turmeric and saffron are two examples of this. Synthetic colors are chemically synthesized colors like tartrazine and indigo carmine[2]. There are many reasons for adding color in food. Adjusting the color loss due to long term storage conditions, correcting the natural variations in color, and providing color to colorless foods are some of them. In fact, color additives are an unavoidable part of most packed foods on the market[1]. It is proven that overexposure to artificial colors beyond the allowed daily intake limit can provoke hyperactivity and other disturbed behavior in children[3]. The Food and Drug Administration (FDA) has regulations to control and ensure the usage of only permitted color additives in food. This underlines the importance of precise analytical techniques to identify and quantify the colorants.

In this Application Note, we developed a reverse phase high pressure liquid chromatography method on an Agilent Poroshell 120 EC-C18 column. The water-solubility of food colorants makes reverse phase HPLC the ideal analysis technique for these substances.

Method

Instruments and software

An Agilent 1260 Infinity Quaternary LC System, consisting of the following modules was used:

- Agilent 1260 Infinity Quaternary Pump and vacuum degasser (G1311B)
- Agilent 1260 Infinity High-Performance Autosampler (G1367E)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B) with Max-Light flow cell (60 mm path length) (G4212-60007)
- Agilent Poroshell 120 EC-C18 column 4.6 x 150 mm, 2.7 µm (693975-902)

The UHPLC analysis was developed and performed using the Agilent 1290 Infinity LC System consisting of:

- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (G4220A) and 100 µL Jet Weaver mixer.
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light flow cell (1.0 µL dispersion volume, 10 mm path length) (G4212-60008)
- Agilent Poroshell 120 EC-C18 columns with internal diameters of 2.1 mm and lengths of 75 mm, packed with 2.7-µm particles (697775-902)

Both systems were controlled using the Agilent ChemStation revision B.04.02.

The dilution series for the linearity levels were prepared using the Agilent 7696A Sample Prep WorkBench.

Reagents and materials

All the chemicals and solvents used were HPLC grade and highly purified water from a Milli Q water purification system (Millipore Elix 10 model, USA) was used. Methanol was of super gradient grade and was purchased from Lab-Scan (Bangkok, Thailand). Disodium hydrogen phosphate and o-phosphoric acid were purchased from Fluka (Germany). Dimethyl sulphoxide (DMSO) was purchased from Qualigens (India). Standards of tartrazine, amaranth, indigo carmine, ponceau 4R, sunset yellow FCF, carmoisine, fast green FCF, acid blue/eryoglaucine, ponceau 3R, and erythrosine B were purchased from Aldrich (India). The sweets for recovery and quantification analysis were purchased locally.

Chromatographic parameters

Chromatographic parameters used for reverse phase liquid chromatography and UHPLC are shown in Table 1.

Colorant standard solution

Standard stock solutions of tartrazine, amaranth, indigo carmine, ponceau 4R, sunset yellow FCF, carmoisine, fast green FCF, acid blue/eryoglaucine, ponceau 3R, and erythrosine B were prepared individually by weighing approximately 20 mg of the standard and transferring it to a 10-mL volumetric standards flask. A 300-µL amount of DMSO was added to each flask and a premixed solution of mobile phase A and B in the ratio 80:20 was used as diluent. Sonication was used when required.

Mixed standard solution and linearity levels

About 100 µL of each standard were precisely mixed with diluent to get a 2,000 µL standard mix of colorants at a concentration of 200 ppm each. Linearity levels were prepared by subsequent serial dilution of this 200 ppm standard mix solution using the Agilent 7696A Sample Prep WorkBench. The linearity standard solutions were covering a range of 0.01 ng/µL to 200 ng/µL (10 levels and 6 replicates).

Parameter	Agilent 1260 Infinity Quaternary LC System	Agilent 1290 Infinity LC System
Column	Agilent Poroshell 120 EC-C18, 4.6 x 150 mm, 2.7 µm, (p/n N693975-902)	Agilent Poroshell 120 EC-C18, 2.1 x 75 mm, 2.7 µm (p/n 697775-902)
Column oven	45 °C	45 °C
Injection volume	5 µL (Needle with wash, flush port active for 5 seconds)	1 µL (Needle with wash, flush port active for 5 seconds)
Sample thermostat	5 °C	5 °C
Mobile phase A	10 mM Na_2HPO_4, pH 7	10 mM Na_2HPO_4, pH 7
Mobile phase B	Methanol	Methanol
Gradient	At 0 min: 5% B	At 0 min: 5% B
	At 4 min: 30% B	At 0.15 min: 5% B
	At 10 min: 40% B	At 0.5 min: 30% B
	At 14 min: 40% B	At 2.3 min: 40% B
	At 18 min: 95% B	At 2.6 min: 40% B
	At 22 min: 95% B	At 3.25 min: 95% B
	At 22.1 min: 5% B	At 4.00 min: 95% B
		At 4.01 min: 5% B
Post run time	5 minutes	1 minute
Flow rate	1.2 mL/min	0.7 mL/min
Flow cell	60 mm path (p/n G4212-60007)	10 mm path (p/n G4212-60008)
Data acquisition	288 nm: Indigo carmine	288 nm: Indigo carmine
	428 nm: Tartrazine	428 nm: Tartrazine
	484 nm: Sunset yellow FCF	484 nm: Sunset yellow FCF
	511 nm: Ponceau 4R and Ponceau 3R	511 nm: Ponceau 4R and Ponceau 3R
	520 nm: Amaranth and Carmoisine	520 nm: Amaranth and Carmoisine
	530 nm: Erythrosin B	530 nm: Erythrosin B
	626 nm: Fast green FCF and Acid blue	626 nm: FastGreen FCF and Acid blue
Aquisition rate	20 Hz, 0.013 min peak width, (0.25 s response time)	80 Hz, 0.003 min peak width, (0.062 s response time)

Table 1
Chromatographic parameters used for the Agilent 1260 Infinity System and the Agilent 1290 Infinity LC System.

Sample preparation for color quantification and recovery studies

Five different types of samples, sweets containing various colors, were used for color quantification and recovery studies. Colors from 2 g sweets were extracted by a simple process using sequential addition of 400 µL DMSO and 20 mL diluent. After sonication and centrifugation at 8,300 rcf for 10 minutes using C0650 rotor on a Beckman Coulter Allegra X22R centrifugation system, the solution was filtered through a 0.25-µm PTFE Agilent Econofilter syringe filter membrane, and used for analysis. Recovery studies were performed using spiked and unspiked samples of sweets. An on-column concentration of 25 ng standard mix was used for sample spiking. The extraction procedure was the same as before.

Precautions

To extend the stability of compounds in solution, all the prepared solutions were wrapped in aluminum foil and stored in a refrigerator at 4 °C in the dark, when not in use. The thermostatted autosampler tray was maintained at 5 °C during the analysis.

Procedure

Calibration levels shown in Table 2, were prepared by subsequent dilution of 200 ng/µL standard mix solution with diluent. The Agilent 7696A Sample Prep WorkBench equipped with a 500 µL syringe was operated in two subsequent sequences to create the linearity levels. In the first sequence, a fixed amount of diluent was added to each vial and in the second sequence, 250 µL of 200 ng/µL solution was added to the vials and vortexed for 15 seconds. Note that instead of running two sequences, the steps can also be programmed in one method and run in one sequence. Serial dilutions were carried out by taking 250/100 µL from the previous level and adding to the next level vial. The syringe parameters used in the setup of the Agilent 7696A Sample Prep WorkBench are given in Table 3. The Agilent 7696A Sample Prep WorkBench[4] setup is well described in the Agilent Application Note, publication number 5990-6850EN.

A 5-µL solution of diluent with DMSO was injected as a blank and followed by each calibration level in six replicates. Area and retention time (RT) information of each level were used to calculate standard deviation (SD) and relative standard deviation (RSD) values. LOD and LOQ were established from the lower linearity level injections. The average area of colorant peaks in each linearity level was plotted against the concentration to construct linearity curves.

Initial concentration (ppm or ng/µL)	Volume taken (µL) (second sequence)	Diluent (µL) prepared (first sequence)	Total vol. (µL)	Concentration of resulting liquid (ng/µL)	On-column with 5 µL injection volume (ng)	Level name
200	250	250	500	100	500	10
100	100	400	500	20	100	9
20	250	250	500	10	50	8
10	100	400	500	2	10	7
2	250	250	500	1	5	6
1	100	400	500	0.2	1	5
0.2	250	250	500	0.1	0.5	4
0.1	100	400	500	0.02	0.1	3
0.02	250	250	500	0.01	0.05	2
0.01	100	400	500	0.002	0.01	1

Table 2
Dilution details for calibration level preparation.

Parameter	Solvent prewash 1	Dispense wash	Dispense pumps	Dispense settings
Number pumps or washes	1	1	2	
Wash volume (µL)	250	250	50	
Draw speed (µL/min)	500	500	500	500
Dispense speed (µL/min)	2500	2500	2500	2500
Needle depth offset (mm)	-1	-1	-1	-1
Viscosity delay (s)	1	1	1	1
Turret solvent	A			
Air gap (%syr.vol)	0			0

Table 3
Agilent 7696A Sample Prep WorkBench syringe parameters.

Six critical method parameters were changed to evaluate the robustness of the method. A standard mix of about 30 ng (on-column) of each colorant was injected in six replicates and data was used for studying the robustness of the method. Recovery studies were performed by injecting with and without spiking 25 ng color additive standard to 2 g sweets. Using the characteristic spectra of all ten color standards, a UV spectral library was created. Along with the retention times this library was used to identify color additives in sweets.

The method was effectively transferred to UHPLC. LOD, LOQ, and linearity of each colorant were evaluated and precision of the method was established by Area and RT RSD. Linearity curves for all colors using the UHPLC method were also plotted. The UHPLC method allows the analysis to be performed much faster without compromising on resolution.

Results and discussion

Separation and detection

Excellent separation of 10 colorants in 20 minutes was achieved using an Agilent Poroshell 120 EC-C18 (150 mm x 4.6 mm, 2.7 µm) column. The absorbance maximum was found to be different for different colors. The chromatographic elution patterns of 10 colors are shown in Figure 1 and the list of colors with individual absorbance maxima are shown in Table 4. We used the peak purity feature in the ChemStation software to check the purity of each peak and thus the specificity of the method was evaluated. Precision, linear range, accuracy, specificity, recovery, and robustness studies were done to validate the method.

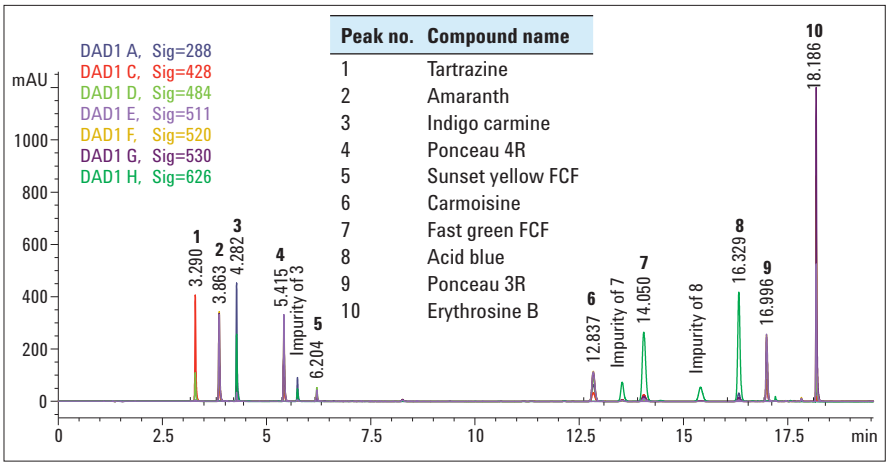

Figure 1
Separation of 10 colorants using a 15-cm Agilent Poroshell 120 EC-C18 column. Traces from seven different wavelengths are overlaid.

Sl no.	Compound name	Molecular formula	Molecular weight	Retention time	Absolute maximum
1	Tartrazine	$C_{16}H_9N_4Na_3O_9S_2$	534.36	3.29	428
2	Amaranth	$C_{20}H_{11}N_2Na_3O_{10}S_3$	604.47	3.86	522
3	Indigo carmine (Indigotine)	$C_{16}H_8N_2Na_2O_8S_2$	466.35	4.28 (imp 5.74)	288 and 612
4	Ponceau 4R (Ponceau SX)	$C_{20}H_{11}N_2Na_3O_{10}S_3$	604.47	5.41	510
5	Sunset yellow FCF	$C_{16}H_{10}N_2Na_2O_7S_2$	452.37	6.20	482
6	Carmoisine	$C_{20}H_{12}N_2Na_2O_7S_2$	502.43	12.83	518
7	Fast green FCF	$C_{37}H_{34}N_2O_{10}S_3Na_2$	808.85	14.04 (imp 13.52)	622
8	Acid blue / Eryoglaucine	$C_{37}H_{34}Na_2N_2O_9S_3$	792.85	16.32 (imp 15.40)	628
9	Ponceau 3R	$C_{19}H_{16}N_2Na_2O_7S$	494.45	16.99	512
10	Erythrosine B	$C_{20}H_8I_4O_5$	835.89	18.18	530

Table 4
List of colors and observed absorbance maxima for each color.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The analyte concentration that provides a signal-to-noise ratio (S/N) of greater than three was considered as LOD and analyte concentration with S/N greater than 10 was considered as LOQ. Observed LOD and LOQ values of each color are shown in Table 5. As an example, the overlay of LOQ chromatograms of ponceau 4R (0.1 ng on-column) with blank is shown in Figure 2.

Linearity

All the prepared linearity levels were injected in six replicates and linearity curves for each color were constructed from the LOQ level to a highest concentration level using area response and concentration values. The observed regression coefficients for all colors are shown in Table 5.

Peak number	Compound name	LOD (ng)	LOQ (ng)	Total levels (n=6)	On–column linearity range (ng)	Linearity equation	R^2 value
1	Tartrazine	0.05	0.1	8	0.1 to 100	y = 15.477x - 5.7137	0.9993
2	Amaranth	0.1	0.25	7	0.25 to 100	y = 12.686x - 5.8682	0.9993
3	Indigo carmine	0.05	0.1	8	0.1 to 100	y = 16.723x - 5.9163	0.9993
4	Ponceau 4R	0.05	0.1	8	0.1 to 100	y = 13.168x - 5.0258	0.9993
5	Sunset yellow FCF	0.25	0.5	8	0.5 to 1000	y = 1.8621x + 7.2227	0.9992
6	Carmoisine	0.25	0.5	8	0.5 to 1000	y = 10.018x + 41.05	0.9993
7	Fast green FCF	0.1	0.25	7	0.25 to 100	y = 31.981x - 14.22	0.9993
8	Acid blue	0.05	0.1	8	0.1 to 100	y = 36.351x - 12.193	0.9994
9	Ponceau 3R	0.1	0.25	9	0.25 to 1000	y = 11.324x + 39.972	0.9992
10	Erythrosine B	0.05	0.1	8	0.1 to 100	y = 40.628x - 10.168	0.9997

Table 5
LOD, LOQ and linearity results of all 10 colors. A 0.25 ng on-column concentration was achieved by injecting 2.5 µL of 0.1 ng/µL standard solution.

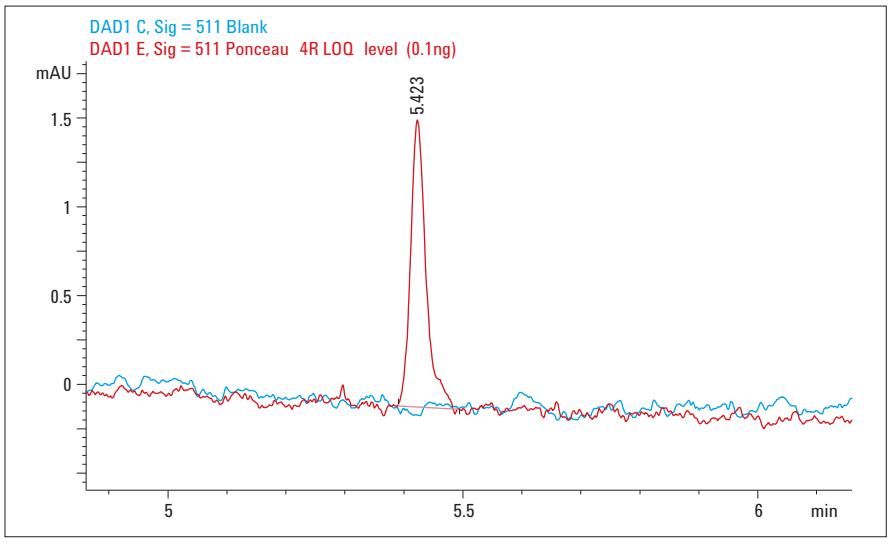

Figure 2
LOQ (0.1 ng) chromatograms of ponceau 4R overlaid with blank.

Precision of retention time and area

To establish the method precision, relative standard deviation (RSD) values for retention time (RT) and area of all 10 colors at 1, 10, and 100 ng (on-column) concentration were calculated. The highest observed area RSD value was 1.19% (for Carmoinsine at 1 ng) and RT RSD was 0.09% (for Tartrazine at 1 ng). Graphical representation of area RSD values of 10 colors is shown in Figure 3 and RT RSD values are shown in Figure 4.

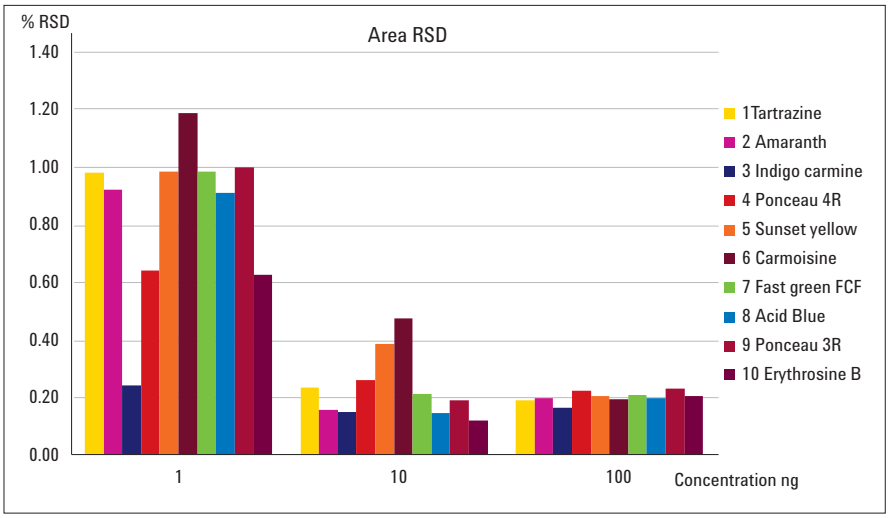

Figure 3
Excellent area RSD values for all colors at 1 ng, 10 ng, and 100 ng (on-column) concentration.

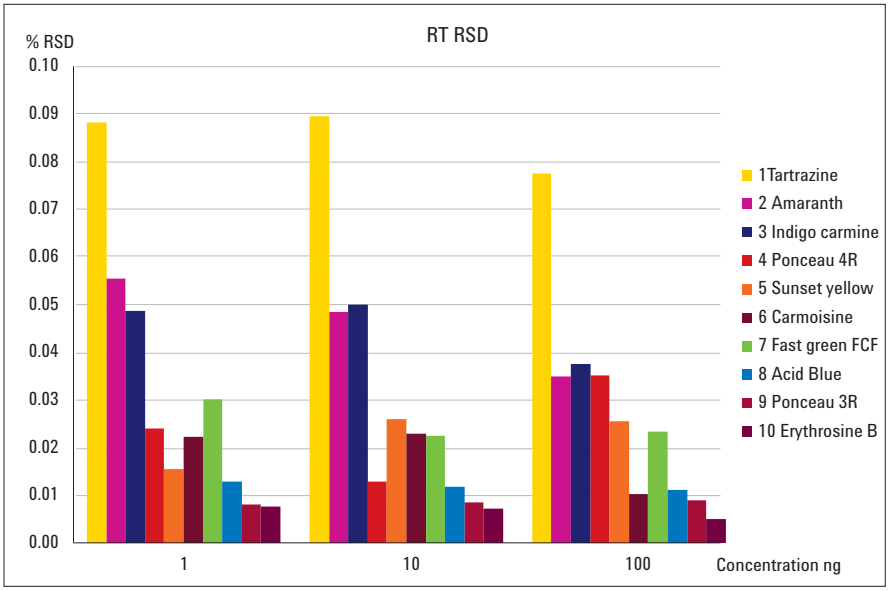

Figure 4
Excellent RT RSD values for all colors at 1 ng, 10 ng, and 100 ng (on-column) concentration.

Robustness

Robustness of the method was evaluated by deliberately varying six critical method parameters. The resulting deviation in area and retention time was calculated and compared to the original method. A standard spike mix solution of color standards was injected in six replicates. Allowed deviations for retention time and area were set to ±3% and ±5% respectively. The robustness test conditions used in this study are noted in Table 6 and results from robustness study are summarized in Figures 5 and 6.

Sl no.	Parameter (actual value)	Measured deviation	Modified value
1	Flow rate (1.2)	2%	1.224 mL/min 1.176 mL/min
2	Injection volume (5 µL)	2%	5.1 µL 4.9 µL
3	Wavelength (288, 428, 484, 511, 520, 530, 626 nm)	(±) 3 nm	Wavelength (291, 431, 487, 514, 523, 533, 629 nm) Wavelength (285, 425, 481, 508, 517, 527, 623 nm)
4	Ph (7.0)	(±) 0.15	10 mm Buffer pH 7.15 10 mm Buffer pH 6.85
5	Column temperature (45 °C)	(±) 2 °C	47 °C 43 °C
6	Gradient steepness (6.25, 5 to 30 in 4 minutes and 13.75, 40 to 95 in 4 minutes)	~10%	6.75, 5 to 32 in 4 minutes and 14.25 for 38 to 95 in 4 minutes 5.75, 5 to 28 in 4 minutes and 13.25 for 42 to 95 in 4 minutes

Table 6
Robustness test conditions used in this study.

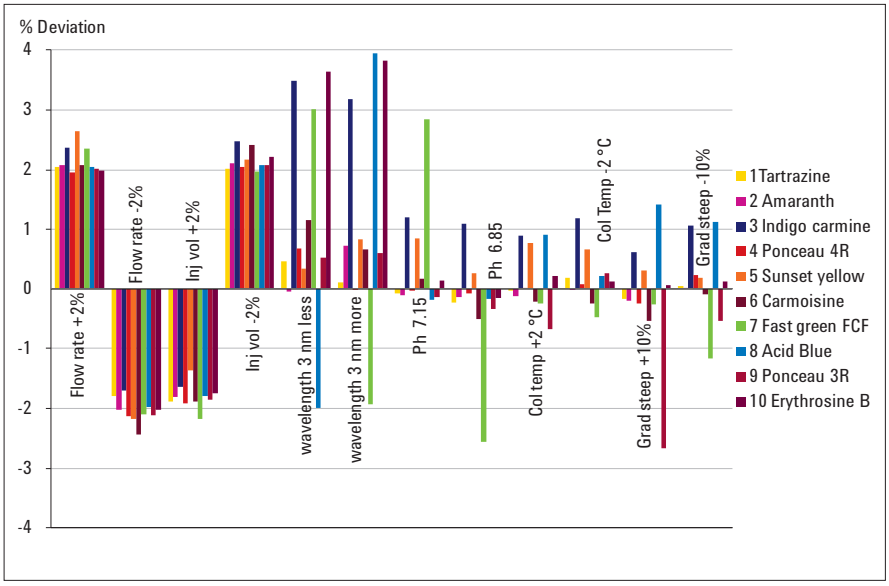

Figure 5
Robustness test result summary for area.

The area deviations for all 10 colors were found to be within the allowed limit for all the varied parameters. Also, retention time deviation for flow rate, injection volume, and pH of mobile phase was found to be within the allowed limit for this robustness study. However, the impact of increased column temperature on RT deviation exceeded the allowed limit for two compounds. With a decrease in column temperature, RT deviation for three compounds crossed the allowed limit. One critical parameter which has considerable impact on retention time was found to be gradient slope. We observed that more than five compounds were showing a RT deviation beyond the allowed limit with a ±10% change in gradient slope. Robustness results indicate that the method is reliable to use for normal usage and the performance remains unaffected to a great extent by deliberate change in parameters.

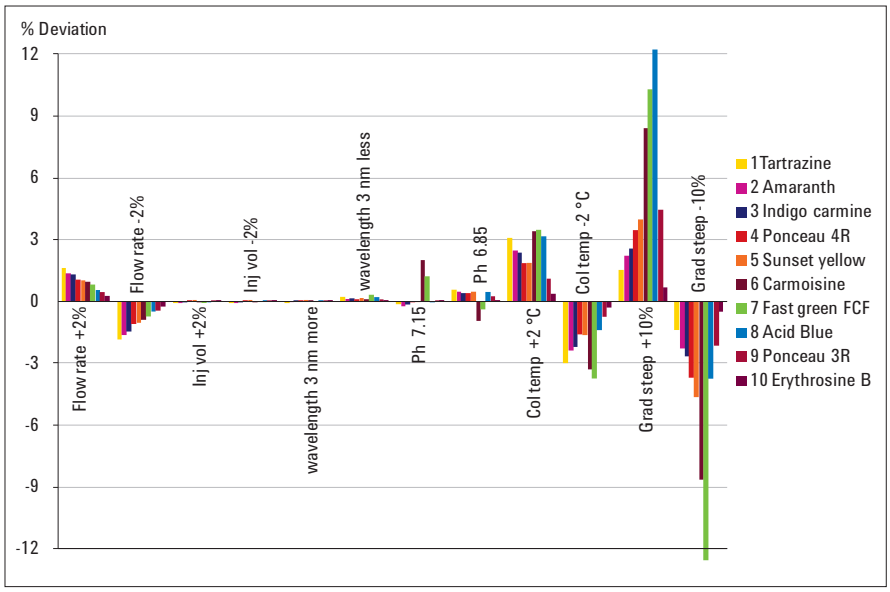

Figure 6
Robustness test result summary for retention time.

Recovery of colorants from sweets

Recovery analyses for various colorants from five different colored sweets were carried out by a standard addition method[5]. A standard mix solution of all ten colorants at 25 ng (on-column) was used for this analysis. The peak area of the individual colorants in the spiked sample, unspiked sample, and standard chromatogram were measured separately. The difference in detector response between spiked and unspiked sample was compared against response observed in standard chromatogram and expressed in percentage as recovery. The recovery for all colorants from sweets were greater than 98%. Chromatograms observed for spiked or unspiked extracted samples from red sweets and standard mix solutions are shown in Figure 7.

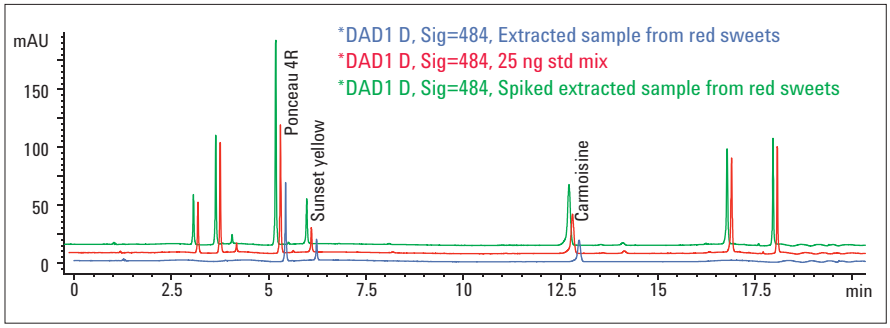

Figure 7
Overlay of spiked, unspiked extracted sample from red sweets and standard mix.

Quantitation of color additives in sweets

Color additives present in various colored sweets were determined using the area response. Linearity equations originating from linearity curves were used for the calculation. In addition, the in-house created UV spectral library was used to identify the compounds using spectral matching. The calculated amounts of colorants from 1 g of five different sweets are tabulated in Table 7. The observed spectral match for Ponceau 4R peak from red sweet with library spectra is shown in Figure 8.

Item number	Color of the sweets	Components	Amount present (µg/g)
Sweet_1	Blue	Acid blue	44.7
Sweet_2	Yellow	Tartrazine	61.7
Sweet_3	Green	Tartrazine	52.5
		Acid blue	10.9
Sweet_4	Orange	Tartrazine	24.8
		Ponceau 4R	26.9
		Sunset yellow FCF	43.3
Sweet_5	Red	Ponceau 4R	27.5
		Sunset yellow FCF	38.3
		Carmoisine	20.6

Table 7
The calculated amounts of colorants from 1 g of sweets.

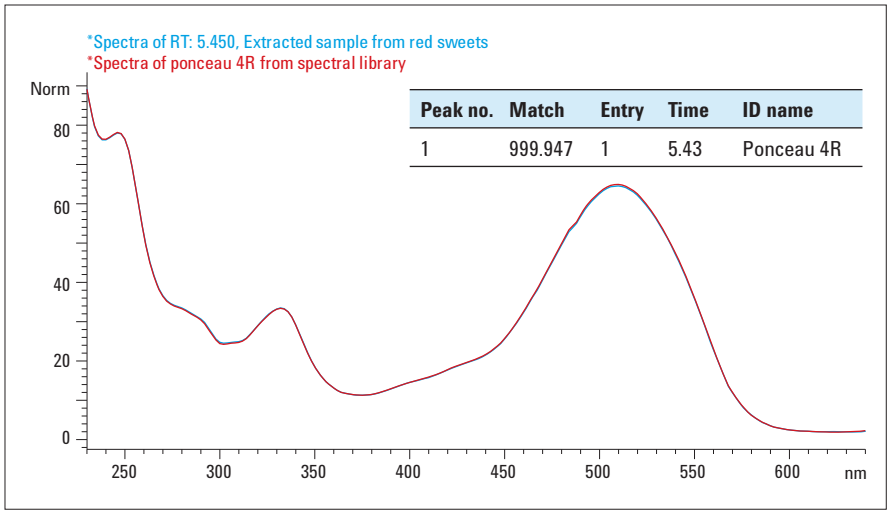

Figure 8
Spectral match for Ponceau 4R peak from red sweet with library spectra.

UHPLC method

A UHPLC method was developed for the separation of ten colorants with diode array detection. The UHPLC method shows excellent resolution and saves about 81% analysis time and 89% solvent compared to the 21-minute HPLC gradient (Figure 9). The resolution value between the fast green FCF peak and its impurity (peak at 13.526) was found to be the lowest of all peaks in the HPLC method, so this resolution was monitored in the UHPLC results to evaluate the overall resolution of peaks in a short run time. With the HPLC method, this resolution was 3.71 and with the short UHPLC method this value was greater than 1.8. The observed LOD, LOQ, and linearity results obtained with the UHPLC method are shown in Table 8. To evaluate the precision of the method, RSD values for RT and area for an on-column concentration of 10 ng were calculated. The highest observed Area RSD was 0.84% and the RT RSD was 0.04% —both for sunset yellow. The results are shown in Figure 10. Low RSD values for area and RT confirmed the precision of the method. These results prove the reliability of the developed UHPLC method. Quick quantification of colors from sweet samples is possible using this method.

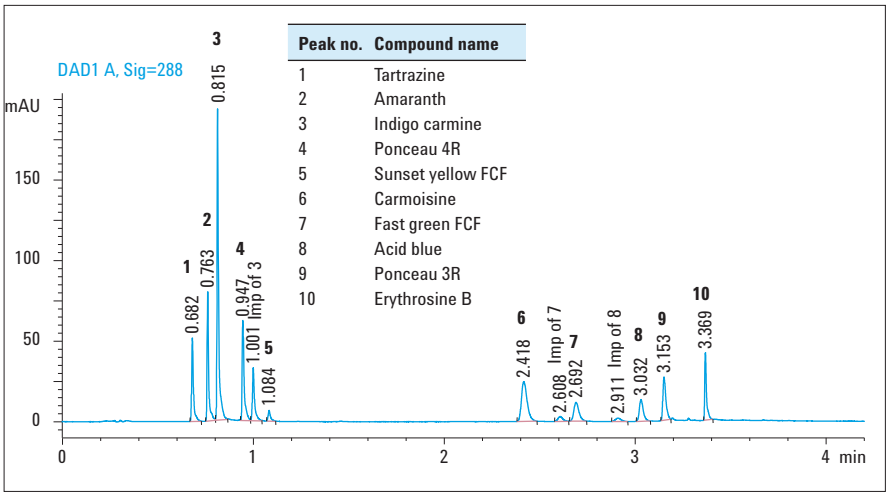

Figure 9
Separation of ten colorants using UHPLC method on the Agilent 1290 Infinity LC System.

Peak number	Compound name	LOD (ng)	LOQ (ng)	Total levels (n=6)	On–column linearity range (ng)	Linearity equation	R^2 value
1	Tartrazine	0.05	0.1	9	0.1 to 200	y = 4.6746x + 2.5573	0.9998
2	Amaranth	0.1	0.25	8	0.25 to 200	y = 3.7682x + 0.585	0.9996
3	Indigo carmine	0.05	0.1	9	0.1 to 200	y = 4.3278x + 3.0266	0.9998
4	Ponceau 4R	0.1	0.25	8	0.25 to 200	y = 3.9616x + 1.4427	0.9997
5	Sunset yellow FCF	0.5	1	6	1 to 200	y = 0.6479x + 0.8958	0.9993
6	Carmoisine	0.25	1	6	1 to 200	y = 3.8231x + 0.5447	0.9996
7	Fast green FCF	0.1	0.25	8	0.25 to 100	y = 9.008x + 3.0979	0.9998
8	Acid blue	0.1	0.25	8	0.25 to 100	y = 10.083x + 14.681	0.9991
9	Ponceau 3R	0.1	0.25	8	0.25 to 200	y = 4.1461x + 0.4156	0.9995
10	Erythrosine B	0.05	0.1	9	0.1 to 100	y = 11.354x + 11.912	0.9996

Table 8
LOD and LOQ values derived from the UHPLC method using the Agilent 1290 Infinity LC System.

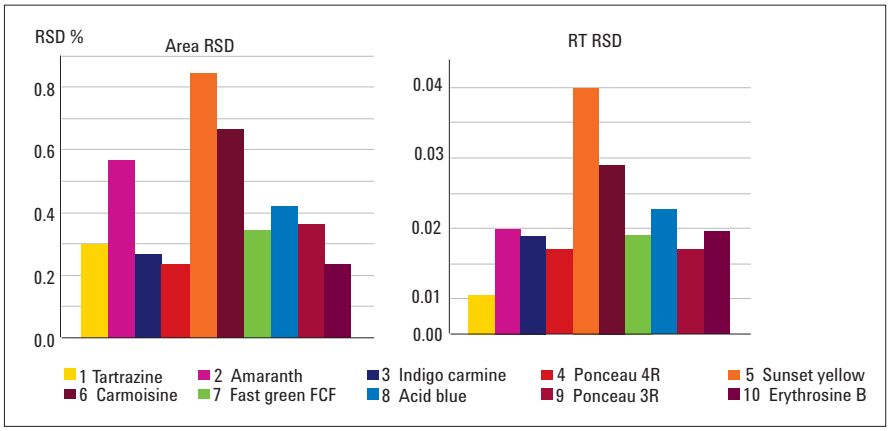

Figure 10
Area and RT RSD values from UHPLC results for all 10 colors at an on-column concentration of 10 ng level. Injection volume is 1 µL (six replicates).

Conclusion

Ten colorants were separated and quantified using an Agilent Poroshell 120 EC-C18 column. With the Agilent 1260 Infinity LC System, a robust, 20-minute HPLC gradient method was developed. The method was partially validated to demonstrate the usability to quantify colors such as tartrazine, amaranth, indigo carmine, ponceau 4R, sunset yellow FCF, carmoisine, fast green FCF, acid blue/eryoglaucine, ponceau 3R, and erythrosine B. The method is simple, specific, sensitive, rapid and also provides good precision, linearity, and recovery values. Efficient usage of this method was established by quantifying colorants from five different colored sweet matrices. Later, this method was transferred to a short 4-minute UHPLC method using the Agilent 1290 Infinity LC System, which saves about 81% analysis time and 89% solvent. These methods using the Agilent 1260 and 1290 Infinity LC systems can be used for accurate routine analysis of colorants. The Agilent 7696A Sample Prep WorkBench simplified the sample preparation for linearity studies. The excellent linearity results confirm that, the result obtained from the Agilent 7696A Sample Prep WorkBench is very precise, and reduces operator error.

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Agilent Technologies

Determination of Fatty Acid Methyl Esters (FAMEs) in Salmon Oil Using Automated Sample Preparation

Application Note

Area

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Introduction

The automated derivatization of fatty acids (FAs) was performed with the Agilent 7696A Sample Prep WorkBench. Since free fatty acids show tailing in gas chromatography, transformation of fatty acids into fatty acid methyl esters (FAMEs) is widely used. Manual sample derivatization is time-consuming and may lead to poor repeatability. Automated derivatization shows significant enhancement of reproducibility and saves time. Especially for highly unsaturated fatty acids, slight variations in reaction temperature and time can negatively affect repeatability when using manual procedures.

Salmon oil is an excellent source of polyunsaturated omega-3 fatty acids. The two main fatty acids–eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - have been identified as important health factors and are correlated with a normal function of the heart. The concentration of EPA and DHA is the crucial quality factor for salmon oil capsules. This application note demonstrates the use of the Agilent 7696A Sample Prep WorkBench for derivatization and subsequent determination of both EPA and DHA from salmon oil capsules.

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Materials and Methods

For sample preparation, 10 mg of salmon oil was weighed into a 2-mL autosampler vial. The sample was diluted in 500 µL of tert-butyl methyl ether (TBME), using the liquid dispensing module of the Agilent 7696A Sample Prep WorkBench and mixed for 90 seconds with the onboard vortex mixer. A 250-µL aliquot of the prepared sample was tranferred to an empty vial and 125 µL of a Trimethylsulfoniumhydroxide (TMSH) derivatization solution [MachereyNagel, Düren] was added and the mixture was again mixed using the vortex mixer of the WorkBench. The mixture was heated for 5 minutes at 80 °C in the single vial heater. The flow diagram for the automated procedure on the Agilent 7696A Sample Prep WorkBench is in shown in Figure 1.

The gas chromatographic conditions were chosen as shown in Table 1.

Table 1. GC/FID Conditions

Peak identification

C14:0	Myristic acid
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C20:0	Arachidic acid
C18:3	γ-Linolenic acid
C20:1	Gadoleic acid
C18:3	Linolenic acid
C22:1	Erucic acid
C20:4	Arachidonic acid
C20:5	Eicosapentaenoic acid
C24:1	Nervonic acid
C22:6	Docosahexaenoic acid

GC Conditions

Instrument	Agilent 6890 Series GC
Column	HP 88, 100 m × 250 µm, 0.20 µm
Injection volume	2 µL
Injector	Split/Splitless, Split 50:1
Carrier gas	H_2
Temperature-program	70 °C–260 °C
Flow	1.4 mL/min
Detector	250 °C, FID
	H_2 flow: 40 mL/min
	Air flow: 450 mL/min
	Makeup flow, N_2: 45 mL/min

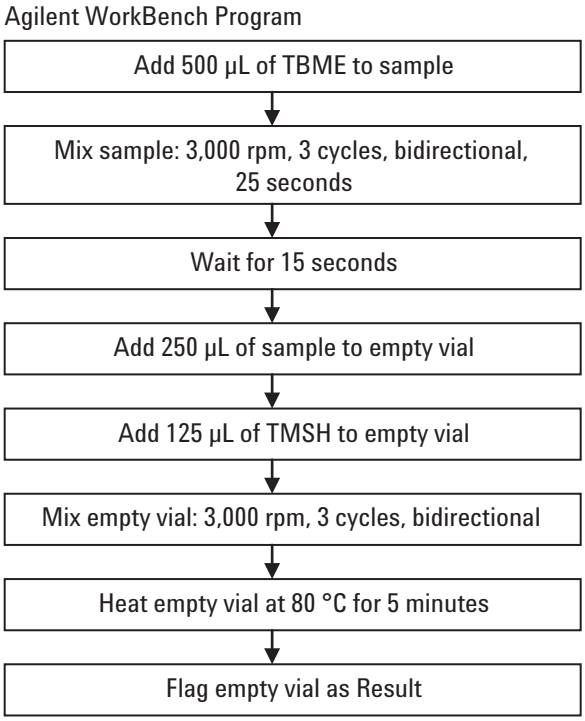

Figure 1. Flow diagram of FAME sample preparation with the Agilent 7696A Sample Prep WorkBench.

Results and Discussion

Figure 2 shows the separation of FAMEs from salmon oil on an Agilent 7696A WorkBench. The separation allows the unequivocal identification of all FAMEs. The two compounds of main interest show retention times of 35.07 minutes (EPA) and 40.55 minutes (DHA). Besides EPA (23.7%) and DHA (20.0%) salmon oil further consists of unsaturated fatty acids oleic (12%), linoic (11%) and palmitoleic (8%) acid. The content of saturated fatty acids, palmitic and stearic acid, is low, 4% and 5% respectively.

For the repeatability test, 10 individual salmon oil samples were derivatized and analyzed to determinate the reproducibility of the automatic sample preparation and chromatography. As shown in Figure 3, excellent repeatability was obtained.

The absolute areas of the EPA and DHA signals showed standard deviations of less than 1% (EPA 0.51%, DHA 0.78%). Moreover, variations of the EPA and DHA relative concentrations were stable. Relative standard deviations of 0.85% for EPA and 1.22% for DHA were achieved. No outliers were observed over the 10 samples.

The total runtime for sample preparation on the Agilent 7696A Sample Prep WorkBench was only 20 minutes per sample, whereas the time for the manual derivatization depends on the skills of the laboratory technician and can take up to 2 hours.

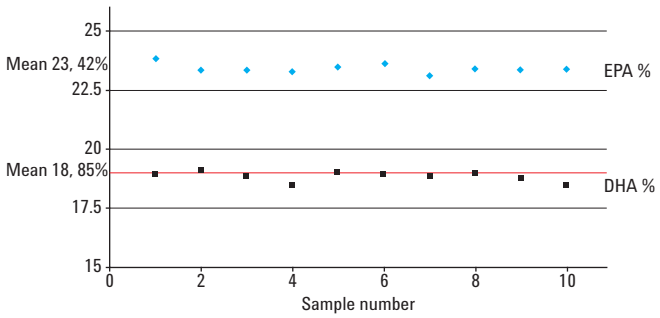

Figure 3. Repeatability data of of automated FAME determination in salmon oil.

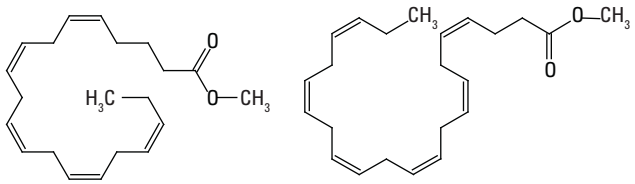

Figure 4. Structure of EPA methyl ester (left) and DHA methyl ester (right).

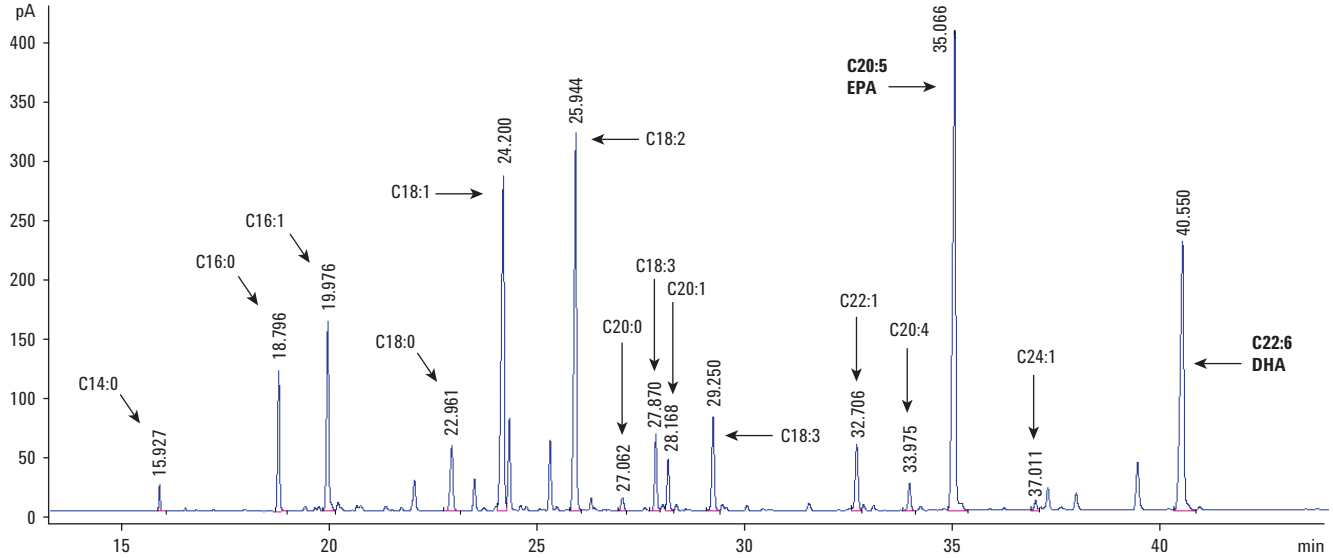

Figure 2. GC/FID chromatogram of a salmon oil sample, prepared using Agilent WorkBench 7696A.

Table 2. Percent Distribution of Fatty Acids in Salmon Oil Sample

C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C18:3	C22:1	C20:4	C20:5	C24:1	C22:6
0.71	4.68	7.95	3.54	12.95	13.86	0.36	2.61	1.71	3.33	3.35	0.87	23.79	0.36	19.93

Conclusion

The automated sample derivatization is easy, fast, and reliable. For samples with high relative concentrations of polyunsaturated fatty acids especially, the automation is significantly more reliable than manual procedures.

Reference

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Agilent Technologies

Quantification of stevioside and rebaudioside A in *Stevia rebaudiana* Bertoni leaves using the Agilent 1260 Infinity LC

Application Note

Food Testing & Agriculture

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Abstract

In this Application Note, we describe the quantification of stevioside and rebaudioside A in *Stevia rebaudiana* Bertoni leaf extracts using the Agilent 1260 Infinity LC. The Agilent ZORBAX carbohydrate column was found to be the most suitable for the separation of the two analytes. The linear dynamic ranges were determined after validating the robustness of critical method parameters. The Agilent 7696A Sample Prep WorkBench was used for the preparation of calibration standards. Both analytes showed good linearity from 1 to 1,000 µg/mL with the R^2 values being > 0.9999. Both the LOD and LOQ values of the two compounds were determined to be 1.0 µg/mL. Good recoveries were obtained for the spiked samples.

Introduction

Diterpene glycosides (stevioside and rebaudioside) extracted from the leaves of *Stevia rebaudiana* Bertoni plants are used in food and beverages as substitutes for synthetic sweeteners. As these compounds are non-nutritive (zero-calorie) and have a high potency (stevioside is 300 times and rebaudioside is 400 times sweeter than sucrose), the leaf extract has been traditionally used in the treatment of diabetes [1, 2]. It is important to characterize the Stevia extract to determine the relative amounts of the various glycosides, which impacts the quality of the product. In a previously published Application Note, the quantification of these two diterpene glycosides using the Agilent ZORBAX carbohydrate column in the range 70 and 700 µg/mL has been demonstrated [3]. In this Application Note, we describe the partial validation and quantification of stevioside and rebaudioside A up to 1,000 µg/mL in *Stevia rebaudiana* Bertoni leaf extract using the Agilent 1260 Infinity LC System.

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Method

Instruments and software

The Agilent LC system consisted of the following modules:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Degasser (G1379B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Agilent 1260 Infinity Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity DAD (G4212B), with Max-Light 60-mm high sensitivity flow cell

Software: Agilent ChemStation revision B.04.03

Sample preparation: Agilent 7696A Sample Prep WorkBench

Reagents and materials

Rebaudioside A, stevioside and *Stevia rebaudiana* Bertoni leaves (Sigma), and acetonitrile (Labscan) were used in this study.

Chromatographic parameters

Column	Agilent ZORBAX Carbohydrate Analysis Column 4.6 × 150 mm, 5 µm (p/n 843300-908)
Mobile phases	A: Water 30% B: ACN 70%
Injection volume	5 µL
ALS thermostat	6 °C
Flow rate	1.0 mL/min (isocratic analysis)
Column temperature	30 °C
Detection	205 nm, 4 nm BW; Ref: No; PW > 0.25 s (20 Hz)

Standards

Stock solutions were prepared in a mix of 30% water and 70% acetonitrile. The method was validated using a solution containing 100 µg/mL of each analyte. To prepare calibration curves, solutions containing 0.5, 1.5, 2.5, 5, 10, 25, 50, 100, 250, 500, 1,000 µg/mL of each standard in 30% water and 70% acetonitrile were used.

The Agilent 7696A Sample Prep WorkBench[4] was used serially to dilute the 2000 µg/mL stock solutions of rebaudioside A and stevioside to obtain a series of calibration standards containing 1.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1,000 µg/mL of each standard in 30% water and 70% acetonitrile mix. Table 1 shows the syringe parameters used in the WorkBench method.

Sample preparation

Stevia rebaudiana Bertoni leaves were crushed and approximately 0.1 g of the powder was weighed into a 20 mL glass vial. Ten milliliters of 30% water and 70% ACN mix was added to the vial which was then vortexed. The extraction was carried out by sonication for 60 minutes. The contents of the vial were centrifuged and the supernatant diluted 10 times with 30% water and 70% ACN mix. To an aliquot of the 10 times diluted sample, we added 2,000 µg/mL stock solutions of rebaudioside and stevioside to test analyte recoveries. The final concentration of each analyte in the spiked sample was 100 µg/mL. Five microliters of the unspiked and spiked sample solutions were injected for analysis.

Results and discussion

Separation

After testing several stationary phases and chromatographic conditions, the Agilent ZORBAX carbohydrate analysis column was found to be the most suitable for the analysis of rebaudioside A and stevioside. A typical chromatogram of the 100 µg/mL calibration standard is shown in Figure 1.

	Solvent prewash 1	Dispense wash	Dispense pump	Dispense setting
Number of washes	1	1	2	-
Wash volume (µL)	100	50	50	-
Draw speed (µL/min)	1,000	200	200	200
Dispense speed (µL/min)	1,000	200	200	200
Needle depth offset (mm)	0	-2	-2	-2
Viscosity delays (s)	-	0	0	0
Turret solvent	A	-	-	-
Air gap (% syringe vol.)	-	-	-	0

Table 1
500 µL syringe parameters.

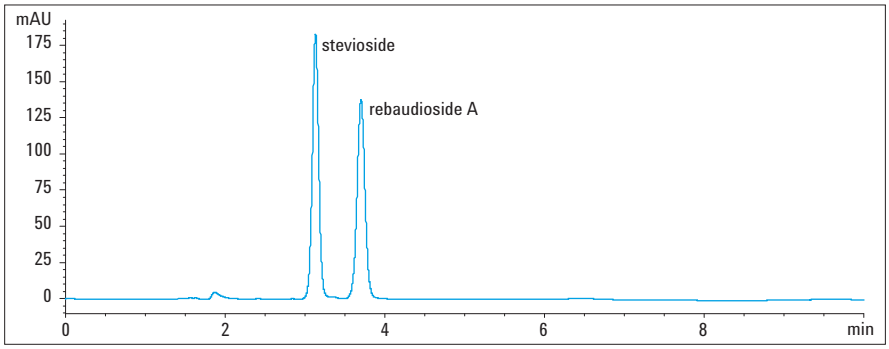

Figure 1
Chromatogram of the 100 µg/mL calibration standard.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ values were determined by dividing the peak heights by peak to peak noise between 0.1–0.4 minutes. It was found that the stevioside and reabudioside A peaks at the 1 µg/mL have signal-to-noise values of 56.2 and 43.7. As no significant peaks were observed for the 0.5 µg/mL level, 1 µg/mL was chosen as the LOD and LOQ for the method.

Linearity

The calibration samples prepared using the Agilent 7696A Sample Prep WorkBench were used to test the method linearity. The area responses plotted against the concentration values were found to be linear between 1.0 and 1,000 µg/mL for both the analytes. The calibration data for stevioside and rebaudioside A are given in Table 2. We observed an improvement in the R^2 values when the calibration standards were prepared using the 7996A Sample Prep WorkBench instead of manual dilution.

Precision of retention times and areas

Calibration samples containing 0.5 to 1,000 µg/mL of both analytes were prepared by manual dilution. Very small peaks that could not be integrated were observed for the 0.5 µg/mL calibration sample. All the other calibration standards were injected 10 times and the last six replicates were used to calculate the RSD values of peak areas and retention times. The RSDs for the retention times of both compounds at all calibration levels were found to be ≤ 0.13%. The peak area RSDs for the two compounds at the various concentration levels were < 4% except for 1.5 and 2.5 µg/mL levels of stevioside at which the RSDs were > 5%.

Robustness

After the preliminary method development, a set of method parameters were systematically varied to test the robustness of the method. As a readout of the impact of parameter variations on the results, we monitored the deviations in retention times and peak areas. Table 3 shows the percentage deviations observed for the retention times and peak areas of the analytes as the parameters were varied. It was observed that the deviations in the retention times were well within the set limit of 3% for all the parameter changes while the deviations in the peak areas were within the set limit of 5% for all the parameters changes except the detection wavelength.

Analyte	R^2	Linear regression
Stevioside	0.99991	Area = 9.86* Amount + 20.18
Rebaudioside	0.99995	Area = 9.50* Amount + 13.30

Table 2
Calibration data for Stevioside and Rebaudioside A standards prepared using the Agilent 7696A Sample Prep WorkBench.

	Stevioside		Rebaudioside A	
Parameter changed	Deviation in the retention time (%)	Deviation in the area (%)	Deviation in the retention time (%)	Deviation in the area (%)
Flow - 2% (0.98 mL/min)	2.51	1.36	2.75	1.79
Flow + 2% (1.02 mL/min)	-2.31	-2.55	-2.48	-2.48
Column temperature - 5% (28.5 °C)	-0.33	0.33	-0.47	-0.22
Column temperature + 5% (31.5 °C)	-1.04	-0.73	-1.5	- 0.96
Injection volume - 5% (4.8 µL)	-0.54	-3.81	-0.93	-3.73
Injection volume + 5% (5.2 µL)	-0.85	4.33	-1.36	3.76
Detection wavelength - 3 nm (202 nm)	-1.16	19.09	-1.78	17.06
Detection wavelength + 3 nm (208 nm)	-1.30	-31.33	-2.01	-31.40

Table 3
Method robustness: Effect of method parameter changes on the retention times and peak areas.

Recovery from sample matrix

The overlaid chromatograms of the unspiked and spiked *Stevia rebaudiana* Bertoni leaf extract is shown in Figure 2. Table 4 shows excellent recoveries of the analytes added to the diluted stevia extract.

Conclusions

In this Application Note, we describe the detection and quantification of two diterpene glycosides, stevioside and rebaudioside A, extracted from the leaves of *Stevia rebaudiana* Bertoni plants. The developed method is robust, sensitive and reproducible. The Agilent 7696A Sample Prep WorkBench was used for diluting samples for preparing calibration standards. The peak areas for both the analytes were found to be linear between 1 and 1,000 µg/mL with the R^2 values being greater than 0.9999. Hence the 7696A Sample Prep WorkBench can be used for routine application in a QC environment which will reduce variability caused by manual errors. The recoveries of the two analytes spiked into the diluted stevia extract were found to be excellent.

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4. Automated Sample Preparation of Headspace Standards Using the Agilent 7696 WorkBench" Agilent Application Note, Publication Number 5990-9025EN, **2011**.

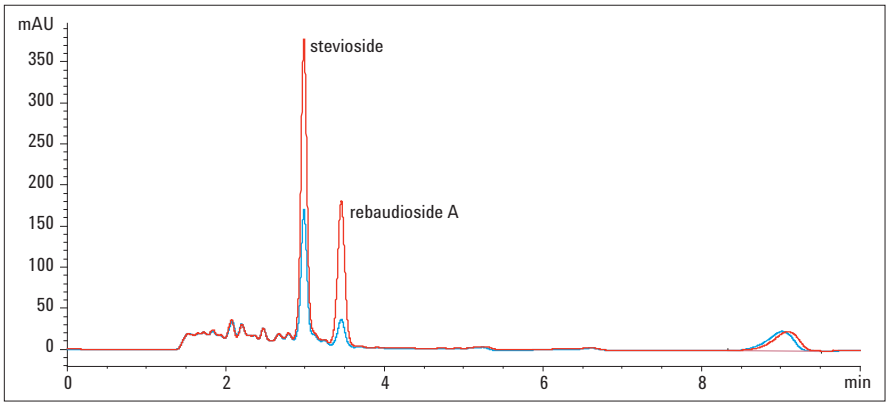

Figure 2
Overlaid chromatograms of the spiked and unspiked Stevia leaf extract.

	Amount in the 10× diluted extract (µg/mL)	Amount spiked during diluting the extract (2nd aliquot) (µg/mL)	Amount found in the diluted and spiked aliquot (µg/mL)	Recoveries
Stevioside	84.30	100	192.83	108.53%
Rebaudioside	21.56	100	114.57	93.01%

Table 4
Calculated concentrations and recoveries in unspiked and spiked diluted stevia extract samples

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Agilent Technologies

Determination of Fatty Acid Methyl Esters (FAMEs) in Olive Oil using Automated Sample Preparation

Application Note

Food Testing

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Abstract

There are different ways to analyze fatty acids (FAs) in oil. This application note shows how to analyze them after a base-catalyzed reaction and the advantages of preparing the samples with the Agilent 7696A Sample Prep WorkBench.

Introduction

The analysis of FAs is very common in olive oil industry and is usually done by gas chromatography. Due to their polar nature and their high boiling points, they generally show poor peak shapes and bad reproducibility. To avoid these problems, most methods use derivatization reactions to convert FAs to fatty acid methyl esters (FAMEs), which are easier to separate and exhibit better peak shapes.

There are a large number of derivatization reactions. One of the most common is the base-catalyzed reaction, which uses hexane and potassium hydroxide (KOH) in methanol. This method is quick, simple, and provides good results although it does not work on free fatty acids.

Agilent Technologies

Experimental

Materials

The materials used were, n-heptane, (hexane could also have been used), methanol (GC grade), and potassium hydroxide from Baker. A solution of KOH 2N was prepared by adding 11.2 g of KOH in 100 mL of methanol.

Heptane and water were used as wash solvents in the 7696A Sample Prep WorkBench. The syringe that extracts KOH solution had to be washed with both solvents, first with water to wash away the potassium hydroxide, and then with heptane. The syringe that extracts the heptane was washed with heptane alone.

Instrumentation

The usual method to analyze fatty acids in olive oil by basic derivatization uses 100 mg of sample, 10 mL of heptane and 100 µL of potassium hydroxide in a 20-mL tube. In this study, the utility of the WorkBench was tested. Therefore, all the quantities had to be divided by 10, because this instrument works with 2-mL vials.

This base-catalyzed reaction happens in a single step within a few minutes.

The WorkBench was used to automatically prepare all the samples injected into the GC/MS system.

The method used is as follows:

The software provides a Resource Manager showing where all the vials and reagents are allocated (see Figure 1).

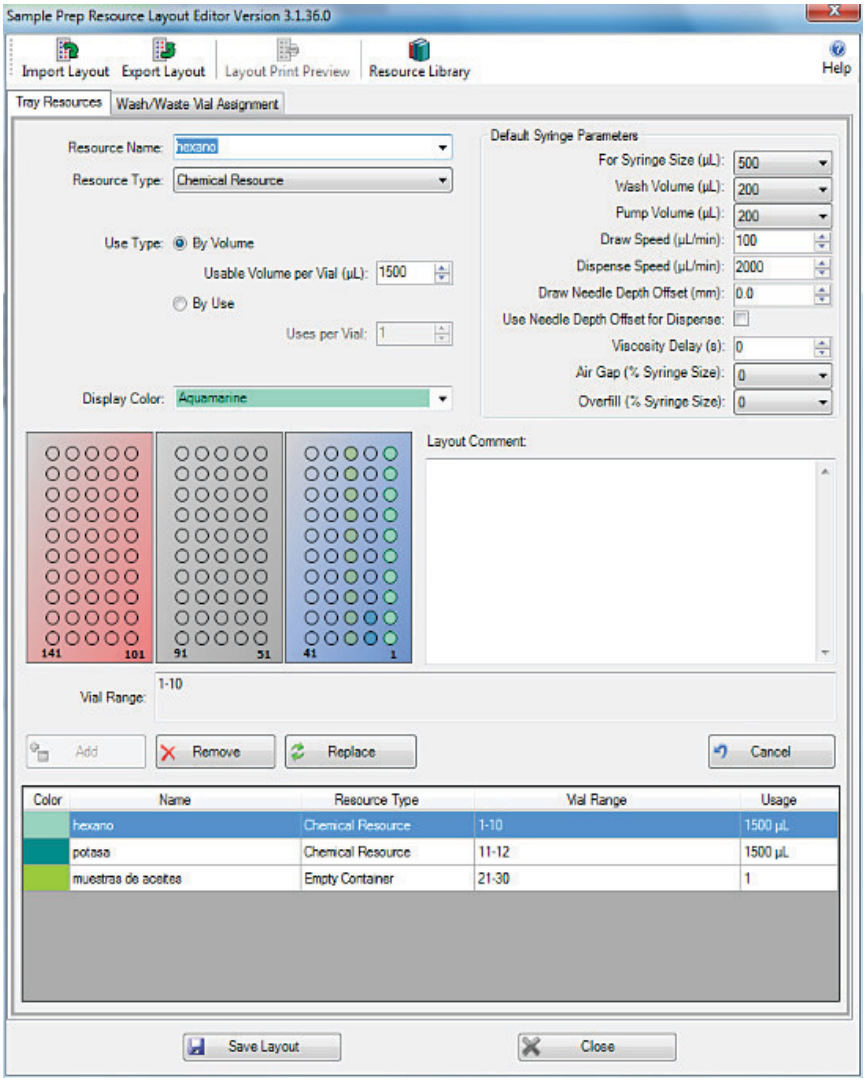

Figure 1. Resource layout.

Figure 2 shows the method used to prepare the samples.

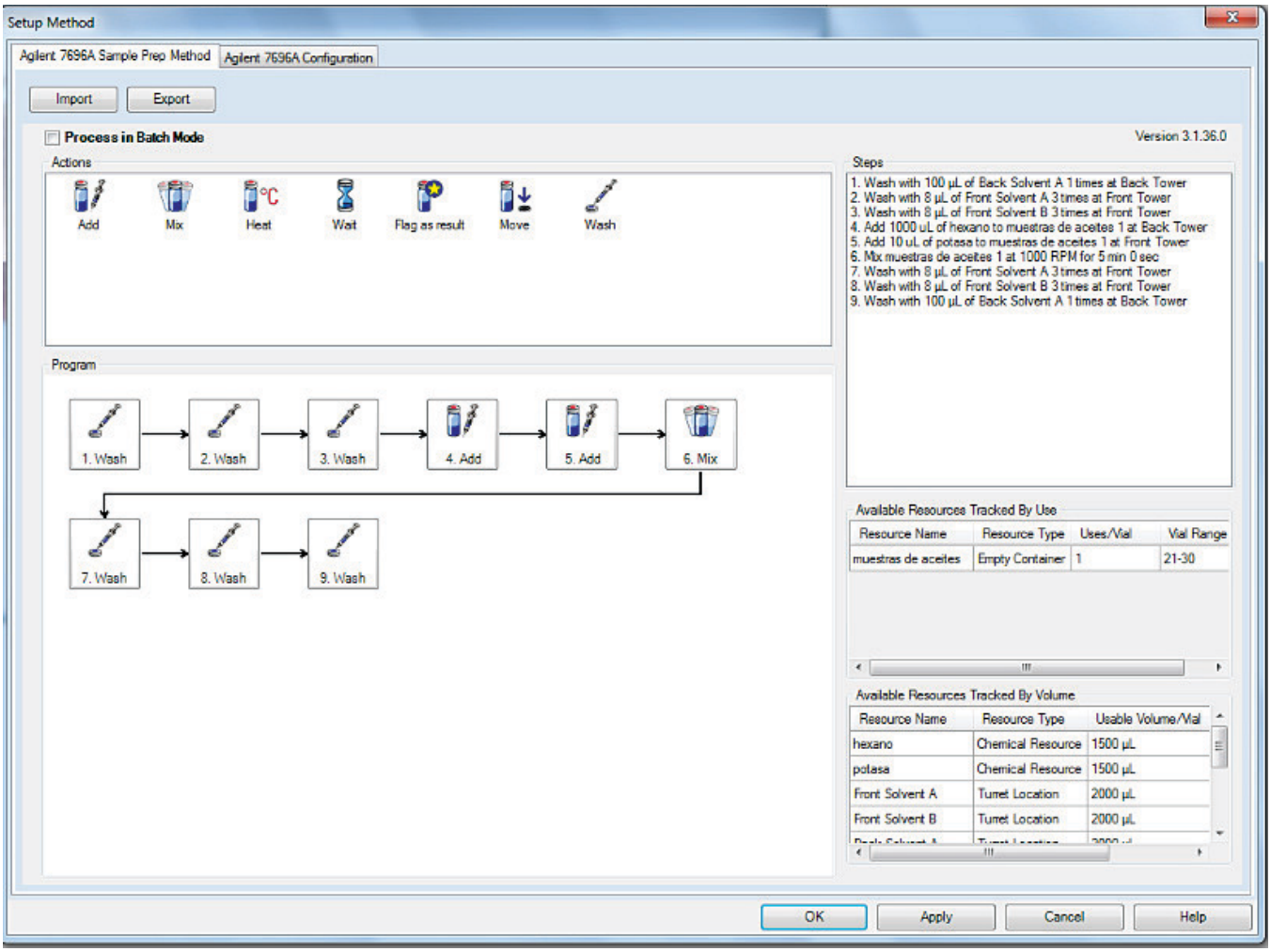

Figure 2. Agilent 7696A Sample Prep method.

In one of the trays, we set three rows of 2-mL vials, one with vials containing heptane, one containing vials with KOH, and the last row containing vials with one drop, about 10 µL, of olive oil (the weight must be noted). The SamplePrep WorkBench uses two syringes to add the necessary amount of each reagent: 1 mL of heptane and 10 µL of KOH. After both additions, the vial was agitated for 10 minutes.

Once the vial was mixed, the upper level was injected in a GC, equipped with a split/splitless inlet at 250 °C, and connected to a MSD. The column used was a HP88 (60 m × 250 µm, 0.2 µm), with a column flow rate of 1 mL/min. A temperature program of 175 °C for 5 minutes and 5 °C/min to 250 °C was used to achieve separation of the fatty acids. The inlet was set to Split mode with a split ratio of 100:1. All the analysis were performed in both SIM and SCAN modes.

Results and Discussion

To evaluate the reproducibility and accuracy of the chromatograms obtained using the WorkBench, 10 vials prepared with the WorkBench were injected on the GC/MS. Table 1 shows the results.

This application note compares the results of the four main compounds of the olive oil. The peak shape in the chromatograms is shown in Figure 3, and the area of the four peaks evaluated is shown in Table 2.

First, 10 vials were weighed after adding a drop of oil into them. Table 1 shows the values obtained.

Table 1. Weight of the 10 Samples Evaluated

Vial	Oil weight/mg
1	12.9
2	13.4
3	14.8
4	14.5
5	14.2
6	14.7
7	13.2
8	14.9
9	13.8
10	13.6

These vials were placed in the WorkBench tray to be automatically filled with the programmed amounts of each reagent.

Once the vials were ready, they were injected in the GC/MS under the conditions described above. Figure 3 shows the results.

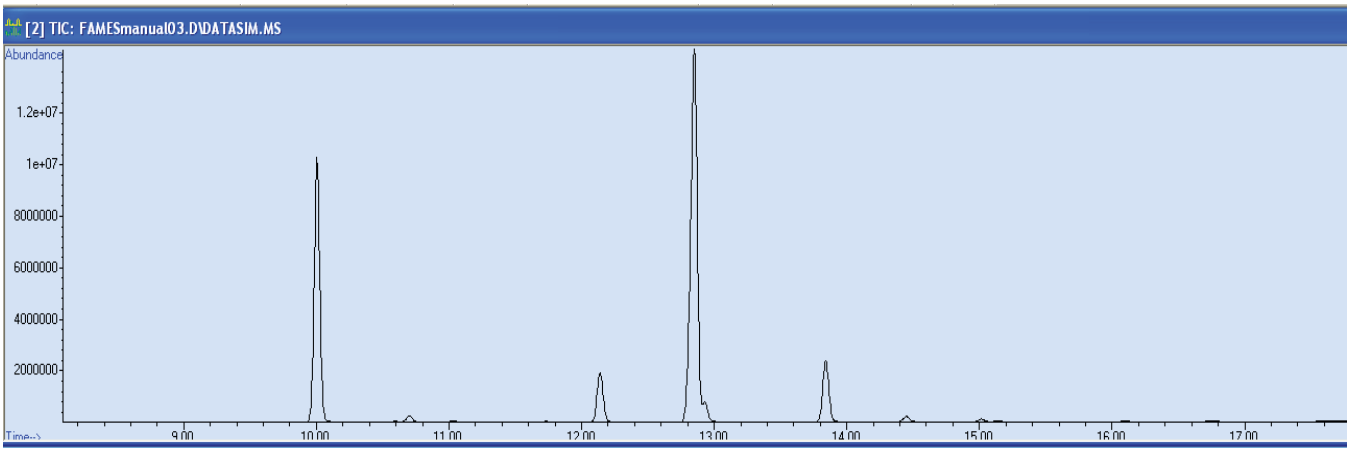

Figure 3. Chromatogram in SIM mode.

Table 2. Area of the Four Main Compounds of the Olive Oil

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	317343837.0	63331226.0	569320584.0	80584679.0
2	373510457.0	74825501.0	660064790.0	94609910.0
3	389137859.0	74174710.0	683431450.0	98106712.0
4	350160186.0	69553324.0	621849766.0	88281829.0
5	350311578.0	69513586.0	622622625.0	88233984.0
6	363692227.0	71973045.0	643859326.0	91639831.0
7	298792007.0	58778562.0	534781631.0	74997383.0
8	376569059.0	74878674.0	666439996.0	95109185.0
9	352698458.0	68424565.0	654254324.0	82569566.0
10	351745852.0	70145747.0	602155656.0	86951448.0
Average	**350409359.2**	**69188856.3**	**622601967.1**	**87561952.4**
Relative standard deviation	**27119463.9**	**5161865.2**	**46358289.5**	**7182432.9**
%RSD	**7.7**	**7.5**	**7.4**	**8.2**

Including the quantity of oil weight in each vial, the area or each compound per milligram is shown in Table 3.

Table 3. Area per mg of Oil

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	24600297.4	4909397.4	44133378.6	6246874.3
2	27873914.7	5583992.6	49258566.4	7060441.0
3	26293098.6	5011804.7	46177800.7	6628831.9
4	24155874.9	4796781.0	42886190.8	6088402.0
5	24669829.4	4895323.0	43846663.7	6213660.8
6	24740967.8	4896125.5	43799954.1	6234002.1
7	22635758.1	4452921.4	40513759.9	5681619.9
8	25273091.2	5025414.4	44727516.5	6383166.8
9	25557859.3	4958301.8	47409733.6	5983301.9
10	25863665.6	5157775.5	44276151.2	6393488.8
Average	**25097420.2**	**4954369.4**	**44585290.7**	**6272059.8**
Relative standard deviation	**1391411.9**	**284657.5**	**2430391.6**	**371694.7**
%RSD	**5.5**	**5.7**	**5.5**	**5.9**

Table 4 shows the area percentage of each FAME for the 10 samples prepared.

Table 4. Area Percentage of Each Peak of the Chromatogram

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	30.8	6.1	55.2	7.8
2	31.0	6.2	54.9	7.9
3	31.3	6.0	54.9	7.9
4	31.0	6.2	55.0	7.8
5	31.0	6.1	55.1	7.8
6	31.1	6.1	55.0	7.8
7	30.9	6.1	55.3	7.8
8	31.0	6.2	54.9	7.8
9	30.5	5.9	56.5	7.1
10	31.7	6.3	54.2	7.8
Average	**31.0**	**6.1**	**55.1**	**7.7**
Relative standard deviation	**0.3**	**0.1**	**0.6**	**0.2**
%RSD	**1.0**	**1.9**	**1.0**	**2.9**

In this experiment, both methods, the original (100 mg of oil) and the method adapted to the WorkBench, are compared. The results from the manual preparation methods are shown in Table 5 and Table 6.

Table 5. Area per mg of Oil Using the Quantities of the Original Method

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	2674181.8	529275.8	4610749.8	674892.3
2	2562129.3	505970.3	4442449.3	648040.5
3	2596966.1	511187.6	4504510.0	655770.4
4	2388663.8	466760.2	4168008.4	601931.7
5	2721157.8	535230.9	4722598.6	688465.5
6	2789232.0	549999.6	4813189.6	704034.8
7	2330855.0	453164.1	4057061.6	589335.4
8	2645696.1	528725.3	4579552.0	669544.2
9	2650632.8	520264.3	4600138.2	668931.5
10	2660736.3	520639.8	4632201.2	671882.6
Average	**2594658.8**	**510416.9**	**4501404.4**	**655410.2**
Relative standard deviation	**142531.1**	**30276.4**	**236121.4**	**36110.5**
%RSD	**5.5**	**5.9**	**5.2**	**5.5**

As seen, the %RSD are similar to the results using the WorkBench.

The same sample preparation used by the WorkBench was performed manually: one weighed drop of oil in a 2-mL vial, plus 1 mL of heptane and 10 µL of KOH in methanol using Agilent syringes, and shaken gently by the operator. The results of the analysis are shown in Table 6.

Table 6. Area per mg of Oil After Manual Sample Preparation using WorkBench Quantities

Sample	Methyl palmitate 9.99 minutes	Methyl stearate 12.128 minutes	Methyl oleate 12.844 minutes	Methyl linoleate 13.83 minutes
1	24414278.4	4280301.6	34483064.7	5405051.1
2	21953969.5	4385041.9	34340981.7	5496525.8
3	25176754.2	4987565.4	39311102.4	6258162.4
4	23806050.0	4723341.1	36249791.4	5917479.9
5	23413864.7	4659269.7	36103230.9	5862013.3
6	22388861.8	4441774.0	34988087.2	5625015.1
7	23345774.4	4655270.9	36540218.6	5654628.9
8	21758664.6	4326697.7	31010500.3	5465899.5
9	22268704.8	4448969.7	34833834.8	5598507.6
10	21726270.7	4324528.6	34099881.5	5188441.0
Average	**22970768.0**	**4513461.5**	**35078976.3**	**5633014.3**
Relative standard deviation	**1194355.0**	**226067.9**	**2129375.1**	**301856.8**
%RSD	**5.2**	**5.0**	**6.1**	**5.4**

As seen, the %RSD are similar to the results using the WorkBench.

Conclusions

The Agilent 7696A Sample Prep WorkBench is a very comfortable, fast, easy and reliable tool to automate some typical laboratory work such as sample preparation. The results detailed in this application note how the reproducibility of the analysis when performed with the WorkBench. The results obtained from the WorkBench preparation are very similar to those obtained with a manual preparation both with original resource quantities and WorkBench-scale quantities.

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Agilent Technologies

PHARMACEUTICAL

Automated precision weighing for confirmation of drug delivery in eye droppers

Drug delivery devices must be able to provide the drug in a consistent manner to ensure the proper amount is applied. In this application the WorkBench with WeighStation provides the mechanism to automate testing the drug delivery, allowing for a precise weight-to-weight calculation.

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PHARMACEUTICAL

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Automated Sample Preparation in Quality Control of Eye-Drop Formulation

Application Note

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Abstract

The Agilent 7696A Sample Prep WorkBench with WeighStation was used in a quality control method for eye-drop formulations. Automated sample preparation included the preparation of calibration standards (four levels) containing two active ingredients, and the preparation of eye-drop formulation samples by weighing and dilution. The two sequences were fully automated. The analyses show excellent repeatability and linearity.

Agilent Technologies

Introduction

The Agilent 7696A Sample Prep WorkBench with WeighStation was used for the determination of two active ingredients, dexamethasone and chloramphenicol, in eye-drop formulations. Dexamethasone is one of the most potent corticosteroids; it is 5 to 14 times more potent than prednisolone and 25 to 75 times more potent than cortisone and hydrocortisone. The addition of chloramphenicol, a broad-spectrum antibiotic, to dexamethasone leads to a combination that yields excellent results in inflammation of the anterior uvea (iritis, iridocyclitis). Eye-drop formulations against inflammation can contain one or both ingredients and are typically offered as a viscous aqueous solution.

The typical analytical QC procedure consists of 1) preparation of a 4-level calibration series containing the active ingredients in water (aqueous mobile phase) and 2) dilution of an amount of the eye-drop formulation in water (aqueous mobile phase). As illustrated in Table 1A, sample preparation is normally done in volumetric flasks, starting from the preparation of two stock solutions, containing respectively dexamethasone and chloramphenicol. The resulting calibration solutions contain both ingredients in a concentration range between 16 and 64 µg/mL dexamethasone and between 80 and 320 µg/mL chloramphenicol. Samples are typically 25-fold diluted in aqueous mobile phase. After sample preparation, calibration standards and samples are analyzed by HPLC with UV detection. Since the standard solutions are not stable, they are typically prepared each time a series of samples has to be analyzed.

In this application note, the automation of the preparation of the calibration standards and the dilution of the samples is described. The general method layout is shown in Table 1B. WeighStation was used to track the exact calibration standard concentrations and to measure sample amounts.

Experimental

Chemicals

Dexamethasone and chloroamphenicol were obtained from Sigma-Aldrich (Beerse, Belgium). Water and acetonitrile (AcCN) were HPLC grade (BioSolve, The Netherlands). Phosphoric acid and sodium hydroxide were from Sigma-Aldrich.

Configuration

The Agilent 7696A Sample Prep WorkBench was equipped with two Agilent 7693A Automated Liquid Samplers. The front injector contained an enhanced syringe carriage with a 500-µL syringe (p/n G4513-60561). The back injector contained an enhanced syringe carriage with a 50-µL syringe (p/n 5183-0314).

WorkBench method

Resource layout

Figure 1 shows the resource layout for the WorkBench. Mobile phase A (0.3% phosphoric acid in water, adjusted to pH = 3 with sodium hydroxide) and acetonitrile are placed as 'Chemical resource' in positions 81-150 and 71-72 respectively. Solvent in vials A1 and B1 for front and back tower was water. All vials in which the standard dilutions are prepared are defined as Empty Container.

WorkBench method program steps

The 7696A Sample Prep WorkBench program for the preparation of the four calibration standards is shown in Figure 2. After weighing the empty vials, the system is placed on pause to allow the addition of solid standards. After this, the automatic process is resumed and solvent is added to give calibration standards at four levels. The lowest level is prepared in six-fold.

Table 1A. Classical Sample Preparation (USP or EP Methods)

	Code	Preparation stock solutions	Target conc (µg/mL)
Calibration standards	DEX Stck	weigh 10 mg dexamethasone in a 25-mL vial + add 25 mL water	400
	CLO Stck	weigh 10 mg chloramphenicol in a 25-mL vial + add 25 mL water	400
		Preparation standard solutions	**Target conc (µg/mL) dexamethasone/chloramphenicol**
	LVL1	1 mL Dex Stck + 5 mL CLO Stck in 25 mL mobile phase	16/80
	LVL2	2 mL Dex Stck + 10 mL CLO Stck in 25 mL mobile phase	32/160
	LVL3	3 mL Dex Stck + 15 mL CLO Stck in 25 mL mobile phase	48/240
	LVL4	4 mL Dex Stck + 20 mL CLO Stck in 25 mL mobile phase	64/320
		Preparation eye-drop formulation samples	
Samples	SAM	1 mL or 1 g eye-drop sample + 25 mL mobile phase	

Table 1B. Agilent 7696A Sample Prep WorkBench Sample Preparation

	Code	Preparation stock solutions	Target conc (µg/mL)
Calibration standards	DEX Stck-1	weigh 4 mg dexamethasone in a 2-mL vial * + add 1 mL AcCN	4,000
	DEX Stck	150 µL DEX Stck-1 + 1,350 µL mobile phase (1/10 dilution)	400
	CLO Stck-1	weigh 4 mg chloramphenicol in a 2-mL vial * + add 1 mL AcCN	4,000
	CLO Stck	150 µL CLO Stck-1 + 1,350 µL mobile phase (1/10 dilution)	400
		Preparation standard solutions	**Target conc (µg/mL) dexamethasone/chloramphenicol**
	LVL1	20 µL Dex Stck + 100 µL CLO Stck + 380 µL mobile phase	16/80
	LVL2	40 µL Dex Stck + 200 µL CLO Stck + 260 µL mobile phase	32/160
	LVL3	60 µL Dex Stck + 300 µL CLO Stck + 140 µL mobile phase	48/240
	LVL4	80 µL Dex Stck + 400 µL CLO Stck + 20 µL mobile phase	64/320
		Preparation finished product samples	
Samples	SAM	1 eye-drop sample* + 1 mL mobile phase (approximately 35–40 mg)	

*solid powder or viscous liquid is added manually while Agilent 7696A Sample Prep WorkBench is on hold. This is followed by a weighing step.

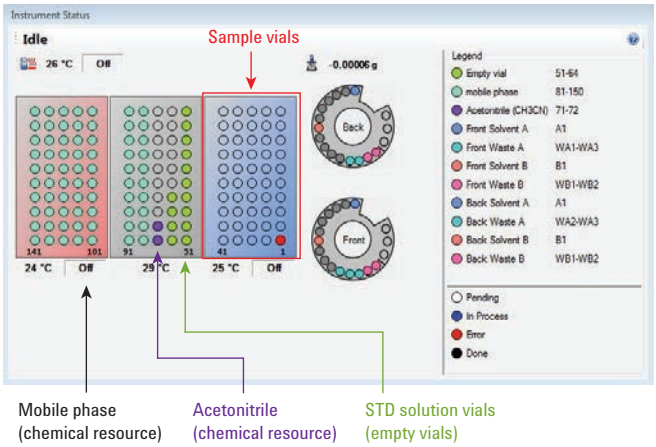


Figure 1. Agilent 7696A Sample Prep WorkBench resource layout for the serial dilution of dexamethasone and chloramphenicol.

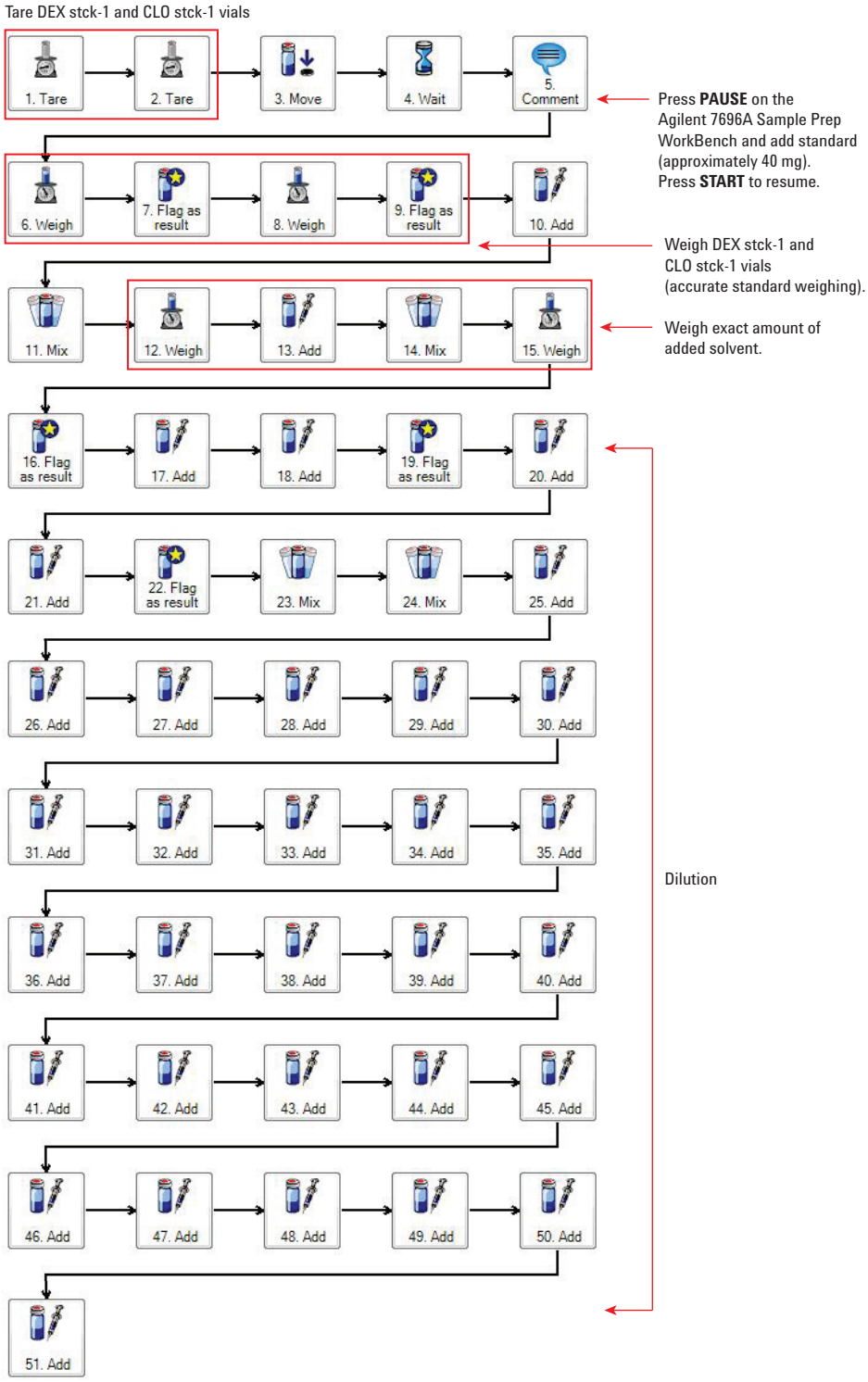


Figure 2. Graphical flow chart used to program Agilent 7696A Sample Prep WorkBench method setup for calibration standards.

An additional sequence is prepared for the dilution of the samples: empty vials are tarred and the system is put on hold. One drop of viscous sample is added to each vial. This requires a manual action since the viscosities of the samples do not allow transferring the liquids using syringes. The workbench is then resumed. The vials are again weighed (Figure 3) and the exact sample amounts are calculated and reported in the sequence report. Finally, solvent is added for sample dilution (see method lay-out in Figure 4).

Both programs for the preparation of the calibration standards and for the preparation of the samples can be programmed in one sequence.

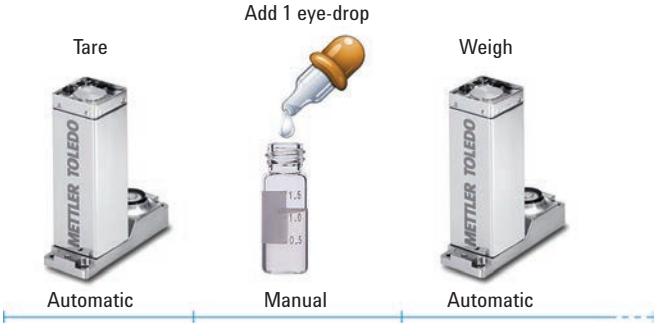
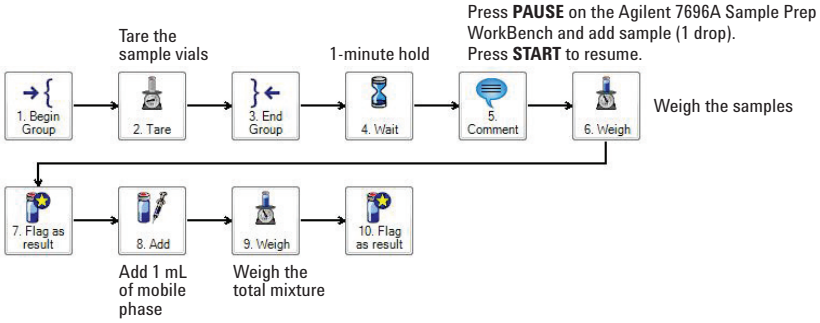


Figure 3. Illustration of taring, sample addition, and weighing of the sample amount.



1. Begin a group.
2. Tare the sample.
3. End the group.
4. Wait 1 minute 0 seconds.
5. Press **PAUSE** on the WorkBench. Press **START** after adding the sample.
6. Weight sample.
7. Flag the sample as RESULT.
8. Add 1,000 µL of the mobile phase to the sample at the front tower (washes, pumps).
9. Weigh the sample.
10. Flag the sample as RESULT.

Figure 4. Agilent 7696A Sample Prep WorkBench method setup for samples.

Experimental conditions for HPLC analysis

Analyses were performed on an Agilent 1290 Infinity HPLC System. Separation was performed on a Poroshell 120 column (2.1 mm × 400 mm, 2.4 µm dp (p/n 695775-902)). Mobile phase A was 0.3% phosphoric acid in water (adjusted to pH = 3 with sodium hydroxide) and mobile phase B was acetonitrile. A gradient from 20% B (0 minutes) to 50% B (5 minutes) was used. Flow rate was 0.5 mL/min. Injection volume was 1 µL and detection was done by UV at 254 nm.

Results and Discussion

The chromatograms corresponding to the four calibration levels are overlaid in Figure 5. The calibration curves are given in Figure 6. The linearities for both dexamethasone and chloramphenicol are excellent ($R^2 > 0.999$).

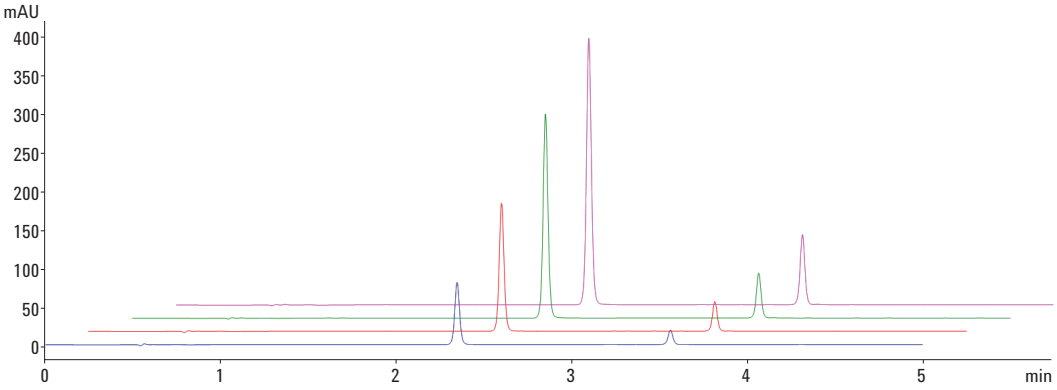


Figure 5. Chromatograms (overlay with X/Y-axis offset) of four calibration levels of chloramphenicol (2.35 minutes) and dexamethasone (3.55 minutes).

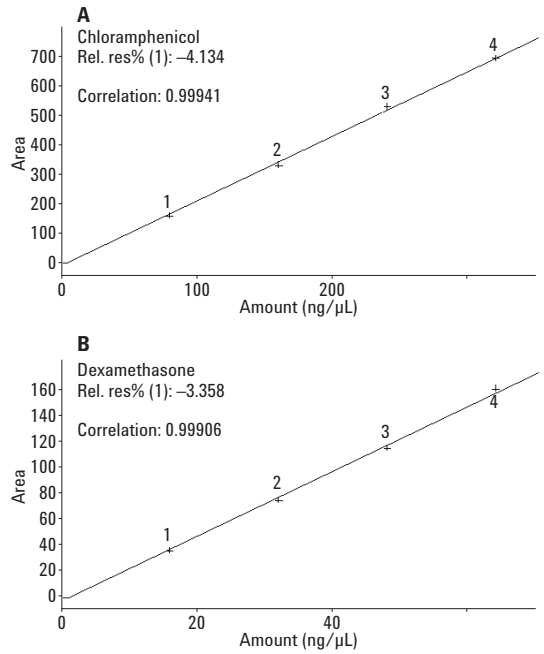


Figure 6. Calibration curves for chloramphenicol (2.35 minutes) and dexamethasone (3.55 minutes) obtained by automated calibration standard preparation using the Agilent 7696A Sample Prep WorkBench.

The repeatability of sample preparation was evaluated by preparing the lowest calibration sample in six-fold. The chromatograms are overlaid in Figure 7 and show excellent repeatability, with RSDs of 1.5% for chloramphenicol and 2.7% for dexamethasone, respectively.

Next, three types of samples were analyzed. The chromatograms are shown in Figure 8. Sample A contains dexamethasone, sample B contains chloramphenicol and sample C contains a combination of both solutes. For all three samples, correct amounts of the active ingredients were detected (values all between 90% and 105% of labelled concentration). The analyses of different sample types that were prepared in the same sequence also revealed that no cross-contamination was observed (no chloramphenicol in sample only containing dexamethasone and *vice versa*).

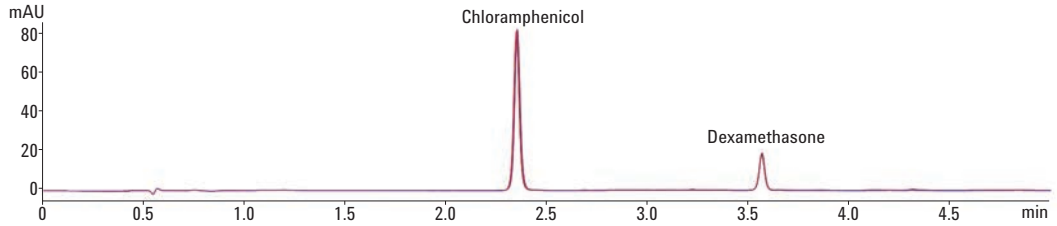


Figure 7. Overlay of 6 chromatograms of six individually prepared calibration solutions of chloramphenicol (2.35 minutes) and dexamethasone (3.55 minutes) obtained by automated calibration standard preparation using the Agilent 7696A Sample Prep WorkBench. The percent relative standard deviation for chloramphenicol and dexamethasone are 1.5% and 2.7%, respectively.

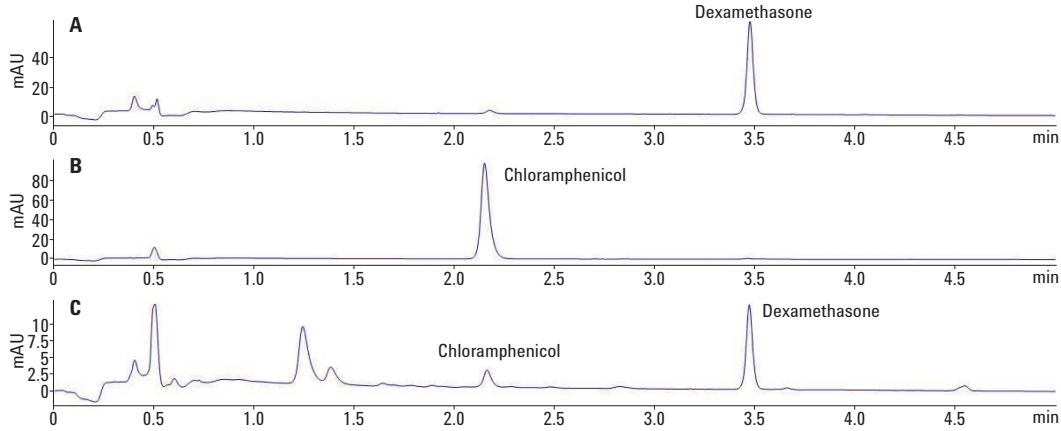


Figure 8. Chromatograms obtained for three eye-drop formulation types.

The repeatability of sample preparation is demonstrated in Table 2, showing the measured concentrations, the sample weights and the final concentrations of chloramphenicol in sample type B. This sample was prepared in 6-fold. The relative standard deviation of the measured amount is lower than 1%.

In addition to these quantitative data, note that the total solvent consumption is drastically reduced, no volumetric glassware is used, and hardly any solutions are wasted.

Conclusions

The Agilent 7696A Sample Prep WorkBench with WeighStation was successfully applied in the automated preparation of calibration samples and sample dilutions. The viscous nature of the samples did not allow volumetric dilution with syringes, but using the weighing station, exact sample masses were determined. After dilution, accurate determination of the active ingredients was possible.

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Table 2. Repeatability of Sample Preparation for Eye-drop Formulation Type B

	Measured conc (µg/mL)	Sample weight (mg)	Corrected conc (µg/mg)
SAM-rep01	124	27.72	4.59
SAM-rep02	121	26.95	4.61
SAM-rep03	114	25.41	4.61
SAM-rep04	103	23.07	4.57
SAM-rep05	143	32.58	4.53
SAM-rep06	138	31.27	4.53
		RSD (%)	**0.74**

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GENERAL

The Agilent 7696A Sample Prep WorkBench automates repetitive and error-prone steps in your sample prep workflow

Precise automation is combined with intuitive software to ensure consistent sample processing, eliminate analyst-to-analyst variability, and allow chemists to work on other, more critical, tasks.

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GENERAL

Maintain sample prep consistency and reproducibility, run after run. Visit agilent.com/chem/workbench

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Agilent 7696A Sample Prep WorkBench: How to Automate Preparation of a Sample Set by Serial Dilution for Measurement of Flame Ionization Detector Performance

Application Note

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USA

Introduction

A challenge that arises more often than the analyst might like, is the need to prepare a set of samples by serial dilution. Serial dilution starts with a single sample of known concentration. It is then used to prepare a set of dilutions, each usually differing from the previous one, by a constant factor. Each sample is made from the previous one in the series. This task may be driven by the need to calibrate an instrument with specific analytes or measure such things as detector performance: linearity, sensitivity and minimum detectable level (MDL). If the samples are not stable over time, they may need to be prepared weekly or even daily. To minimize errors in manual preparations or reduce the frequency of tiresome dilutions, the user will often prepare larger volumes of sample than needed, which leads to unnecessary waste and expense.

The Agilent 7696A Sample Prep WorkBench provides a solution to this problem by automating the serial dilution process precisely so that small volumes of sample can be routinely prepared when needed over as large a concentration range as desired. The preparative method for serial dilution starts with a measured volume of solvent in an empty vial followed by a measured volume of sample. After mixing, this step is repeated using a new vial of solvent and an aliquot from the last dilution. For example, measuring the performance of a flame ionization detector (FID) requires a set of samples, each diluted by a factor of ten from the previous sample. The starting sample is a normal hydrocarbon such as n-tridecane (C_{13}). Each dilution consists of 90% solvent and 10% previous sample (v:v). A set of seven or eight samples, as prepared in this application, are required to demonstrate the normal seven orders of magnitude of FID linearity. As described below, eight sets of test samples were prepared over a two week period. Three were prepared manually and five with the Agilent 7696 Sample Prep Workbench at a total volume per sample of either 1 mL or 0.5 mL. Repeatability over all sets was excellent whether measured by sample weight in each set or by FID performance.

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Experimental

The Agilent 7696A Sample Prep WorkBench was used to prepare a set of eight samples, each diluted by a factor of ten from the previous sample. Two sequences were used so that samples could be weighed after each addition. The first used a method that added a fixed amount of solvent to each vial. The second started with a manually-prepared 10% solution of C_{13} in solvent, then added enough solution to the next vial to make a tenfold less concentrated solution. After mixing, an aliquot of the freshly made sample was used to make the next dilution in the series until the eight sample set was complete. The empty vials were tared, and then weighed after each sequence to measure reproducibility of transfers across the series. The same preparations were also done manually for comparison.

Hardware Configuration

The Agilent 7696A Sample Prep WorkBench was equipped with two Agilent 7693A Automated Liquid Samplers. The back injector contained an enhanced syringe carriage containing a 500-µL syringe (p/n G4513-60561). The front injector used a standard syringe carriage containing a 100-µL syringe (p/n 5183-2042). The back injector was used for solvent delivery to each of the empty vials (first sequence) and the front injector was used for sample transfer from one sample to the next (second sequence).

Sample Preparation

Two protocols were used that differed only in the volume of the prepared dilution. The first used 900 µL solvent + 100 µL sample and the second used half these amounts: 450 µL solvent + 50 µL sample.

A single Agilent 7696A Sample Prep WorkBench resource layout was used for both sequences:

Resource Layout:

Vial Range	Name	Type	Usage
2-9	MT vial	Empty container	1 use/vial
12-19	Solvent	Chemical resource	1 use/vial

The single sample required was a solution of 10% C_{13} in isooctane. It was prepared by adding 100 µL C_{13} to a 1 mL volumetric and diluting to mark.*

The first sequence prepared the 1 mL sample (900 µL + 100 µL) by adding 900 µL solvent to an empty vial (see Appendix for syringe parameters). The sequence specified vials 2 through 9.

* I started with the 10% C_{13} instead of 100% C_{13} to avoid any volume shrinkage that might occur when mixing two neat compounds by volume.

The second sequence specified sample dilutions according to the following steps. (see Appendix for syringe parameters):

Step	Function
1	Add 100 µL of Sample (Front) to vial #2
2	Mix vial #2 at 1500 RPM for 0 min 5 sec
3	Add 100 µL of vial #2 to vial #3
4	Mix vial #3 at 1500 RPM for 0 min 5 sec
5	Add 100 µL of vial #3 to vial #4
6	Mix vial #4 at 1500 RPM for 0 min 5 sec
7	Add 100 µL of vial #4 to vial #5
8	Mix vial #5 at 1500 RPM for 0 min 5 sec
9	Add 100 µL of vial #5 to vial #6
10	Mix vial #6 at 1500 RPM for 0 min 5 sec
11	Add 100 µL of vial #6 to vial #7
12	Mix vial #7 at 1500 RPM for 0 min 5 sec
13	Add 100 µL of vial #7 to vial #8
14	Mix vial #8 at 1500 RPM for 0 min 5 sec
15	Add 100 µL of vial #8 to vial #9
16	Mix vial #9 at 1500 RPM for 0 min 5 sec

Results

Over a period of two weeks, eight serial dilution runs were made: Three manual (two at 1 mL and one at 0.5 mL); five with the Agilent 7696A Sample Prep WorkBench (three at 1 mL and two at 0.5 mL).

Table 1. Reproducibility for Solvent Delivery (Average of Eight Samples)

Type	Manual	Manual	Manual	7696A	7696A	7696A	7696A	7696A
Volume (mL)	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5
Average weight (g)	*	0.6165	0.6151	0.3089	0.6176	0.6195	0.6180	0.3088
%SD	*	0.17	0.26	0.11	0.16	0.09	0.06	0.17

* Not measured.

Reproducibility for the second step was ±1 µL, for all but the last sample. Each sample except the last was used to prepare the next. The weight should not change because the same volume is added to and then removed from each sample. The average weight change regardless of whether a 1 mL or 0.5 mL preparation was involved was equivalent to ±1 µL. The volume increase of the last sample was 100 µL or 50 µL for the 1 mL and 0.5 mL volumes, respectively.

The total Agilent 7696A Sample Prep WorkBench runtime was 49 min for the 1 mL set of samples and 41 min for the 0.5 mL set. The time for the manual preparations was not measured.

Reproducibility of FID performance

The protocol used for FID linearity, sensitivity and MDL followed the ASTM protocol closely [1]. The major difference was the use of liquid samples rather than gas samples as specified by ASTM. All preparations were tested on the same FID. The linearity results (Figure 1) are essentially indistinguishable whether the samples were prepared by the Agilent 7696A Sample Prep WorkBench or manually. The average sensitivity and % SD were 26.3 and 2.4, respectively. This is very good performance for repeat runs on a single FID. The large spread in the MDL (Table 2) is caused by day-to-day variability in average detector noise in the region where C_{13} elutes. MDL is a sensitive function of noise. Table 2 and Figure 1 summarizes the results.

Table 2. FID MDL

Prep Type	Manual	Manual	Manual	7696	7696	7696	7696	7696
Volume (mL)	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5
Sensitivity (ma-s/gC)	27.2	25.7	25.8	26.8	26.8	25.5	26.6	25.5
MDL (pgC/s)	0.96	1.14	1.66	0.92	0.68	1.31	1.23	1.15

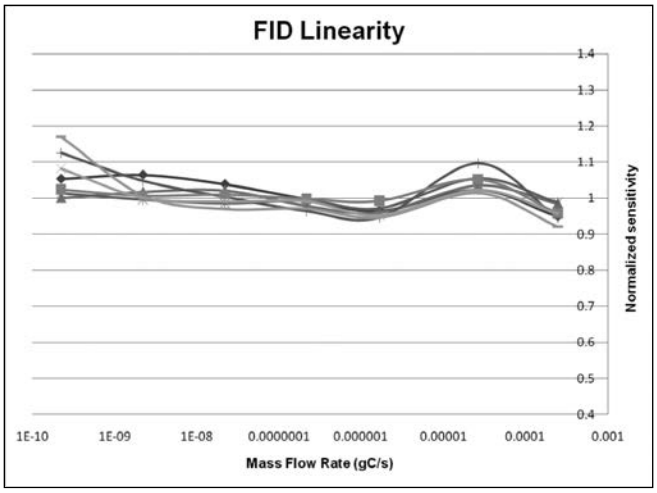

Figure 1. Linearity Plots for all eight runs overlaid.

Conclusion

The Agilent 7696A Sample Prep WorkBench simplifies the preparation of a set of samples by serial dilution. The user can prepare fresh samples only when needed at volumes no larger than necessary to satisfy the analytical requirements. The result is less boredom, less chance for operator error, less consumption of reagents, less waste disposal expense and better repeatability.

Appendix

500 µL syringe parameters:

	Tower Back	Solvent Prewash1	Solvent Prewash 2	Dispense wash	Dispense pumps	Dispense settings	Solvent postwash1	Solvent postwash2
Number pumps or washes					3			
Wash volume (µL)					50			
Draw speed (µL/min)					1250	1250		
Dispense speed (µL/min)					3000	3000		
Needle depth offset (mm)					0	0		
Viscosity delay(s)					2	2		
Turret solvent								
Air gap (% syr.vol.)						0		

100 µL syringe parameters:

	Tower Back	Solvent Prewash1	Solvent Prewash 2	Dispense wash	Dispense pumps	Dispense settings	Solvent postwash1	Solvent postwash2
Number pumps or washes		1		1	2			
Wash volume (µL)		10		20	10			
Draw speed (µL/min)		300		300	300	300		
Dispense speed (µL/min)		6000		6000	6000	6000		
Needle depth offset (mm)		0		0	0	0		
Viscosity delay(s)		2		2	2	2		
Turret solvent		A						
Air gap (% syr.vol.)						0		

Reference

1. ASTM E594-96 (2006) Standard Practice for Testing Flame Ionization Detectors used in Gas or supercritical Fluid Chromatography

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Automation of QC Testing Using the Agilent Sample Prep WorkBench

QUALITY CONTROL

The accuracy, reliability and ease of use of the Agilent 7696A Sample Prep WorkBench provide accurate and error-free preparation of QC samples for the production of standards.

Reliable and accurate calibration of reference standards are a must for any laboratory running GC/MS. ULTRA Scientific provides such certified analytical standards, using stringent QC procedures to ensure their accuracy, reliability, and now adherence to ISO Guide 34 requirements as a certified reference material producer.

The ULTRA Scientific procedures require selection of random samples from the unitizing process to monitor homogeneity and ensure proper concentrations for each analyte in a standards mix. The concentrations of the components used to prepare the standard set are determined by constructing a calibration curve for each component, using a procedure that requires precise dilutions as well as the addition of an internal standard. The same internal standard is added in prescribed amounts to each vial sampled from the unitizing process. The actual concentration of each standard compound in the vial is determined by GC analysis, measuring the ratio of the area of the compound peak relative to that of the internal standard. This relative response is compared to the same ratio determined in a calibration run at the beginning of the QC sequence and comprised of known amounts of each analyte present in the standard being packaged. The result is a highly accurate determination of the actual concentrations of the compounds in the vial.

Traditionally, ULTRA Scientific has performed this QC procedure by manually pipetting the internal standards. However, the company recently tested the Agilent Sample Prep WorkBench to automate pipetting, using both a volatile and a semi-volatile standards mix. The WorkBench provided accuracy measurements in most cases equal to or better than those determined for the manual method. This was true for the volatile standard set, even though WorkBench punctures the vial seal to add the internal standard.

The Sample Prep WorkBench is a valuable tool for automating applications requiring reliable and accurate pipetting, including QC in a manufacturing environment.

Key Benefits

- Eliminates the opportunity for error inherent in manual methods
- Accuracy equal to or better than that of manual methods
- QC methods can be stored in the WorkBench for rapid access
- User-friendly, templated software that is easy to master
- Bar code reading for easy sample tracking

This work was performed as a collaboration between Scott A. Lorimer of ULTRA Scientific and Jared Bushey of Agilent Technologies.

Method Used to Generate the QC Samples on the Agilent WorkBench

Method steps

1. Add 315 µL of methylene chloride (volatile standards set) or methanol (semivolatile standards set) to Empty Vials 1 at Back Tower.
2. Add 35 µL of sample to Empty Vials 1 at Front Tower.
3. Add 35 µL of biphenyl (volatile standards set) or fluorobenzene (semivolatile standards set) internal standard to Empty Vials 1 at Front Tower.
4. Mix Empty Vials 1 by vortex at 2,000 RPM, 5 sec spin, bidirectional, 2 cycles.
5. Flag Empty Vials 1 as 'Results'.

WorkBench configuration

Front injector syringe size	100 µL
Rear injector syringe size	500 µL
Barcode heater	enabled at 50 °C

The amount of each analyte in the QC sample was calculated according to the following formula:

$$\left(\frac{\left(\frac{\text{Analyte area}}{\text{istd area}} \right)_{\text{Sample}}}{\left(\frac{\text{Analyte area}}{\text{istd area}} \right)_{\text{Calibration}}} \right) \times (\text{istd concentration})_{\text{Sample}}$$

Accuracy results for the QC of vials removed from a production run*

Volatile Standards Set

Standard component	Manual % difference	WorkBench % difference
2-picoline	-1.1960	0.6202
Acetophenone	-3.2765	0.3384
N-nitrosopiperidine	-3.3925	-2.6848
a,a-dimethylphenethylamine	0.9115	1.0354
N-nitrosodi-n-butlamine	-0.2920	0.2560
1,2,4,5-tetrachlorobenzene	-0.8785	0.8129
1-chloronaphthalene	9.4015	0.1805
Pentachlorobenzene	-2.2000	0.7535
Diphenylamine	-0.0380	-0.4711
Phenacetin	-2.1165	2.0917
4-Aminobiphenyl	-9.6435	5.1187
Pentachloronitorbenzene	-2.5130	1.0443
Pronamide	-3.7865	2.5000
p-(dimethylamino)azobenzene	2.1465	3.1419
7,12-dimethylbenz[A]anthra	-1.5785	0.0849
3-methylcholanthrene	1.6965	-0.4717
Dibenz[A,J]acridine	2.2478	1.7602

Semi-volatile Standards Set

Standard component	Manual % difference	WorkBench % difference
1,1-dichloroethene	-7.7578	-1.0566
trans-1,2-dichloroethene	-1.4115	-3.8288
cis-1,2-dichloroethene	3.0163	-1.6658
Benzene	3.1771	-0.5289
Trichloroethene	3.2273	-5.9651
cis-1,3-dichloropropene	5.7795	-4.1192
Toluene	4.5507	-1.5242
trans-1,3-dichloropropene	6.4737	-2.8953
Tetrachloroethene	4.1876	-4.7692
Chlorobenzene	7.0025	-1.8823
Ethylbenzene	6.4856	-0.9086
Meta+para-xylene	6.5132	-1.9300

*Accuracy is defined as the % difference between the amount of a given analyte determined to be in the sample versus the amount that should have been present, based on the calibration run data.

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Agilent Technologies

Increase Capabilities Using Batch Enabled Sample Preparation

Application Note

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Abstract

With the implementation of batch enabled sample preparation, additional time and resource savings are realized using the Agilent 7696A Sample Prep WorkBench. A common sample preparation task was performed using both non-batch and batch mode sample processing. The amount of wash solvent used and the time required to complete each sample was compared.

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Introduction

The Agilent 7696A Automated Sample Prep WorkBench can perform many sample preparation tasks for either gas chromatographic (GC) or liquid chromatographic (LC) analyses. WorkBench consists of two liquid dispensing modules, a single vial heater capable of reaching 80 °C, a single vial vortex mixer, and bar code reader (Figure 1). This enables dilutions/aliquoting, liquid addition, sample heating, liquid/liquid extractions, and sample mixing. Individual racks can also be heated or cooled. This sample preparation instrument can perform tasks with the same accuracy and precision as the 7693A Automatic Liquid Sampler [1] in an offline setting instead of on top of a GC.

Figure 1. Agilent 7696A Sample Prep WorkBench

The Agilent 7696A Sample Prep WorkBench uses the Easy SamplePrep paradigm to greatly simplify sample prep programming. Easy SamplePrep (ESP) features icon based programming and a resource manager. Using a drag-and-drop editor, users can create a sample prep method in a manner similar to following a protocol or instructions in a laboratory notebook. ESP also gives a textual display of the sample prep steps. There are two modes of operation for ESP, batch and non-batch mode. Non-batch mode processes each sample singularly and in series, that is all steps are performed for one sample, thus completing the sample preparation before moving on to the next sample. Conversely, batch mode processes samples in parallel, that is each step is performed on all samples before moving to the next sample preparation step, thus completing all the samples at approximately the same time.

An automated method for the esterification of fatty acids [2] was performed using both non-batch and batch mode processing. The amount of wash solvent used was determined for each operative mode as well as the time required to complete sample preparation.

Results and Discussion

Batch mode processing allows significant time and resource savings (Table 1). To complete six samples using the method outlined in [2], non-batch mode processing required 45 minutes per sample (270 minutes to complete all six samples). When using the batch mode available in the software, all six samples were completed in 138 minutes, averaging to 23 minutes per sample. A large time saver was the ability to move all samples to the heated rack, wait for the 20 minute reaction time, then return all samples to the original location. With batch mode, all samples can be reacted (heated) at the same time compared to non-batch mode which took advantage of the single vial heater, but reacted/heated each sample separately for 20 minutes.

Table 1. Time and Resource Savings

Batch size n = 6	Non-batch	Batch	Improvement
Number of programming steps	12	12	n/a
Wash steps	9	9	n/a
Total number of washes	54	9	1/n (n times)
Total time	4.5 h	2.3 h	~50%
Time per sample	45 min	23 min	~50%
Wash volume	15.3 mL	2.55 mL	1/n (n times)

Likewise, when comparing the amount of wash solvent used, the advantages of batch mode were clear. Using batch mode to process the samples, only nine wash steps totaling 2.6 mL were used. To process the six sample using non-batch, nine wash steps were again employed, but for six samples, totaling 54 wash steps and using 15.3 mL. By using batch mode to process the samples, the amount of wash solvent used was reduced six-fold for this particular comparison.

Conclusions

Comparing batch and non-batch mode processing of a sample preparation method developed for the Agilent 7696A Sample Prep WorkBench demonstrated the benefits of using the batch feature to process samples. Batch processing reduces the time per sample. For the example given here, batch mode allowed the sample to be processed twice as fast. Additionally, significant wash solvent can be saved using batch processing. Using the batch feature, the wash steps are reduced n-fold (where n is the number of samples) and the amount of wash solvent used is also reduced n-fold. For six samples, this equates to a six-fold savings in wash solvent usage, 2.6 mL versus 15.3 mL for non-batch mode operation. This will result in significant savings, especially as the throughput of the instrument is increased.

References

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

2. Rebecca Veeneman, "Improving the Analysis of Fatty Acid Methyl Esters Using Automated Sample Preparation Techniques," Agilent Technologies Publication 5990-6873EN

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Agilent 7696A Sample Prep WorkBench Supplies

Quick reference guide

Agilent supplies for Agilent instruments

Agilent Technologies is committed to optimizing your laboratory's productivity, so we have produced this list of the most commonly ordered supplies and parts for the Agilent 7696A Sample Prep WorkBench. WorkBench automates tedious sample preparation steps for HPLC, GC, LC/MS and GC/MS in one standalone instrument.

Vials

Description	Color	Unit	Part No.
2 mL wide opening screw top vial	Clear	100/pk	5182-0714
		1000/cs	5183-2067
	Clear, write-on spot	100/cs	5182-0715
		1000/cs	5183-2068
	Amber	100/pk	5188-6535
		1000/cs	5188-6536
	Amber, write on spot	100/pk	5182-0716
		1000/cs	5183-2069

Screw caps

Description	Septa type	Unit	Part No.
Multicolor screw-cap pack	PTFE/silicone	50/pk of each color: blue, green, red, light turquoise, purple	5040-4682
Pre-slit septa	PTFE/white silicone	100/pk	5183-2074

Syringes

Description	Volume	Part No.
Syringe, PTFE tip, fixed needle, 23/42/HP	500	G4513-60561
Syringe, PTFE tip, fixed needle, 23-26s/42/HP	100	G4513-80222
Syringe, PTFE tip, fixed needle, 23-26/42/HP	10	G4513-80203

Labels and ribbon

Description	Unit	Part No.
Ribbon plus label for 2 mL vials	2500/ roll	5190-3177
Label only, for 2 mL vials	2500/ roll	5190-3180
Barcode label printing bundle. Includes printer, software, templates, and labels to print barcode labels	1	G9201AA

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Your results are only as reliable as your sample prep

That is why the Agilent 7696A Sample Prep WorkBench combines precise automation with an intuitive software interface to help you:

- Automate repetitive manual sample preparation steps
- Save money on glassware, solvents, reagents, and solvent disposal – without sacrificing precision and reproducibility
- Reduce the need for rework due to variability between analysts
- Increase productivity and lower your cost per sample
- Minimize exposure to hazardous chemicals
- Automate gravimetric confirmation of liquid dispensing

For more information

To learn more about the Agilent 7696A Sample Prep WorkBench, visit
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