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Analysis of Bisphenol A in Food by Solid Phase Microextraction Using an Overcoated Fiber

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ISSUE 4 | 2019

Analytix Reporter

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The Life Science Business of Merck has recently undergone some updates to align our offering with the requirements of you, our customers. There are now 6 individual portfolio brands within our Life Science Business: Sigma-Aldrich®, SAFC®, BioReliance®, Milli-Q®, Millipore® and, of course, Supelco®. The Supelco® Portfolio is now a key pillar in the business and the trusted, go-to destination for customers for all our analytical products.

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The articles in this issue of Analytix Reporter cover a range as wide as our Supelco® offering. There is a particular focus on reference materials, including new certified reference material solutions for both paralytic shellfish toxins and ^{19}F Quantitative NMR, new phytochemical testing standards in collaboration with PhytoLab, as well as information on the benefits of our new proficiency testing portal. Additionally, we consider mobile technology with a look at how smartphone-based analysis can be applied to soil testing.

Coincidentally, we also have pieces on two topics that are becoming increasingly part of the public consciousness: methods and standards for the analysis of cannabinoid compounds in edibles and extraction techniques for the detection of BPA in packaged food products, as well as much more.

I hope you find this issue insightful and informative.

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FOOD & BEVERAGE

Analysis of Bisphenol A in Food by Solid Phase Microextraction Using an Overcoated Fiber

Katherine Stenerson, Principal Scientist, **Katherine.stenerson@milliporesigma.com**

Introduction

Bisphenol A (BPA) is commonly used for food packaging applications such as polycarbonate bottles and the linings of metal cans used for soups, juices, etc. It is a suspected endocrine disruptor, and therefore, low level, long term exposure as a result of migration into food from packaging materials is a concern. The use of BPA in food contact applications is regulated by the Food and Drug Administration (FDA), and in 2013 was prohibited for use in the packaging materials of infant formula.[1] For other food contact applications, margins of safety were published by FDA as "NOAEL", which stands for "no observed adverse effect level". This NOAEL was set at 5 mg/kg body weight per day, which is well above the estimated dietary intake.[2] Similarly, tolerable daily intake or "TDI" was set by the European Union (EU) at 4 µg/kg body weight per day.[3] While exposure to BPA through diet is thought to be low, testing continues in order to assess its migration into food from can and lid linings, plastic containers, etc. In the case of the EU, the specific migration limit (SML) for BPA from packaging into food has been amended in Sept 2018 to 0.05 mg/kg (formerly 0.6 mg/kg) food. In case of contact materials for food products with intended use for infants or young children, no BPA migration from coatings or varnishes is permitted at all.[4]

Extraction methods for determination of BPA in food include both solvent extraction (SE) and solid phase extraction (SPE), with the latter more commonly used with liquid samples and the former for solid samples. Analysis can be done by either LC or GC, and both have been used throughout the literature. Solid phase microextraction (SPME) has been used for the determination of BPA in water, but has not been widely used for this application in food matrices due to sensitivity and fiber ruggedness issues associated with exposure to matrix components such as fats and proteins.[5,6]

The purpose of this application was to revisit the use of SPME to develop a quick, easy, and sensitive method for analysis of BPA in a variety of food products. The issues mentioned previously related to food matrices and SPME were addressed through the use of an overcoated (OC) divinylbenzene (DVB) fiber. The overcoating, which consists of polydimethylsiloxane (PDMS), protects the DVB layer from contamination and increases the physical robustness of the fiber.

In addition, material adhering to the overcoating is more easily removed during the wash step typically performed for such samples. SPME extraction using the OC-DVB fiber was followed by GC-MS/MS analysis for optimum sensitivity. The steps taken in method development and optimization as well as durability comparison are outlined here. For more detailed information, please refer to the online version of this article under SigmaAldrich.com/Analytix (Issue 4).

Experimental

The final, optimized SPME method using the OC fiber is described in **Table 1**. After extraction, the fiber was desorbed in the inlet of a 7890/7000C GC-MS/MS system, and analysis proceeded following the conditions

Table 1. Optimized SPME procedure for extraction of BPA from food samples

sample/matrix:	10 mL vial containing 0.5 g of sample and 6.5 mL of water at pH 4 containing 25 % sodium chloride
SPME fiber:	Overcoated PDMS-DVB, 23 gauge
incubation:	10 min, 50 °C, 400 rpm
extraction:	immersion, 50 min, 50 °C, 250 rpm, vial penetration 34 mm
wash:	0.5 min, 250 rpm, vial penetration 34 mm
desorption:	3 min, 260 °C
post bake:	6 min, 270 °C

(continued on next page)

Table 2. GC-MS/MS conditions

column:	SLB®-PAHms, 30 m x 0.25 mm I.D., 0.25 µm (28340-U)
oven:	100 °C (3 min), 15 °C/min to 300 °C (10 min)
inj.temp.:	260 °C
carrier gas:	helium, 1 mL/min constant flow
detector:	MRM: BPA: 213/119, 213/91, 119/91
	BPA-d16: 224/125, 224/97, 125/97
MSD interface:	325 °C
liner:	0.75 mm I.D. SPME

listed in **Table 2**. The samples analyzed (canned pumpkin, pureed carrot baby food, cream of chicken soup and canned energy drink) were obtained from a local grocery store and refrigerated prior to testing. They were prepared for SPME by weighing 0.5 g into a 10 mL autosampler vial. Spiked samples for determination of accuracy and repeatability were spiked at 10 ng/g by direct addition of 5 µL of a 1 µg/mL solution of BPA in methanol to the 0.5 g sample. Samples were then allowed to equilibrate for 30-60 minutes. 6.5 mL of SPME diluent (LC-MS/MS grade water containing 25 % NaCl by weight, and adjusted to pH=4 with H_3PO_4) was added to each, followed by 7 µL of a 1 µg/mL methanolic solution of BPA-d16 internal standard. To decrease BPA background from the laboratory, all measuring glassware and pipettes used were glass, and were triple rinsed with methanol prior to use. The salt used to make the SPME diluent was treated in a muffle furnace and stored in a glass jar.

Samples (spiked and unspiked) were quantitated against matrix-matched calibration curves prepared as described previously and extracted following the method in **Table 1**.

Results and Discussion

Method Optimization

A primary goal of method development was to determine a single set of SPME parameters that could be used with multiple sample types. In the following paragraphs, optimization of critical parameters is outlined (more details in the online version under SigmaAldrich.com/Analytix).

Salt, pH and dilution. Addition of salt and lowering of pH increased response significantly of both BPA and BPA-d16 (not shown). The samples to be analyzed were mostly very viscous, and required dilution prior to SPME; thus, a water diluent at pH 4 containing 25 % salt was chosen. After experimentation with different sample sizes/dilutions, 0.5 g diluted to 7 mL was found to work adequately for all the matrices evaluated, which included canned pumpkin, pureed carrots, condensed cream of chicken soup, and a fruit flavored energy drink (latter one probably could had been analyzed undiluted).

Post-extraction wash. Since the method was to be used with food samples, incorporation of a post-extraction wash step was critical in removing residual matrix on the surface of the fiber prior to desorption in the GC inlet. Past work found this step to be more effective with the overcoated rather than the standard DVB fiber.[7] To maximize washing, a 30-second time was chosen for the method. Since good response was still obtained, the loss in response compared to no/shorter wash time was not expected to severely impact the sensitivity of the method.

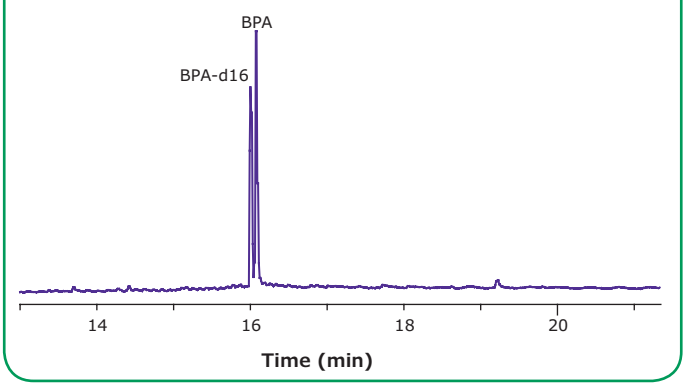
Extraction & equilibration conditions. Extraction times from 10 to 60 minutes were studied using 25 % saltwater at pH 4, spiked at 0.1 ng/mL (10 min equilibration @ 400 rpm agitation). Response steadily increased from 10 to 50 minutes and then leveled off from 50 to 60. Thus, 50 minutes was chosen as the extraction time.

Since temperature can influence the kinetics of the extraction, especially when working in heavy matrix like pureed carrot baby food, the effect of extraction temperature was studied at 30 °C, 40 °C, and 50 °C. Absolute response of BPA increased with temperature; thus 50 °C was chosen as the temperature for extraction and equilibration.

BPA background. BPA is a common laboratory contaminant, thus a major challenge in its analysis by any approach is managing background. SPME requires minimal sample preparation steps and materials, reducing sources of contamination compared to liquid-liquid extraction or SPE. Some steps taken to reduce background for the SPME method included methanol rinsing of pipettes and glassware, muffle furnace treatment of the sodium chloride, and use of LC-MS/MS water in a glass bottle for the diluent solution. Some background was generated upon injection in the GC, and increasing the septum purge setting from 3 to 6 mL/min reduced this. Some BPA background was still present from the SPME process; however, low level detection from samples was still possible.

Calibration. Since SPME is an equilibrium extraction technique, quantitation must be done against standards extracted using the same method as the samples. In the case of BPA, extraction efficiency varied by matrix, thus matrix calibration had to be used for accurate quantitation. An unspiked sample for each was included as a "0" concentration point.

Figure 1. GC-MS analysis (full scan) of BPA and BPA-d16, underivatized, on the SLB-PAHms 10 ppm standard in methanol



GC-MS/MS Analysis. A common approach to the GC analysis of BPA is derivatization using silylation or acetylation. This improves peak shape and response, allowing for better quantitation.[5,6] For this method, using an SLB®-PAHms column, as seen in **Figure 1**, derivatization was not necessary to obtain sufficient chromatographic performance and response.

SPME Method Performance

Analysis of spiked and unspiked samples. For all four matrices studied, both BPA and the internal standard, BPA-d16, could be detected free of interferences. An example is shown for the heaviest matrix, canned pumpkin, in **Figure 2**. As seen in **Figure 3**, the SPME method showed good linearity from the different matrices. The units are reported as ng/mL, which reflects the concentration from 0.5 g of sample diluted to a final volume of 7 mL prior to analysis, and translates to 7 to 140 ng/g BPA in the original sample. Accuracy and reproducibility of the method from these same matrices was determined by analysis of samples spiked at 10 ppb. The results of these evaluations are summarized in **Table 3**. Accuracy was >80% for all four matrices, with reproducibility as percent relative standard deviation (% RSD) or relative percent difference (%RPD) of <15 %. Since matrix-matched calibration curves were used to quantitate spiked samples, the level of BPA present in each sample prior to spiking could be determined with the "0" concentration or unspiked analysis using a standard addition approach. These values are reported in **Table 4**. The BPA detected in the carrot/baby food in the glass jar was probably a result of leaching from the lined cap, and the level detected is in the range found by others in the analysis of baby food in glass jars with metal lids.[8] In the canned samples, the highest level of BPA was detected in the cream of chicken soup. However, this level was still lower than past BPA levels determined by others in canned chicken soup.[9] It should also be noted that the soup was analyzed directly without water dilution. Normal preparation for consumption requires a 1:1 dilution with water, which would essentially cut the BPA level by 50 %.

As mentioned previously, when doing immersion SPME into heavy matrices, a post extraction wash step is essential in removing residual matrix prior to the desorption step in the GC inlet. A comparison of fiber durability and method ruggedness between the OC and standard, non-overcoated versions of the PDMS/DVB fiber was conducted by subjecting both to multiple extractions of canned pumpkin samples. Separate samples of pumpkin were weighed out and spiked at 10 ppb with BPA-d16. These were then run in a continuous sequence with 1 ng/mL BPA/BPA-d16 spiked water samples run every 6th extraction. The results

Figure 2. SPME-GC-MS/MS analysis of BPA spiked at 10 ppb in canned pumpkin

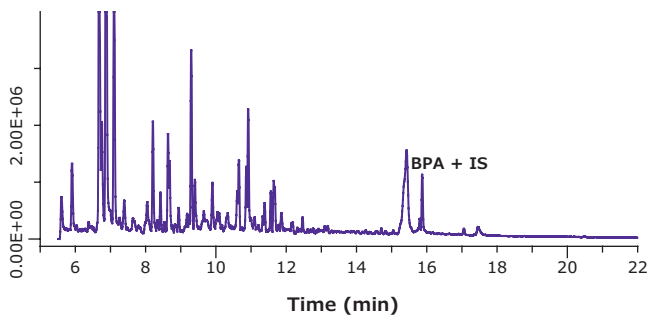


Figure 3. BPA / SPME method linearity from different matrices

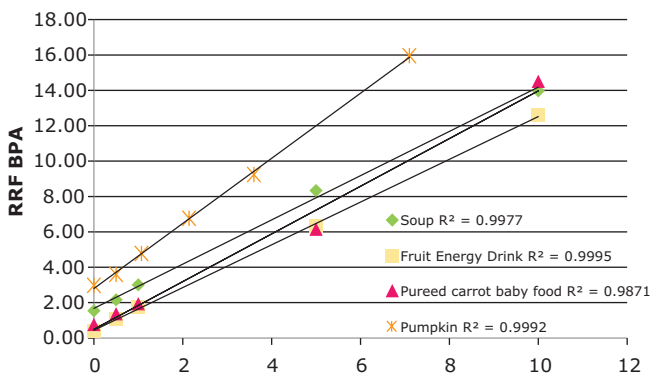


Table 3. Accuracy and reproducibility for SPME method applied to spiked samples

Sample	Spike Level	Avg. Amount Measured	Accuracy	RSD
fruit flavored energy drink	10 ng/mL	11.5 ng/mL	115 %	1 % (*n*=3)
baby food, carrots	10 ng/g	11.7 ng/g	117 %	2 %*
cream of chicken soup (condensed)	10 ng/g	8.2 ng/g	82 %	9 %*
pumpkin	10 ng/g	11.0 ng/g	110 %	13 % (*n*=6)

*%RPD, 2 replicates

Table 4. Level of BPA in unspiked samples; calculated using standard addition

Sample	Container Type	BPA Level - measured in unspiked sample
fruit flavored energy drink	can	0.8 ng/mL
baby food, carrots	glass jar with metal lid	0.65 ng/g
cream of chicken soup (condensed)	can	12.7 ng/g
pumpkin	can	1.6 ng/g

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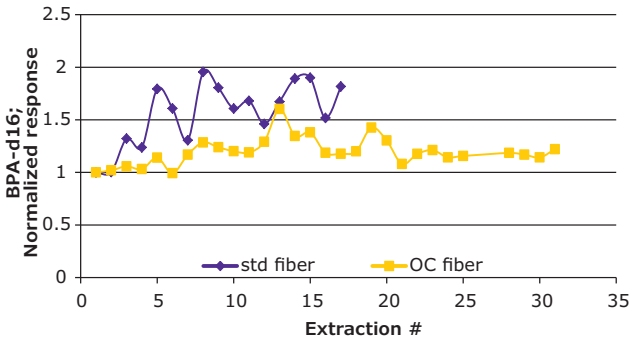
Figure 4. Response trend of BPA-d16 from spiked pumpkin samples over repeated extractions. Response is normalized to the first sample.



are shown in **Figure 4**. For purposes of studying the trend in response, area counts were normalized to the first sample extraction. In 25 extractions of pumpkin using the OC fiber, response did not show a significant decline. The condition of the fiber after the testing sequence was fairly good, with some discoloration but no evidence of physical damage. By comparison, the standard fiber run was stopped after 18 extractions, as the coating had stripped completely off the fiber core. The response trend was erratic, as seen in **Figure 4**. The findings of the durability testing indicate that either the overcoating is protecting the phase from damage as a result of exposure to the pumpkin matrix, and/or the post-extraction wash step is more effective for the OC fiber at removing residual matrix. This then helps to prolong fiber life.

Summary and Conclusions

An immersion SPME-GC-MS/MS method using an overcoated PDMS/DVB fiber was developed for the low level analysis of BPA from various food products. Method linearity from different matrices - a fruit flavored beverage, canned pumpkin, pureed carrot baby food, and cream of chicken soup - was in the range of 0.9871 (carrots) to 0.9995 (beverage). Method accuracy and reproducibility at a 10 ppb spiking level was between 80-110%, with RSD/RPD values of <15%. Durability testing showed the OC fiber to be more physically robust, with more consistent response compared to a standard fiber; the SPME method had only a few steps and was easy to automate. In addition, it was highly sensitive, and when combined with GC-MS/MS, provided the selectivity necessary to be used with different matrices.

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Featured Products

Description	Cat. No.
SPME OC Fiber Assembly (PDMS/DVB), Pk.3	**57439-U**
SPME fiber holder for use with CTC autosampler	**57347-U**
SLB®-PAHms capillary GC column, 30 m x 0.25 mm I.D., 0.25 µm	**28340-U**
Bisphenol A, certified reference material, TraceCERT®, 100 mg	**42088**
Bisphenol A-d16, analytical standard, 50 mg	**442876**

Related Products

Description	Cat. No.
Sodium chloride, ACS reagent grade	**746398**
Clear vial, screw top, 10 mL, for CTC autosampler, Pk.100	**SU860099**
Magnetic screw cap, with 1.3 mm septa, for autosampler vial, Pk.100	**SU860101**
0.75 mm I.D. direct (SPME) liner for Agilent®	**2637501**
Molded Thermogreen™ LB-2 septa with injection hole, 11 mm, Pk.50	**28336-U**

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FOOD & BEVERAGE

Analysis of Active Cannabis Compounds in Edible Food Products: Gummy Bears and Brownies

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Margaret Wesley, 2016 R&D Summer Intern from Pennsylvania State University, State College, PA

Introduction

Potency testing in marijuana-infused edibles is a problematic task that analytical labs are facing due to the complexity of the involved matrices. Among the most popular matrices are gummy bear candies and brownies. According to one laboratory site, the concentration of active ingredients in the edibles can range from a few parts per million to 3.5 parts per thousand.[1] In this application, a procedure was developed to extract active cannabinoid compounds from gummy bears and brownies. The procedure included a simple and fast extraction of the active compounds from the studied foods, and analysis by HPLC-UV using a biphenyl stationary phase chemistry.

Experimental

Cerilliant® cannabinoid standards, available as 1 mg/mL solutions in either methanol or acetonitrile, were used for this experiment. The concentration of cannabinoids allowed for the spiking of both gummy extract and brownies at about 40 ppm with all compounds. The following compounds were included in this study: cannabidivarinic acid (CBDVA), cannabidivarin (CBDV), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidiolic acid (CBDA), cannabidiol (CBD), tetrahydrocannabivarin (THCV), cannabinol (CBN), (-)-Δ^9-Tetrahydrocannabinol (Δ^9-THC), (-)-Δ^8- Tetrahydrocannabinol (Δ^8-THC), and (-)-Δ^9-Tetrahydrocannabinolic acid A (THCAA). This list of 11 different cannabinoids includes several acidic forms; thus HPLC analysis was used in order to quantitate these in their native forms.

The HPLC column used was Ascentis® Express Biphenyl, 2.7 µm particle size, which gave the best separation of all 11 compounds in under 13 minutes. The use of this column with Fused-Core® particle architecture resulted in low back pressure, thus a standard pressure HPLC system could be used during this experiment.

Sample Preparation

One gummy bear candy, non-spiked, (2.3 g) was dissolved in 20 mL of warm water. This solution was then spiked with cannabinoids and extracted using a QuEChERS procedure. The average spiking level in each gummy bear was 45 ppm for each compound. Bears of four different colors were tested – orange, yellow, red, and green. After spiking, the water/candy solution was

transferred to a 50 mL plastic QuEChERS extraction tube (55248-U). Acetonitrile (10 mL) was added, and the tube was shaken for one minute by hand. Supel™ QuE non-buffered salts (55295-U) were added, and the samples were shaken for 5 minutes on an automated QuEChERS shaker. Post-shaking, the samples were centrifuged for 5 minutes at 5000 rpm. The top layer was collected and injected directly into the HPLC.

For brownies, a 2.5 g sample of a non-spiked brownie with frosting was added to the QuEChERS extraction tube. This sample was spiked with cannabinoids and allowed to sit for 30 minutes prior to extraction. The average spiking level for the brownies was 40 ppm. The QuEChERS extraction was performed as previously described for gummy bears. Post-extraction, the top acetonitrile layer was collected into a vial and kept under refrigeration for a minimum of 3 hours to remove fats prior to HPLC analysis.

A calibration curve was constructed in acetonitrile bracketing the expected concentration of 10 µg/mL in the final extracts. The following calibration points were included: 2 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL and 25 µg/mL.

Results and Discussion

For the gummy bear samples, it was found that neither the red, yellow, nor green color interfered with detection of cannabinoids at 220 nm. The red color was partially extracted into acetonitrile, while the green and yellow colors stayed in the aqueous layer upon extraction. However, the orange color from the gummy bear, when extracted into acetonitrile, was found to have an interfering peak that co-eluted with CBDVA. Thus, for the orange gummy bear, quantitation of CBDVA was done at 280 nm, where CBDVA has significant absorbance free of interference. Quantitation was done at 220 nm for the rest of compounds in this study (**Figure 1**).

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While no cleanup was required for gummy bear samples post-extraction, the co-extractives in the brownie were found to decrease the recoveries of the analytes if the brownie extract was injected into HPLC without further processing. The brownie extract was cleaned by refrigeration to remove the co-extracted fats.

The ruggedness of the method for brownies was tested by injecting the brownie extract (**Figure 2**) multiple times followed by the injection of the 10 µg/mL standard. After 7 injections of the brownie extract, it was found that the peak retention times were not affected, indicating that the column was being thoroughly cleaned between injections. The peak areas for the standards showed a slight decrease of 4 %.

Excellent recovery values of above 90 % for gummies and above 80 % for brownies were achieved with good accuracies (**Table 1**).

Table 1. Recoveries From Spiked Gummy Bears and Brownies

Peak No.	Compound	Yellow Gummy	Orange Gummy	Red Gummy	Average Gummy and RSD	Average Brownie and RSD
1	CBDVA	90 %	92 %*	92 %	91 % (2 %)	91 % (1 %)
2	CBDV	93 %	100 %	100 %	98 % (3 %)	93 % (5 %)
3	THCV	87 %	93 %	90 %	90 % (3 %)	87 % (1 %)
4	CBDA	94 %	90 %	95 %	94 % (3 %)	95 % (1 %)
5	CBGA	87 %	91 %	89 %	91 % (4%)	90 % (2 %)
6	CBD	95 %	100 %	98 %	97 % (3 %)	89 % (5 %)
7	CBG	93 %	99 %	98 %	96 % (4 %)	91 % (5 %)
8	CBN	88 %	95 %	97 %	95 % (6 %)	84 % (4 %)
9	Delta-9-THC	93 %	99 %	100 %	97 % (3 %)	82 % (4 %)
10	Delta-8-THC	91 %	97 %	98 %	95 % (3 %)	80 % (4 %)
11	THCA-A	89 %	89 %	89 %	92 % (7 %)	91 % (2 %)

*The orange gummy was done at 280 nm due to the interfering background peak quantitation.
Note: THCA is the abbreviation used by AOAC

Conclusion

A method was developed for analysis of active cannabinoid compounds in both brownies and gummy bears. The extraction procedure involved a salting out step into acetonitrile and did not require intensive cleanup. The separation of eleven compounds was achieved on a biphenyl stationary HPLC phase and was completed in 13 minutes. The active compound CRMs are available from Cerilliant® through SigmaAldrich.com.

Reference

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Figure 1. HPLC Chromatogram of Orange Gummy Bear Extract at (a) 220 nm and (b) 280 nm Figure 2. HPLC of a Brownie Extract at 220 nm. The peak elution order is listed in **Table 1**.

column:	Ascentis® Express Biphenyl, 10 cm × 2.1 mm I. D., 2.7 µm particles (64065-U)
mobile phase:	(A) 0.1 % TFA in water; (B) 0.1 % TFA in acetonitrile
gradient:	at 47 % B, to 50 % B in 13 minutes, to 100 % B in 0.1 min, 100 % B for 3 minutes, to 47 % B in 0.1 min, at 47 % B for 2.5 minutes
flow rate:	0.70 mL/min
column temp.:	35 °C
detector:	UV, 220 nm and 280 nm
injection:	5 µL
pressure:	340 bar
instrument:	Agilent® 1200, with UV detector

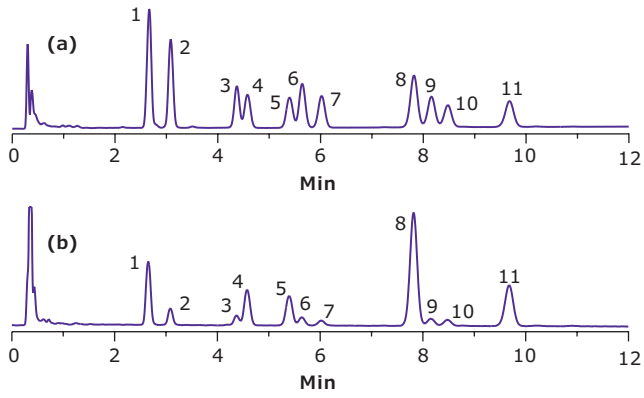
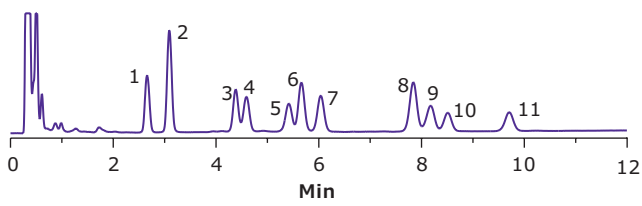


Figure 2. HPLC of a Brownie Extract at 220 nm
The peak elution order is listed in **Table 1**.

Conditions same as **Figure 1**.



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Description	Cat. No.
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Non-buffered Extraction Tube 2, 12 mL, pk of 50	**55295-U**
Empty Centrifuge Tube, 50 mL, pk of 50	**55248-U**
Ascentis® Express Biphenyl HPLC Column	
10 cm × 2.1 mm I.D., 2.7 µm particle size	**64065-U**
Cerilliant® Certified Reference Materials	
Cannabidivarinic acid (CBDVA), 1 mg/mL in acetonitrile, CRM	**C-152**
Cannabidivarin (CBDV), 1 mg/mL in methanol	**C-140**
Cannabigerolic acid (CBGA), 1mg/mL in acetonitrile	**C-142**
Cannabigerol (CBG), 1 mg/mL in methanol	**C-141**
Cannabidiolic acid (CBDA), 1 mg/mL in acetonitrile	**C-144**
Cannabidiol (CBD), 1 mg/mL in methanol	**C-045**
Tetrahydrocannabivarin (THCV), 1 mg/mL in methanol	**T-094**
Cannabinol (CBN), 1 mg/mL in methanol	**C-046**
(-)-Δ^9-Tetrahydrocannabinol (Δ^9-THC), 1 mg/mL in methanol	**T-005**
(-)-Δ^8-Tetrahydrocannabinol (Δ^8-THC), 1 mg/mL in methanol	**T-032**
(-)-Δ^9-Tetrahydrocannabinolic acid A (THCA-A), 1 mg/mL in acetonitrile	**T-093**
Accessories	
QuEChERS Shaker and Rack Starter Kit, USA compatible plug	**55278-U**
QuEChERS Shaker and Rack Starter Kit, EU Schuko plug	**55438-U**
Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100	**29653-U**

FOOD & BEVERAGE

New Phytochemical Standards

Phytolab reference materials are now available through SigmaAldrich.com

Matthias Nold, Global Product Manager Reference Materials, **matthias.nold@merckgroup.com**

Plants are an important source for both dietary supplements and traditional medicinal products. Natural high levels of variation in the composition of plants and the increasing problem of adulteration necessitate thorough quality control for plant derived products. An unambiguous identification, as well as accurate quantification of plant constituents, are crucial and require the availability of reliable reference materials.

We are happy to announce our collaboration with PhytoLab, a global leader in the testing of phytochemicals and in manufacturing phytochemical reference materials. By making their phyproof® reference materials available globally through our web portal, we are significantly increasing our offering of reference materials for phytochemicals. Included in this article you will find a list of the initial products (**Table 1**). Many more will be introduced in the coming months. Please visit **SigmaAldrich.com/phytolab** for an up-to-date list.

Our entire offering of phytochemical reference materials, including standards and certified reference materials in neat and solution form, and reference materials of plant extracts and essential oils, can be found on our website. All products can be viewed either by compound class or by plant genus.

Table 1. Phytopharma Standards manufactured by PhytoLab, now available through SigmaAldrich.com

Product Name English	Qty.	Cat. No.
Amarogentin	10 mg	**PHL80178**
Anemonin	10 mg	**PHL80346**
Apiin	10 mg	**PHL89161**
Atropine sulfate	20 mg	**PHL80892**
Bacoside A	10 mg	**PHL89576**
Berberine chloride dihydrate	100 mg	**PHL89487**
Caffeoylmalic acid	10 mg	**PHL89238**
Californidine perchlorate	10 mg	**PHL89592**
(-)-Catechin 3-gallate	10 mg	**PHL82497**
α-Chaconine	5 mg	**PHL80075**
Chelidonine	20 mg	**PHL89600**
Cimifugin	10 mg	**PHL89387**
Cinnamtannin A2	5 mg	**PHL83372**
Convallatoxin	10 mg	**PHL80834**
8-p-Coumaroylharpagide	10 mg	**PHL89178**
Crocin	10 mg	**PHL80391**
Cucurbitacin B	10 mg	**PHL82226**

(continued on next page)

Product Name English	Qty.	Cat. No.
Cucurbitacin E	10 mg	**PHL80013**
Cucurbitacin E-2-O-glucoside	10 mg	**PHL89613**
Cucurbitacin I	10 mg	**PHL89464**
(-)-Curine	10 mg	**PHL80979**
Cyanidin 3,5-diglucoside chloride	10 mg	**PHL89615**
Cymarin	20 mg	**PHL89838**
23-epi-26-Deoxyactein	10 mg	**PHL89183**
3,5-Dicaffeoylquinic acid	10 mg	**PHL80426**
3,4-Dicaffeoylquinic acid	10 mg	**PHL80425**
4,5-Dicaffeoylquinic acid	10 mg	**PHL80427**
Digitoxin	25 mg	**PHL82516**
Echimidine	10 mg	**PHL89553**
Echimidine N-oxide	5 mg	**PHL83590**
(-)-Englerin A	10 mg	**PHL82530**
Erucifoline	5 mg	**PHL83432**
Erucifoline N-oxide	5 mg	**PHL83433**
Eupatilin	10 mg	**PHL80337**
Europine hydrochloride	10 mg	**PHL83237**
Europine N-oxide	10 mg	**PHL83238**
Gelsemine	10 mg	**PHL80457**
Gentiopicroside	10 mg	**PHL89512**
Ginkgotoxin hydrochloride	10 mg	**PHL82638**
Ginsenoside Rc	10 mg	**PHL89210**
Glucoraphanin potassium salt	10 mg	**PHL89215**
Guaiazulene	100 mg	**PHL89697**
Harpagide	10 mg	**PHL89703**
Heliotrine	10 mg	**PHL80403**
Heliotrine N-oxide	10 mg	**PHL83236**
Hyoscyamine sulfate	20 mg	**PHL82389**
Indicine hydrochloride	10 mg	**PHL83234**
Indicine N-oxide	10 mg	**PHL83235**
Indirubin	10 mg	**PHL89716**
Intermedine	10 mg	**PHL82424**
Intermedine N-oxide	5 mg	**PHL83446**
Isorhamnetin 3-rutinoside	10 mg	**PHL83337**
Isoxanthohumol	10 mg	**PHL89234**
Jaceosidin	10 mg	**PHL80786**
Jacobine	5 mg	**PHL83434**
Jacobine N-oxide	5 mg	**PHL83435**
Kahweol	20 mg	**PHL82293**

Product Name English	Qty.	Cat. No.
Lasiocarpine	10 mg	**PHL80412**
Lasiocarpine N-oxide	10 mg	**PHL83220**
Z-Ligustilide solution	10 mg	**PHL89242**
Lycopsamine	10 mg	**PHL89726**
Lycopsamine N-oxide	5 mg	**PHL83447**
Malvidin 3,5-diglucoside chloride	10 mg	**PHL89727**
Marrubiin	10 mg	**PHL89830**
Miliacin	10 mg	**PHL82261**
Mitraphylline	10 mg	**PHL89736**
Monocrotaline N-oxide	10 mg	**PHL82629**
Muscimol	10 mg	**PHL89495**
Nordihydrocapsaicin	10 mg	**PHL89254**
Pelargonidin 3-glucoside chloride	10 mg	**PHL89753**
Petunidin 3-glucoside chloride	5 mg	**PHL89755**
Primulic acid I	10 mg	**PHL89259**
Primulic acid II	10 mg	**PHL89260**
Protodioscin	10 mg	**PHL80522**
Punicalagin (A + B mixture)	10 mg	**PHL80524**
Quercetin	20 mg	**PHL89262**
Retrorsine N-oxide	10 mg	**PHL82630**
Ruscogenin	10 mg	**PHL89268**
Sabinene	100 mg	**PHL82342**
Sanguinarine chloride	10 mg	**PHL89327**
Sarsasapogenin	20 mg	**PHL80531**
Scopolamine hydrobromide	5 mg	**PHL80046**
Scopolin	10 mg	**PHL82649**
Sempervirine nitrate	10 mg	**PHL83105**
Senecionine N-oxide	5 mg	**PHL82631**
Seneciphylline N-oxide	5 mg	**PHL82632**
Senecivernine	5 mg	**PHL83436**
Senecivernine N-oxide	5 mg	**PHL83437**
Silybin (A + B mixture)	25 mg	**PHL89280**
α-Solanine	10 mg	**PHL80074**
γ-Strophanthin	20 mg	**PHL80207**
α,β-Thujone	100 mg	**PHL82669**
Trichodesmine	10 mg	**PHL83438**
Trifolirhizin	10 mg	**PHL80547**
Tryptanthrine	10 mg	**PHL83458**
Urushiol (15:2)	10 mg	**PHL80181**

A complete list of PhytoLab Standards available from us can be found on **SigmaAldrich.com/phytolab**

For our comprehensive portfolio of analytical standards and certified reference materials (CRMs) visit us at **SigmaAldrich.com/standards**

An overview of plant extract reference materials can be found at **SigmaAldrich.com/plantextracts**

FOOD & BEVERAGE

New CRM Solutions for Paralytic Shellfish Toxins

Matthias Nold, Global Product Manager Reference Materials, **matthias.nold@merckgroup.com**

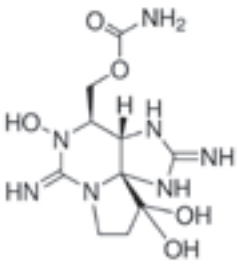
Paralytic Shellfish Toxins (PSTs) are a group of highly toxic, naturally occurring alkaloids produced by certain species of marine algae. During harmful algae blooms, these toxins can accumulate in bivalve mollusks and cause severe poisoning after consumption. The occurrence of PSTs in seafood, therefore, needs to be monitored. We are introducing a series of native and isotope labeled Certified Reference Material (CRM) solutions for paralytic shellfish toxins, suitable for calibration of LC-MS / LC-IDMS (isotope dilution MS) food testing methods.

The CRM solutions are manufactured in-house under our ISO/IEC 17025 and ISO 17034 accreditation, and feature:

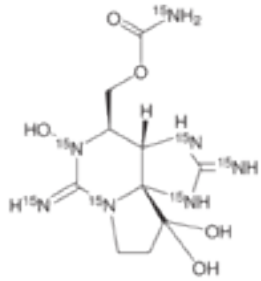
- Certification by qNMR or IDMS with traceability to SI unit via NIST or NRC primary standards
- Solutions produced gravimetrically
- Homogeneity, accelerated and long-term stability tested by LC-MS
- A comprehensive certificate including the overall uncertainty

For more information, and an up-to-date list of marine toxin CRMs, please visit:

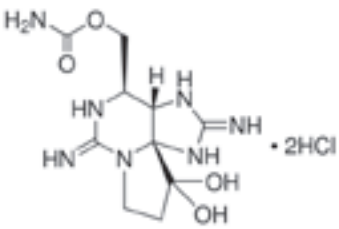
SigmaAldrich.com/marinetoxins



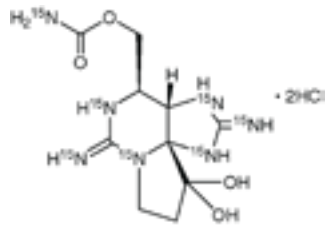
41619
Neosaxitoxin solution
20 µg/g in 3 mmol/L HCl



41206
Neosaxitoxin $^{15}N_7$ solution
10 µg/g in 3 mmol/L HCl



93665
Saxitoxin dihydrochloride solution
20 µg/g in 3 mmol/L HCl



30929
Saxitoxin $^{15}N_7$ dihydrochloride solution
10 µg/g in 3 mmol/L HCl

As a first set of products, we are introducing the PSTs Neosaxitoxin and Saxitoxin dihydrochloride as CRM solutions both in native and ^{15}N labeled form.

Description	Qty.	Cat. No.
Neosaxitoxin, 20 µg/g in hydrochloric acid, certified reference material, TraceCERT®	0.5 mL	**41619**
Neosaxitoxin-$^{15}N_7$, 10 µg/g in hydrochloric acid, certified reference material, TraceCERT®	0.5 mL	**41206**
Saxitoxin dihydrochloride, 20 µg/g in hydrochloric acid, certified reference material, TraceCERT®	0.5 mL	**93665**
Saxitoxin-$^{15}N_7$ dihydrochloride, 10 µg/g in hydrochloric acid, certified reference material, TraceCERT®	0.5 mL	**30929**

FOOD & BEVERAGE

Life Science Award in Food & Beverage Safety

Ilona Matus, Analytical Sciences Liaison, **ilona.matus@merckgroup.com**

Our Life Science Awards recognize the innovation that the academic community brings to the future of life science. It is our goal to foster the development of post-graduate students interested in a career in life science and help advance technologies which can transform the industry.

Following the success of the 2017 awards, in 2018 we called for applications to win a life science award for innovative and effective strategies to promote safety in the food and beverage arena.

From numerous applications covering a wide range of technical and scientific areas, all of them at a high scientific level, a jury of experts from our research and marketing selected four, and these candidates were invited to present their work in an award ceremony at the company headquarters in Darmstadt, Germany.

During the event, the young scientists also had the chance to visit various production areas and have discussions with R&D and marketing staff as well as meet the head of Applied Solutions, Jean-Charles Wirth, who gave some insights and answered questions from the participants.

The jury had the pleasure to listen to four excellent presentations:

Immanuel Yüce, Justus Liebig University of Giessen (Germany)

"Development of in situ and in silico tools as well as improvement of classical techniques for structure elucidation in planar chromatography" guiding us through analytical chemistry, highlighting innovative proposals of planar chromatography for impurity identification.

Shaokang Zhang , University of Georgia (USA),

"Whole genome sequencing (WGS), a one-stop platform for foodborne pathogen subtyping, characterization and detection" taking the jury on a ride of the benefit of Whole Genome Sequencing for outbreak surveillance and the future potential of this technology for pathogen detection.

Anna Sophia Harrand, Cornell University (USA)

"Effects of strain diversity and growth conditions on subsequent bacterial growth" a very well presented Whole Genome Sequencing based approach outlining a proposal to improve process validation in production.

The candidates with the jury

Yanqi Qu, University of Massachusetts, Amherst (USA)

"Safety and quality analysis of alcoholic and non-alcoholic beverages using surface-enhanced Raman scattering (SERS)" demonstrated how self-assembled nanoparticle mirrors enable implementation of Surface Enhanced Raman Scattering in beverage safety and quality.

All four candidates gave inspiring presentations of their topics, followed by open discussions. It was not an easy task for the jury to make a decision.

The winner of the 2018 Life Science award in F&B safety and €10,000 is Yanqi Qu. The other finalist all received €2,000 for their contributions

We congratulate the winner, but also thank the other candidates for their contributions.

From left: Jean-Charles Wirth (Head of Applied Solutions), Yanqi Qu (2018 winner), Ian Jennings (Head of Biomonitoring) and Heike Petri (Head of Advanced Analytical & Industrial Testing)

Certified Reference Materials for ^{19}F Quantitative NMR Ensuring Traceability to "The International System of Units" (SI)

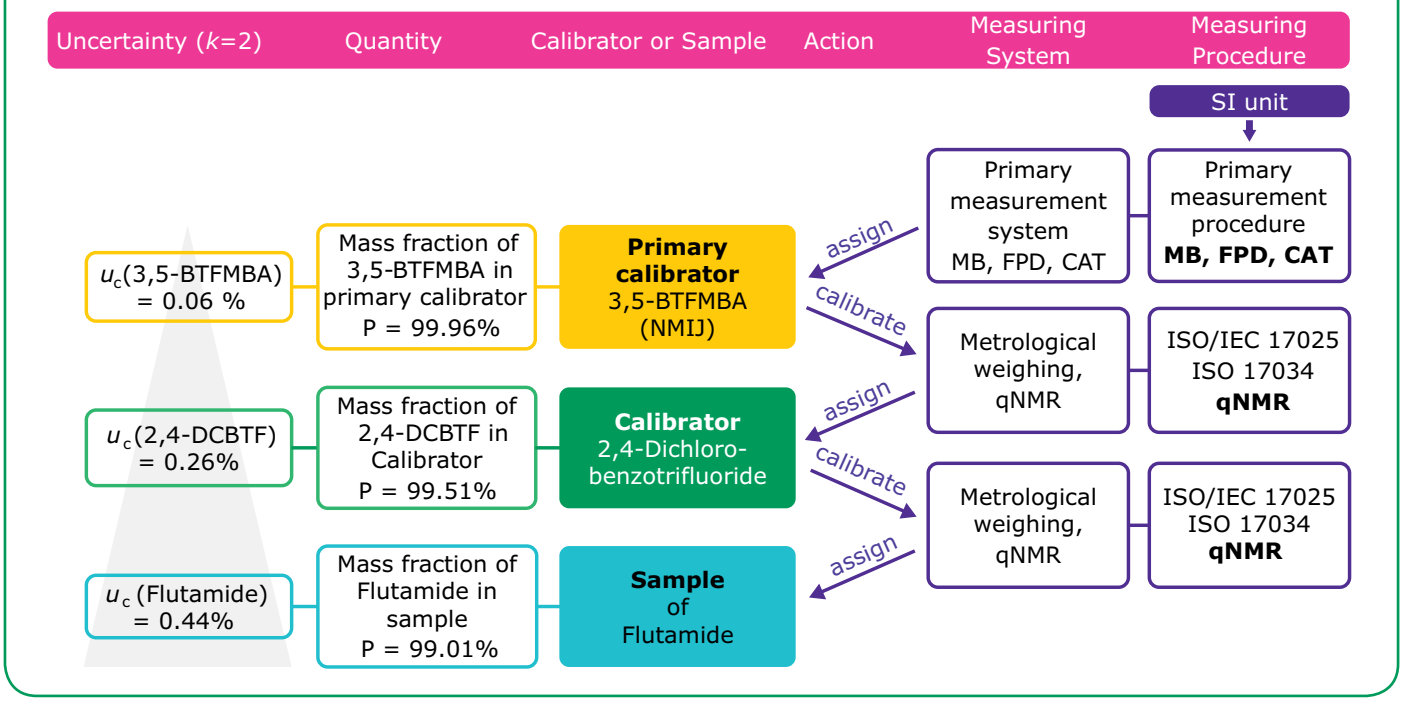
Romana Rigger, Alexander Rück, Christine Hellriegel, Robert Sauermoser, Fabienne Morf, Kathrin Breitruck, Markus Obkircher

Markus Obkircher, Head of Reference Materials R&D, **markus.obkircher@merckgroup.com**

In recent years quantitative NMR (qNMR) spectroscopy has become one of the most important tools for content determination of organic substances and quantitative evaluation of impurities. The implementation of qNMR for new application fields, e.g., metabolomics, environmental analysis and physiological pathway studies, brings along more complex molecules and systems, thus making the use of ^{1}H-qNMR challenging. A smart workaround is possible through use of other NMR active nuclei, namely ^{31}P and ^{19}F.

At our manufacturing site in Buchs (Switzerland), we have been using qNMR since 2009 to produce certified reference materials (CRM) traceable to the SI unit, under ISO/IEC 17025 and ISO Guide 34 (since 2017: ISO 17034) accreditation (an example of a traceability chain is shown in **Figure 1**). The *Trace*CERT® product range of organic CRMs suitable for HPLC or GC is certified using this technique and comprises over 200 products including pesticides, vitamins, amino acids, plasticizers, PAHs, antibiotics, FAMEs and many other product groups. In addition to this product range, we also provide a toolkit of qNMR standards traceable to primary material from NIST (National Institute of Standards and Technology, USA) or NMIJ (National Metrology Institute of Japan), see SigmaAldrich.com/qnmr. The expansion of this qNMR standard product line with new, interesting CRMs is ongoing and up-to-date 16 different ^{1}H qNMR CRMs with known purity values and small expanded measurement uncertainties have been developed. They cover the whole spectral and solubility range, enabling access to the qNMR certification of hundreds of organic products.

Figure 1. Traceability chain of Flutamide. Certification was done by comparison with 2,4-DCBTF (secondary calibrator) and 3,5-BTFMBA (primary calibrator) and finally to the SI unit. MB = mass balance, FPD = freezing point depression, CAT = coulometric acidimetric titration.



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In certain cases, ^{1}H qNMR reaches its limits, especially regarding the certification of complex and larger molecules. However, new fields of application often also bring along the presence of heteroatoms, namely ^{31}P and ^{19}F. Thus we introduced 4 CRMs for ^{31}P qNMR with traceability to the SI.

In the following section, the development of CRM for the use in ^{19}F qNMR is described. This article is an excerpt from our AOAC paper published in 2017. Please refer to this reference for further information.[1]

3,5-Bis(trifluoromethyl)benzoic acid (3,5-BTFMBA, NMIJ CRM 4601-a) is a primary CRM for use in ^{1}H and ^{19}F qNMR certified by NMIJ. The NMR shift range of ^{19}F is very large but the window for linear excitation, which is necessary for ^{19}F qNMR, is quite small and depends on field strength and NMR parameter. Techniques to counter this dilemma were published earlier including the use of new NMR experiments. Therefore, we set out to develop qNMR CRMs with peaks in different shift regions which can further be chosen corresponding to the analytes' shift and employed in standard ^{19}F qNMR experiments. Two of the most common structure elements are CF_3 groups and fluorine atoms bound directly to substituted aromatic compounds. Shifts of ^{19}F in CF_3 groups arise around -55 to -90 ppm, while shifts of fluorine atoms bound to aromatics can be found between approximately -110 and -180 ppm. Further structure elements show signals between -70 and -140 ppm (CF_2) or between -120 and -240 ppm (fluorine atoms in saturated and unsaturated aliphatic compounds).

Recently three different ^{19}F qNMR CRMs were developed by us. They were selected based on various parameters including solubility, stability, homogeneity, purity and shift range. As a prerequisite to show traceability to the SI and the certification concept, we selected molecules that carry both ^{1}H and ^{19}F nuclei. 2,4-Dichlorobenzotrifluoride (2,4-DCBTF, cat. no. 53396) is liquid and the CF_3 group shows a singlet at -61.2 ppm in the ^{19}F spectrum, depending on the solvent (DMSO-d_6). The three aromatic protons show analyzable signals between 7.5 and 8.5 ppm in the ^{1}H spectrum (DMSO-d_6). 2-Chloro-4-fluorotoluene (2Cl4FT, cat. no. 80730) is also liquid and the fluorine atom bound to the aromatic ring shows a multiplet at -115.3 ppm (DMSO-d_6) in the ^{19}F spectrum. In the ^{1}H spectrum, again three aromatic protons show peaks between 7.0 and 8.0 ppm and an additional peak can be found for the methyl group at around 2.3 ppm (DMSO-d_6). 4,4'-Difluorobenzophenone (4,4'-DFBP, cat.no. 07563) is solid and the two symmetrical fluorine atoms show a multiplet at around -106.5 ppm (DMSO-d_6) in the ^{19}F spectrum. Eight aromatic protons give signals between 7.0 and 8.0 ppm (DMSO-d_6). All three compounds are soluble in common organic NMR solvents. Molecular weights are 215 g/mol (2,4-DCBTF), 144.57 g/mol (2Cl4FT) and 218.2 g/mol (4,4'-DFBP). Purity values, expanded measurement uncertainties, NMR solvent specific shifts and relaxation times (T1) can be found in **Table 1**.

Technical aspects of ^{19}F qNMR

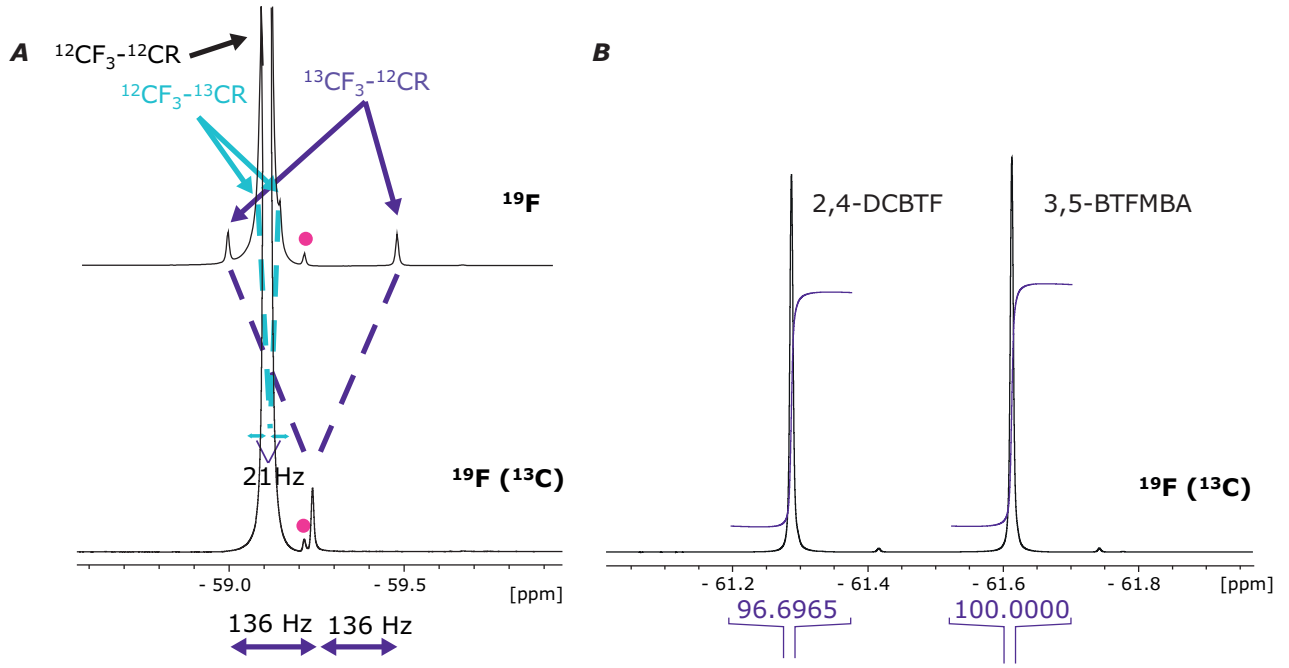
A characteristic of ^{19}F NMR is given by ^{13}C and ^{12}C satellites that are present in the NMR spectrum. The interaction of ^{19}F with ^{12}C and ^{13}C leads to an isotopic effect and thereby to unsymmetrical satellites on the one hand and to multiple satellites around the main peak in non-decoupled spectra on the other hand. Additionally, peak shapes are different depending on the structure element. In general, CF_3 peaks show singlet signal pattern and aromatic bound ^{19}F atoms multiplet signal pattern.

As with ^{31}P qNMR, inverse gated decoupling was used during ^{19}F qNMR data acquisition. Using this method instead of an e.g., power-gated decoupler minimizes NOE (Nuclear Overhauser Effect) build-up. With this experiment, decoupling is applied only during data acquisition and thus allows the spin system to reach equilibrium between decoupling steps. By applying inverse gated decoupling, only one satellite appears that is on only one side of the main peak (**Figure 2**). When performing pretests, a set of decoupled and coupled spectra was recorded to distinguish between satellites and impurities. Integration of decoupled spectra (**Figure 2**) was done either including both satellites, only the ^{12}C satellite, or if possible no satellite. No matter which possibility was chosen, integration was performed in the same way for the internal standard and the sample compound with regard to the line width. Similar to ^{13}C, the

Table 1: Summarized data of ^{19}F qNMR CRMs. Solubility tests were done at room temperature using commercially available NMR solvents. T1 relaxation times were recorded for the CRM only (c = 10 to 20 mg/mL at 25 °C). u_c(CRM) is the combined measurement uncertainty of the CRM and d(ppm) is the chemical shift in the ^{19}F spectrum (k=2).

					$CDCl_3$		DMSO-d_6		CD_3OD		CD_3CN	
Substance	Cat. No.	Package Size	Purity (%)	u_c (CRM) (%)	δ (ppm)	T1 (s)	δ (ppm)	T1 (s)	δ (ppm)	T1 (s)	δ (ppm)	T1 (s)
4,4'-Difluoro-benzophenone	**07563**	1g	99.82	0.30	-105.8	2.4	-106.5	1.4	-108.1	2.8	-108.3	2.3
2,4-Dichloro-benzotrifluoride	**53396**	1g	99.51	0.26	-62.5	2.3	-61.2	1.2	-65.4	3.3	-63.0	2.9
2-Chloro-4-fluorotoluene	**80730**	1g	99.57	0.24	-115.8	4.4	-115.3	3.3	-117.7	4.8	-117.3	4.7

Figure 2. A: ^{19}F NMR Signal of Flutamide in coupled (^{19}F) and decoupled ($^{19}F(^{13}C)$) spectra. The pink point is an impurity. Satellites are assymetrically arranged around the main peak in coupled spectra. B: Example for the integration of 2,4-DCBTF (analyte) and 3,5-BTFMBA (internal standard). Both signals were integrated without the outer satellite.



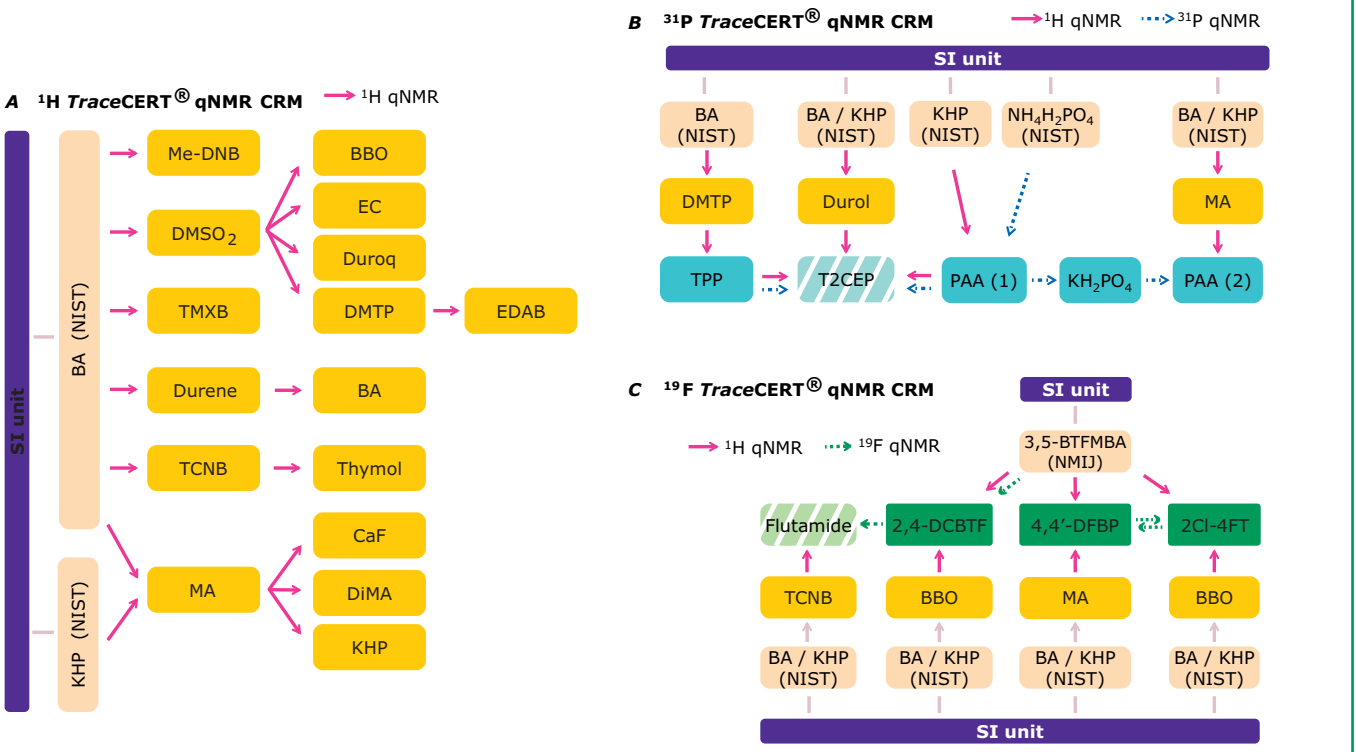
^{19}F nucleus has a wide chemical shift range. To perform quantitative measurements, broadband excitation over the full spectral width is required. Due to insufficient available radiofrequency power for pulsed excitation, signal intensities and thus signal integration can be error-prone. The effect leads to relatively narrow ranges of frequencies (15 - 30 kHz, 600 MHz NMR, 90 ° pulse) where an accurate quantification can be guaranteed. This requires sound pretesting, followed by accurate adjusting of spectral width and transmitter frequency offsets. Furthermore it is important to set the acquisition time as short as possible to avoid NOE build up, but long enough to avoid loss of spectral quality by truncation of the Free Induction Decay (FID). That requires an additional analysis of the FID prior to quantitative measurements. All ^{19}F NMR experiments were performed on a Bruker Avance III 600 MHz NMR instrument equipped with a Prodigy TCI probe head. Even though a standard probe (instead of a dedicated ^{19}F probe) was used, a good spectral quality could be ensured. Background distortions by probe head and sample tube materials, pulse breakthrough and ringing artifacts influence the spectral quality, especially the baseline (rolling baseline), which is typical for ^{19}F, ^{11}B and ^{29}Si and increases when measuring over large spectral width. This can be counteracted by either applying additional processing steps (FID repair by cutting the first data points before data transformation) or by increasing the pre-scan delay. For ^{19}F qNMR experiments during the development of our CRMs, an increased pre-scan delay was used and no FID cutting was done. T1 times were determined by inversion recovery experiments. Typical T1 times for ^{19}F qNMR CRMs are between 1.2 and 4.8 s depending on the concentration, of the mixture and solvent. Multiplying T1 times by a factor of 7-10 gives D1 times between 20 and 35s.

CRM for ^{19}F qNMR - traceability to the SI through primary CRM

Similar to the study published for ^{31}P, a traceability scheme for ^{19}F qNMR CRMs was elaborated to guarantee the traceability to the SI unit and show the comparability of ^{1}H and ^{19}F qNMR experiments and thus the independency of the result of the measured nucleus (**Figure 3, C**). As primary reference material, 3,5-BTFMBA of the National Metrology Institute of Japan was selected. This reference is highly pure (99.96 %), has a very small expanded measurement uncertainty (0.06 %, k=2) and the two symmetrical CF_3 groups show a sharp ^{19}F signal at -61.3 ppm (in DMSO-d_6). The three aromatic protons give signals around 8.2 - 8.6 ppm (DMSO-d_6), depending on the solvent. 3,5-BTFMBA is soluble in all common organic solvents and is specified by NMIJ for ^{1}H and ^{19}F qNMR.

(continued on next page)

Figure 3. Traceability chains for ^{1}H (A), ^{31}P (B) and ^{19}F (C) qNMR CRMs. Pink arrows symbolize ^{1}H qNMR measurements, blue arrows ^{31}P measurements and green arrows ^{19}F qNMR measurements. Light grey boxes indicate primary reference material, dark grey boxes ^{1}H *Trace*CERT® qNMR CRM, dark blue and dark green boxes ^{31}P and ^{19}F *Trace*CERT® qNMR CRM and light blue and light green boxes testing substances (chromatography *Trace*CERT® CRM).



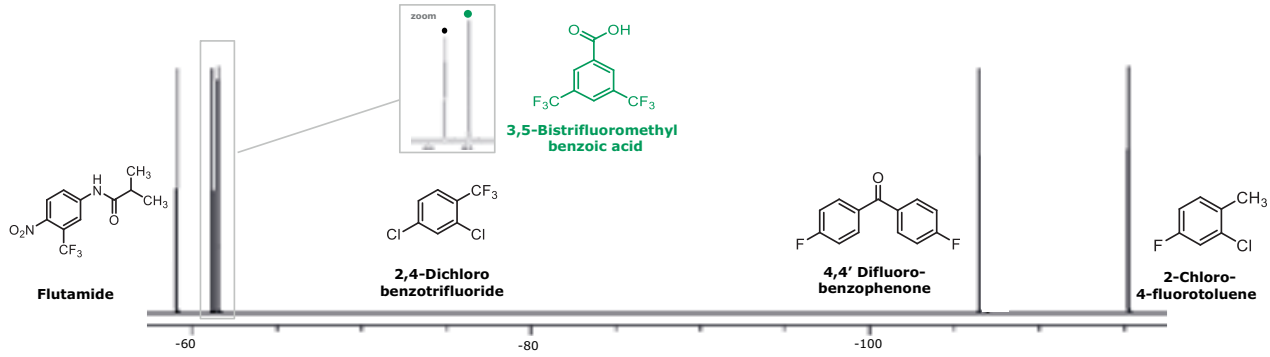
The purity value of 2,4-DCBTF was certified by ^{19}F and ^{1}H qNMR using 3,5-BTFMBA. In a second way, certification was done with ^{1}H qNMR using 1,2,4,5-Tetrachloro-3-nitrobenzene (TCNB, cat. no. 40384) with traceability to the primary CRM BA (NIST SRM® 350b). The three purity values and their expanded measurement uncertainties are in perfect accordance (SD = 0.015, **Figure 4**). Values for u_c(CRM) (k=2) are also comparable between the different experiments (0.25 – 0.29 %).

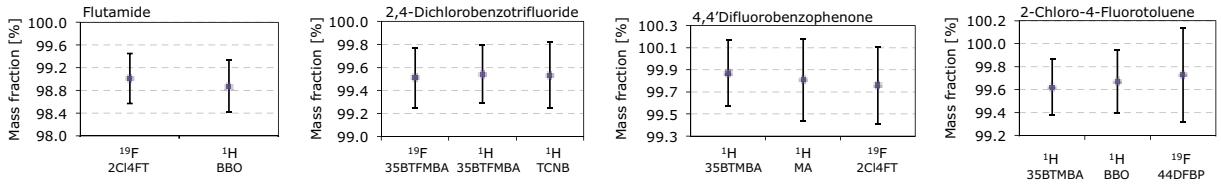
Due to different signal shapes and spectral regions of peaks, 2Cl4FT and 4,4'-DFBP were certified by another route. Traceability to the SI for 2Cl4FT was achieved by determining a mass fraction via ^{1}H qNMR using 3,5-BTFMBA. In a second way, Benzyl benzoate (BBO) was used as internal standard. A third value is assigned by ^{19}F qNMR using 4,4'-DFBP as internal standard. The purity values from the three different measurements are overlapping within their expanded measurement uncertainties and again show good accordance (SD = 0.053, **Figure 4**). The uncertainty values u_c(CRM) (k=2) are similar to that of 2,4-DCBTF (0.24 – 0.41 %).

The purity value of 4,4'-DFBP was certified via ^{1}H qNMR using 3,5-BTFMBA, and in a second way Maleic acid (MA, cat.no. 92816), as internal standard. ^{19}F qNMR certification was performed using 2Cl4FT, showing again the independency of the result of the observed nucleus. All three values are comparable and the SD of the three results is small (SD = 0.055, **Figure 4**). The values of u_c(CRM) are slightly higher compared with the other two ^{19}F qNMR CRMs (0.30 to 0.37 %). The increased uncertainties (e.g., 0.41 %, 2Cl4F2 and 0.37 % 4,4'-DFBP) do not result from the measurement procedure but are caused by a higher uncertainty contribution by the internal standard (4,4'- DFBP, MA) and homogeneity of the material. In all other ^{19}F certifications the overall repeatability of the measurement represents the most significant uncertainty contribution.

A last experiment was done to assign a purity value to the *Trace*CERT® Flutamide CRM. It was possible to show, that via ^{19}F qNMR and using 2,4-DCBTF as internal standard, comparable results were achieved as by the common route via ^{1}H qNMR using an established CRM (BBO). Again, overall repeatability of the measurement represents the most significant uncertainty contribution, which is in the same order for certification via ^{1}H and ^{19}F. The purity values are overlapping within their expanded measurement uncertainties (**Figure 4**), which is again a clear indicator that ^{19}F qNMR can be used routinely as a stand-alone method to assign the purity of fluoroorganic substances.

Figure 4. ^{19}F spectrum showing shifts of different secondary CRMs and the primary CRM 3,5-BTFMBA. Depending on the structure element (CF_3 or aromatic bound F) peaks are shifted to different regions. Results for the purity determination of secondary CRMs via ^{19}F and ^{1}H qNMR and using different internal standards are shown. Different values for purity of an analyte (P_{CRM}) are within their expanded measurement uncertainties u_{CRM}. The certified values for 2,4-DCBTF, 4,4′-DFBP and 2Cl4FT are shown in bold, and CRM 4601-a was selected as the primary CRM for the three. Due to chemical shifts in the NMR spectrum, direct comparison on the basis of ^{19}F was only possible in the case of 2,4-DCBTF. In the cases of 4,4′-DFBP and 2Cl4FT, ^{1}H qNMR had to be used, but also referencing to CRM 4601-a. Flutamide could be measured in both ways.





P_{CRM} (%)	99.01	**98.87**
u_{CRM} (%)	0.44	**0.46**

P_{CRM} (%)	**99.51**	99.54	99.53
u_{CRM} (%)	**0.26**	0.25	0.29

P_{CRM} (%)	**99.82**	99.76	99.71
u_{CRM} (%)	**0.30**	0.37	0.35

P_{CRM} (%)	**99.57**	99.62	99.68
u_{CRM} (%)	**0.24**	0.28	0.41

Conclusion

In summary, qNMR using ^{1}H, ^{31}P, or ^{19}F TraceCERT® CRMs is a very valuable method. We outlined sensitive aspects that are important for an accurate qNMR certification and need particular awareness by the operator. The presented set of ^{1}H, ^{31}P, and ^{19}F qNMR CRMs is produced fulfilling the requirements for a reference material producer under ISO 17034 accreditation, covering additional data such as homogeneity of the material and short-term and long-term stability.

Reference

1. Rigger R, Rück A, Hellriegel C, Sauermoser R, Morf F, Breitruck K, Obkircher M (2017) Journal of AOAC International, Vol. 100, No. 5, 1365-1375.

For an overview on our qNMR products visit us at **SigmaAldrich.com/qnmr**

The full portfolio of organic TraceCERT® certified reference materials (CRMs) can be found at **SigmaAldrich.com/organiccrm**

Featured Products

Description	Qty.	Cat.No.
TraceCERT®, certified reference material for ^{19}F-qNMR		
4,4′-Difluorobenzophenone	500 mg,1 g	**07563**
2,4-Dichlorobenzotrifluoride	500 mg	**53396**
2-Chloro-4-fluorotoluene	500 mg	**80730**

Related Products

Description	Qty.	Cat.No.
TraceCERT®, certified reference material for ^{31}P-qNMR		
Triphenyl phosphate	1 g	**05498**
Potassium phosphate monobasic	1 g	**92214**
Phosphonoacetic acid	1 g	**96708**
Triethyl phosphate	1 g	**90999**

Description	Atom %	Cat.No.
Deuterated solvents		
Chloroform-d6	99.96	**151858**
Dimethyl sulfoxide-d6	99.96	**156914**
Methanol-d4	99.96	**444758**
Acetonitrile-d3	99.96	**233323**

CLINICAL & FORENSIC

Online Solid Phase Extraction and LC-MS Analysis of Thyroid Hormones in Human Serum

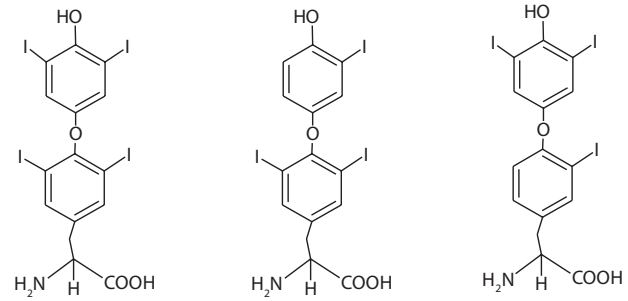
Hillel Brandes, Analytical Technology Specialist, **hillel.brandes@milliporesigma.com**

Introduction

Thyroid hormones play critical roles in the regulation of biological processes, such as: growth, metabolism, protein synthesis, and brain development. Specifically, both 3,3′,5,5′-tetraiodo-L-thyronine (thyroxine or T4) and 3,3′,5-triiodo-L-thyronine (T3), are essential for development and maintenance of normal physiological functions. For a clinical laboratory, measurements of total T4 and total T3, along with estimates of free T4 (FT4) and free T3 (FT3), are important for the diagnosis and monitoring of thyroid diseases. Most clinical laboratories measure thyroid hormones using immunoassays. The immunoassay-based methods offer a relatively rapid, high patient sample throughput that lends itself to automation, but are significantly compromised by problems with assay interference and are complicated by changes in protein levels that alter the free hormone availability.[1]

Liquid chromatography mass spectrometry (LC-MS) has been reported to offer superior specificity and speed over the immunoassays for determination of thyroid hormones in biological matrices such as serum and tissues. Nevertheless, the reported sample preparation procedures, typically liquid-liquid extraction followed by solid phase extraction (SPE), involve multiple time consuming steps, and are less compatible with automation.[2,3] The present work demonstrates successful online SPE with LC-MS for rapid determination of T4, T3, and 3,3',5'-triiodo-L-thyronine (rT3) from biological matrices.

Figure 1. Chemical structures of the thyroid analytes



Thyroxine (T4), $C_{15}H_{11}I_4NO_4$, Monoisotopic mass: 776.686 Da

3, 3', 5-Triiodothyronine (T3), $C_{15}H_{12}I_3NO_4$, Monoisotopic mass: 650.790 Da

3, 3', 5'-Triiodothyronine (Reverse T3), $C_{15}H_{12}I_3NO_4$, Monoisotopic mass: 650.790 Da

Note T3 and rT3 are isobaric

Experimental

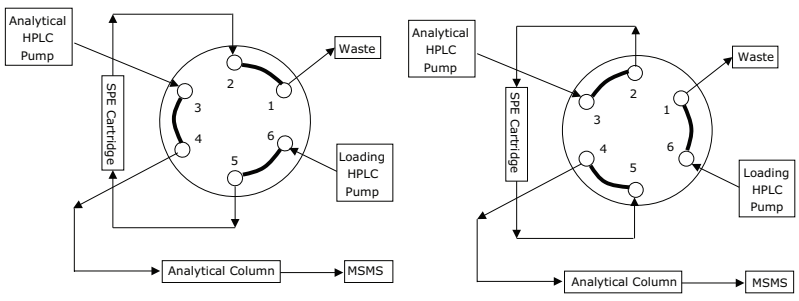
Materials: Supel™ Genie C8 and RP-Amide (RPA) online cartridges (2 cm × 4.0 mm I.D.), human serum (Cat. No. H1388), protein precipitation solvent: methanol with 1 % (w/v) ammonium formate.

Sample Processing Procedure: the human serum spiked with analytes was protein precipitated by vortex mixing with the precipitation solvent at a 1:3 ratio. Then the mixture was centrifuged at 10,000 x g for 3 min and the resulting supernatant was collected and directly injected for LC-MS analysis.

Online SPE-LC-MS Setup: As shown in **Figure 2.**, the setup consists of a 6-port switching valve and two

Figure 2. Configuration of the online SPE-LC-MS system

column:	Ascentis® Express Biphenyl, 10 cm × 2.1 mm I.D., 2.7 µm (64065-U)
mobile phase:	(A) water; (B) methanol, each with 0.1 % acetic acid
isocratic:	70 % B for 10 min
flow:	0.3 mL/min
column temp:	35 °C
SPE online cartridge:	Supel™ Genie C8 and RP-Amide, 2 cm x 4 mm I.D.
sample loading/ washing:	Equilibrate in Pos-1: 2.5 min (10 % MeOH). Load sample by injecting 2 µl in Pos-1. Leave to wash for 2 min. Switch to Pos-2 for online analytical separation (70 % MeOH, reverse flow)
sample loading solvent:	10 % methanol
injection vol:	2 µL injection
detection:	MS, ESI(+), MRM mode
instrument:	Shimadzu® LCMS-8030 with 2DLC setup



Position-1: Sample loading/washing

Position-2: Online SPE → LC-MS analysis

pumps; one for sample loading and washing, the other for sample elution. To minimize the potential peak broadening from the cartridges, the flow of sample loading/washing and the subsequent elution are in reversed directions.

Results and Discussion

The conventional (off-line) sample preparation by SPE typically involves multiple labor-intensive and time-consuming steps, including: conditioning, sample loading, washing, elution, and finally evaporation and reconstitution of the sample in mobile phase. The Supel™ Genie C8 and RPA online cartridges have been developed to automate the sample preparation process, minimize hands-on time and human error, and reduce overall sample processing time. The present work utilized the C8 and RPA online cartridges with LC-MS for the detection of thyroid hormones from human serum. **Figures 3** and **4** show the representative LC-MS chromatograms of T3, rT3, and T4 spiked in human serum with C8 and RPA online cartridges, respectively. The human serum samples were simply protein precipitated with methanol containing ammonium formate and then directly injected for online SPE and LC-MS analysis. The sample loading/washing were carried out entirely by the instrument, without any hands-on effort. Additionally, the time-consuming solvent evaporation and reconstitution steps were eliminated.

As can been seen from **Figures 3** and **4**, both C8 and RPA were capable of capturing a trace amount (100 ng/mL x 2 µL in this case) of thyroid hormones from complicated human serum. All three analytes are resolved from each other, with a peak width at half height <6s and tailing factor from 1.4-1.8. The total run time is within 6 min.

Tables 1 and **2** show the ruggedness of the online SPE-LC-MS with C8 and RPA cartridges, respectively, from 120 consecutive injections of human serum samples. As can be seen, the retention times of the analytes with C8 or RPA are very reproducible, with RSD's of 0.1 % - 0.2 %. Reproducibility (%RSD) of the peak areas for both C18 and RPA were very good, with %RSDs of 6.2 % - 7.0 % and 5.1 % - 7.7 %, respectively.

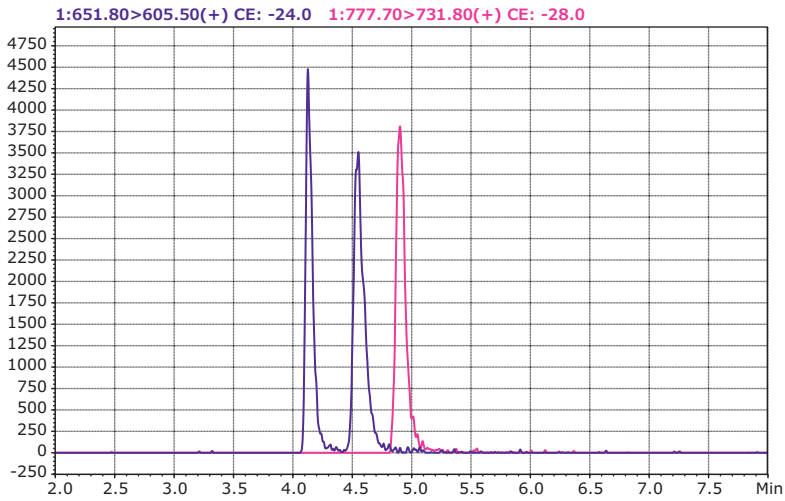
Table 1. Ruggedness of the System with C8 Cartridge

Analyte	MRM Quantifier	Retention Time (Min) (Avg. n = 120)	Retention Time Reproducibility (%RSD, n=120)	Peak Area (Avg. n = 120)	Peak Area Reproducibility (%RSD, n = 120)
3,3',5-triiodo-L-thyronine (T3)	651.8 / 605.5	4.13	0.1	17711	6.9
3,3',5-triiodo-L-thyronine (rT3)	651.8 / 605.5	4.53	0.2	22081	7
3,3',5,5'-tetraiodo-L-thyronine (T4)	777.7 / 731.8	4.89	0.1	22233	6.2

Table 2. Ruggedness of the System with RPA Cartridge

Analyte	MRM Quantifier	Retention Time (Min) (Avg. n = 120)	Retention Time Reproducibility (%RSD, n=120)	Peak Area (Avg. n = 120)	Peak Area Reproducibility (%RSD, n = 120)
3,3',5-triiodo-L-thyronine (T3)	651.8 / 605.5	4.03	0.2	27046	5.1
3,3',5-triiodo-L-thyronine (rT3)	651.8 / 605.5	4.43	0.2	33723	6.2
3,3',5,5'-tetraiodo-L-thyronine (T4)	777.7 / 731.8	4.79	0.2	23766	7.7

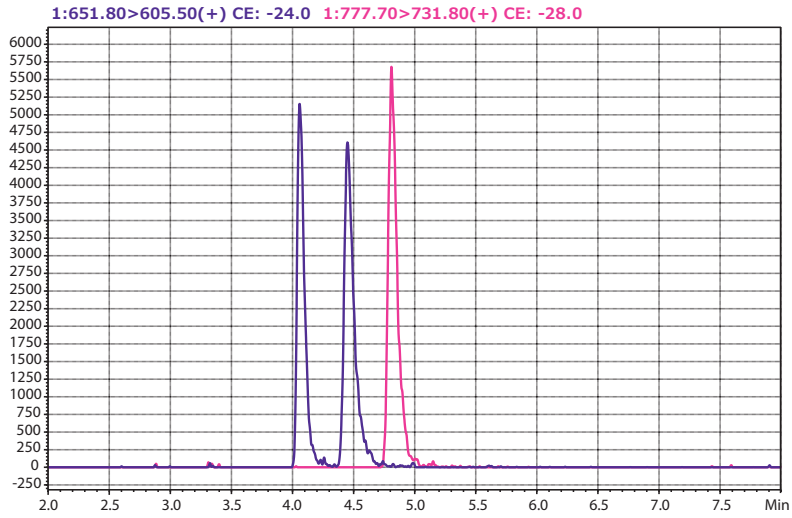
Figure 3. Representative LC-MS chromatogram of thyroid hormones in human serum with C8 online cartridge



Peak	Analyte	Peak Width at 50 % Height (s)	Tailing Factor
1	T3	3.7	1.6
2	rT3	5.2	1.5
3	T4	4.9	1.4

(continued on next page)

Figure 4. Representative LC-MS chromatogram of thyroid hormones in human serum with RPA online cartridge



Peak	Analyte	Peak Width at 50% Height (s)	Tailing Factor
1	T3	3.7	1.6
2	rT3	5.2	1.5
3	T4	4.9	1.4

Comparing the two online cartridges, RPA appears to deliver better signals (peak height and area) for all three thyroid analytes compared with the C8 cartridge. The mechanism behind this is not clear, however, the RPA is known to offer better retention for analytes with polar moieties which form hydrogen bonds. Otherwise C8 and RPA provide similar results in terms of peak shape and reproduciblity of peak area.

Summary

An online SPE-LC-MS method has been developed for the rapid detection of thyroid hormones in human serum with minimal hands-on effort and time-consuming steps. Both C8 and RP-Amide online cartridges were shown to be capable of capturing a trace amount of analyte from protein precipitated human serum samples. All three analytes, T3, rT3 and T4 were resolved on a Biphenyl column, with sharp and symmetric peak shapes. In addition, reproducibility (%RSD) of the retention times of the thyroid hormones from 120 consecutive injections is between 0.1% and 0.2%, with either C8 or RPA online cartridges, while the peak area reproducibility (%RSD) is between 5.1% and 7.7%. These RSD's indicate great ruggedness of the online SPE-LC-MS system.

References

1. Kahric-Janicic N, Soldin SJ, Soldin OP, West T, Gu J, Jonklaas J, Tandem mass spectrometry improves the accuracy of free thyroxine measurements during pregnancy. *Thyroid*. 2007;*17(4)*: 303-11.
2. Susan S-C. Taia, Lorna T. Sniegoski and Michael J. Welch, Candidate Reference Method for Total Thyroxine in Human Serum Use of Isotope-Dilution Liquid Chromatography-Mass Spectrometry with Electrospray Ionization. *Clinical Chemistry*, 2002; *48(4)*: 637-642.
3. Dongli Wang and Heather M. Stapleton, Analysis of thyroid hormones in serum by liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2010; *397(5)*: 1831–1839.

Featured Products

Description	Cat. No.
Ascentis® Express Biphenyl HPLC Column, 10 cm × 2.1 mm I.D., 2.7 µm	**64065-U**
Supel™ Genie RP-Amide Online Starter Kit*	**55516-U**
Supel™ Genie RP-Amide Online SPE Cartridge, pk. of 2	**55519-U**
Supel™ Genie RP-Amide Online SPE Cartridge, pk. of 6	**55522-U**
Supel™ Genie C8 Online Starter Kit*	**55274-U**
Supel™ Genie C8 Online SPE Cartridge, pk. of 2	**55512-U**
Supel™ Genie C8 Online SPE Cartridge, pk. of 6	**55515-U**

Related Products

Description	Cat. No.
Supel™ Genie HybridSPE® Online Starter Kit*	**55324-U**
Supel™ Genie HybridSPE® Online SPE Cartridge, pk. of 2	**55326-U**
Supel™ Genie HybridSPE® Online SPE Cartridge, pk. of 6	**55327-U**
Thyroid Hormones	
L-Thyroxine (T4), 100 µg/mL in 0.1 N NH_3 in methanol, 1 mL	**T-073**
3,3',5-Triiodo-L-thyronine (T3), 100 µg/mL in 0.1 N NH_3 in methanol, 1 mL	**T-074**
3,3',5'-Triiodo-L-thyronine (reverse T3) 100 µg/mL in 0.1 N NH_3 in methanol), 1 mL	**T-075**
3,3',5-Triiodo-L-thyronine-$^{13}C_6$ (T3-$^{13}C_6$), 100 µg/mL in 0.1 N NH_3 in methanol, 1 mL	**T-077**
3,3´,5'-Triiodo-L-thyronine-$^{13}C_6$ (reverse T3-$^{13}C_6$), 100 µg/mL in 0.1 N NH_3 in methanol, 1 mL	**T-078**

* 1 Holder, 1 cartridge

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For an overview on our LC-MS solvents, visit us at
SigmaAldrich.com/LC-MS
SigmaAldrich.com/UHPLC-MS

ENVIRONMENTAL

Proficiency Testing from Merck
A New Critical Tool for Assessing Performance

Nick Hauser, Global Product Manager Reference Materials **nick.hauser@milliporesigma.com**

We are pleased to introduce our new proficiency testing data entry portal. The new portal, released in mid-July 2018, can be accessed at **Merck-pt.com**. The new portal has been streamlined and will offer superior speed, ease of use, and data handling. We have been committed to updating and providing a user interface that is efficient, simple, and meets the evolving needs of the end user. The result is our new data entry portal which is easy to learn, easy to remember, and easy to use.

Some of the features of the new PT portal:

- One-click design that is easy to navigate, even for first time users
- All open studies prominently displayed on the home screen
- Easily add new personnel/analysts to your lab
- Add accreditors and enter data all on a single page
- Easily copy down method/analyst/analysis date to all analytes in a sample
- Data is actively saved as you report
- A confirmation screen that shows exactly what you have entered and will be submitted for evaluation
- Methods saved as default along with the option to add in-house methods
- Calendar which will have open and close dates for studies in which customers are enrolled
- A newly designed Frequently Asked Questions page
- Automated email reminders sent to customers 7 days before study closes
- Option to send results to a third party (i.e., Corporate QA Manager)
- Accreditors will be able to log in and download results at any time
- Expand/collapse items on the data entry page for quick loading times

Data Tracking and Analysis Using MyTrends and MyStats

All study data that was previously entered into the former PT portal has been transferred over to the new system. If you are a new user to the updated portal, you also have the ability to import your historical data in order to perform data trending regardless of your previous PT provider. The addition of graphs to reports also provides a new enhancement for all data processing needs.

Training on the New Portal

A webinar has been offered to demonstrate the new portal and field any questions that you might have. If you didn't have the opportunity to attend the webinar, a copy has been posted in the FAQ section of the PT Portal login page (**Merck-pt.com**) to view at your convenience.

To learn more about our solutions for proficiency testing, visit us at **SigmaAldrich.com/PT**

SCIENCE & TECHNOLOGY INNOVATIONS

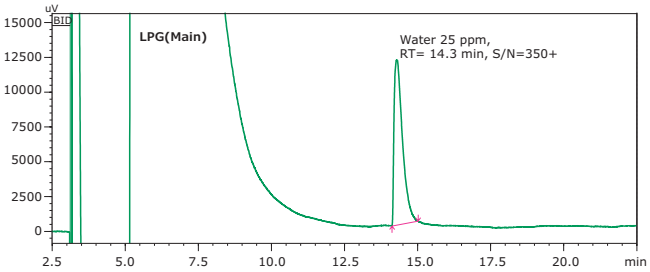
Innovative Solutions for Your Toughest Separations – Ionic Liquid Capillary GC Columns

Supelco® Innovative Gas Chromatography Solutions

Lisa Battle (McCombie), Product Manager, GC & Carbons, **lisa.mccombie@milliporesigma.com**

Gas chromatography has been used for decades with continuous phase development. Most updates have been based on polymeric materials, but a new page was turned with the introduction of the Ionic Liquid (IL) columns, which offer new selectivity and application options. Arguably one of the most exciting Ionic Liquid Capillary columns is the Watercol™ column. This column allows the qualitative and quantitative determination of water in a variety of matrices by GC, eliminating the need for special testing. It is particularly handy for gaseous samples (**Figure 1**).

Figure 1. Chromatogram for water determination (25 ppm) in LPG. Limit of Quantification (S/N=10) and Limit of Detection (S/N=3.3) can be down to 0.66 ppm and 0.22 ppm respectively (courtesy of Shimadzu, see also Analytix Reporter Issue 2).



Instrument	
gas chromatograph:	Tracera (GC-2010 Plus A + BID-2010 Plus)
sample injection:	Valco Internal Liquid Sample Injector with Splitter Injection Unit
gas purifier:	Supelco High Capacity Gas Purifier (Cat. No. 29541-U)
Analysis Conditions	
column:	Watercol™ 1910, 30 m x 0.25 mm I.D., 0.20 µm (29711-U)
oven:	35 °C (2 min), 5 °C/min to 150 °C (15 min)
carrier gas:	Helium 45 cm/sec (Column flow rate 3.78 mL/min)
inj. volume:	2 µL
split:	5:1
transfer line temp.:	175 °C (After internal liquid sample injector to GC column oven)
detector temp.:	200 °C
discharge gas vol.:	50 mL/min (He)

Looking into ASTM D3606: Standard Test Method for Determination of Benzene and Toluene in Spark Ignition Fuels by Gas Chromatography, an IL column alternative is available, the SLB®-ILD3606 column. This column allows the complete resolution of aromatics and oxygenates in reformulated gasoline on one column (**Figure 2**), and by that, significantly reduces analysis time and improves results.

Supelco also provides several other IL columns, which include the SLB®-ILPAH for high resolution PAH analysis (**Figure 3**) and also the new improved i Series (SLB®-IL60i, SLB®-Il76i, SLB®-IL111i) with unique selectivity paired with higher inertness. These i-series columns have applications in various fields such as petroleum, food and chemical analysis, and can provide reliable and reproducible results. The SLB®-IL60i has a polarity similar to PEG/Wax phases, while the SLB®-IL111i is the highest polarity GC phase currently available, and can offer benefits in the determination of unsaturated fatty acid methyl ester (FAME) isomers.

Background on Ionic Liquid Technology

In 2005, **Prof. Daniel W. Armstrong** (University of Texas at Arlington) showed that dicationic and polycationic ionic liquids could successfully be used as viable GC stationary phases. These phases consist of two or more organic cations joined by a linkage and associated with anions, which can be either inorganic or organic. Ionic liquid phases differ physically and chemically from traditional GC stationary phases:

- The molecules in stationary phase are much smaller compared to big, bulky polysiloxane polymer and polyethylene glycol phases, plus there are no active hydroxyl groups. These features lead to **greater stability**, even in the presence of **moisture and/or oxygen**.
- Many modifications are possible in order to **alter selectivity**. The base structure can be dicationic or polycationic. There are numerous cation, linkage, and anion choices. Pendant groups can be added to cations and/or linkages.

Figure 2. Oxygenate resolution in reformulated gasoline

column:	SLB®-ILD3606, 60 m × 0.25 mm I.D., 0.20 µm (29691-U)
oven:	50 °C (6 min), 15 °C/min to 265 °C (10 min)
inj. temp.:	250 °C
detector:	FID, 250 °C
carrier gas:	helium, 21 cm/sec
injection:	1 µL, 100:1 split
liner:	4 mm I.D., split type, cup design
sample:	reformulated gasoline (contains 10% ethanol) with 7 other oxygenates added (at 2.5-5%)

1. C5-C11 aliphatic hydrocarbons
2. Methyl tert-butyl ether (MTBE)
3. tert-Amyl butyl ether (TAME)
4. Ethanol
5. Benzene
6. sec-Butanol
7. n-Propanol
8. Isobutanol
9. Toluene
10. n-Butanol
11. Ethylbenzene
12. Methyl isobutyl ketone (MIBK)
13. p-Xylene
14. m-Xylene
15. o-Xylene

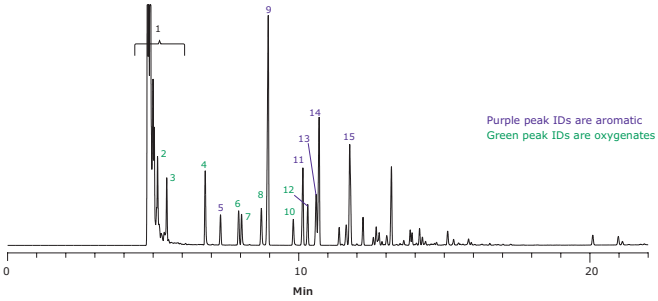
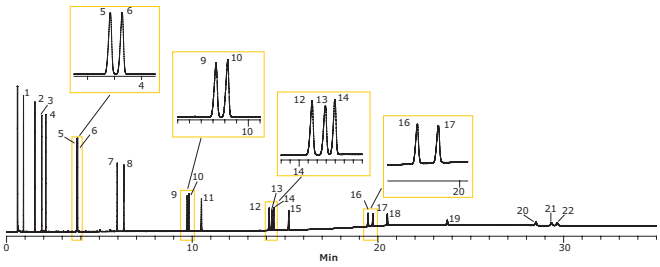


Figure 3. ILPAH resolution of critical groups in a 22-component PAH mix

column:	SLB®-ILPAH, 20 m × 0.18 mm I.D., 0.05 µm (29799-U)
oven:	90 °C (6 min), 20 °C/min to 225 °C, 5 °C/min to 300 °C (10 min)
inj. temp.:	300 °C
detector:	FID, 310 °C
carrier gas:	hydrogen, 1.3 mL/min, constant flow
injection:	1 µL, 50:1 split
liner:	4 mm I.D., split type, cup design
sample:	10 PAHs, each at 100 µg/mL in methylene chloride

1. Naphthalene
2. Acenaphthene
3. Acenaphthalene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo[a]anthracene
10. Chrysene
11. 5-Methylchrysene
12. Benzo[b]fluoranthene
13. Benzo[k]fluoranthene
14. Benzo[j]fluoranthene
15. Benzo[a]pyrene
16. Dibenz[a,h]anthracene
17. Indeno[1,2,3-cd]pyrene
18. Benzo[g,h,i]perylene
19. Dibenzo[a,l]pyrene
20. Dibenzo[a,e]pyrene
21. Dibenzo[a,i]pyrene
22. Dibenzo[a,h]pyrene



Merck has patented this technology under the Supelco® brand and is expanding the Ionic Liquid GC Column technology to many different application areas.

These columns have the opportunity to impact current practices in several ways:

- Columns can be engineered with **similar selectivity & polarity** to non-ionic liquid columns, but with **higher operating temperatures** and **less susceptibility to be damaged** by moisture and/or oxygen, hence, they are ideal for GC-MS.
- Columns can be engineered with **completely unique selectivity** to non-ionic liquid columns, or polarities not available before (e.g., SLB®-IL111i, which is more polar than TCEP), and produce good peak shape and resolution for compounds of varying functionality.
- Columns can be used in **multidimensional separations**, due to their engineered orthogonality and high thermal stability.

Featured Products

Description	Cat. No.
Watercol™ 1910, 30 m x 0.25 mm ID, 0.20 µm	**29711-U**
SLB®-ILPAH, 20 m × 0.18 mm I.D., 0.05 µm	**29799-U**
SLB®-ILD3606, 60 m × 0.25 mm I.D., 0.20 µm	**29691-U**

More details on the Watercol & i-series columns can be found at
SigmaAldrich.com/watercol
SigmaAldrich.com/il-gc-inert

For additional information on this innovative technology, visit us at **SigmaAldrich.com/il-gc**

SCIENCE & TECHNOLOGY INNOVATIONS

High Purity Carbon Adsorbents for Sample Preparation and Chromatographic Applications

Leidy Peña Duque, Senior R&D Scientist Gas Separations, **leidy.penaduque@milliporesigma.com**

Wiliam Betz, Principal Scientist Gas Separation R&D, **wiliam.betz@milliporesigma.com**

Introduction

The first high purity Supelco® carbon adsorbents were made for gas chromatographic packed columns. Then, specialty carbons were developed for thermal desorption tubes followed by the introduction of carbons in solid phase extraction (SPE) cartridges. In later developments, Supelco® carbons were used to make porous layer open tubular (PLOT) columns as well as solid phase microextraction SPME fibers. Current Supelco carbon technologies include nanocarbons for electronic applications.

Merck, under the Supelco® brand, has a total of 75 carbon intermediates that are utilized combined or stand-alone in different analytical devices. These carbons are highly customizable, high capacity, synthetic, and reusable which differentiates them from activated charcoal. These carbons are categorized below.

- Carboxen® and Carbosieve® are amorphous carbon molecular sieves suitable for permanent gas analyses and small molecule analysis at high pressures (16,000-20,000 psi), volatile organic compound analysis using air sampling tubes, and for extracting small molecules from aqueous samples for SPE analyses.
- Carbopack™ and Nanocarbons are graphitized carbon blacks. These carbons are effective for separating volatile, semi-volatile, and non-volatile compounds for gas chromatographic analyses, and for extraction of semi-volatile and non-volatile compounds from aqueous samples for SPE applications. Carbopacks typically can only withstand 400 psi, but are effective adsorbents for separation of semi-volatiles and non-volatiles in air sampling applications.
- Graphsphere™ are graphitized polymer carbons, and represent an additional benefit over the graphitized carbon blacks due to their spherical particle shape in packed bed systems. Graphsphere™ is also non-friable; it can withstand superior pressures (16,000-20,000 psi) when used in packed bed systems in gas and liquid applications. The uniformly defined graphite surface provides unique selectivity for both chromatography and sample preparation.

All four families of carbons are processed at the Supelco® site in Bellefonte, USA where all carbon containing devices are manufactured. For those research groups that are investigating new applications, these carbons are also available in family kits containing either carbon molecular sieves, graphitized carbon blacks or graphitized polymer carbons.

Carbon Properties

Particle Size Distribution

The particle size range of all the carbons is 200 nm to 850 µm; therefore, the particle size distribution can be tailored to a specific application.

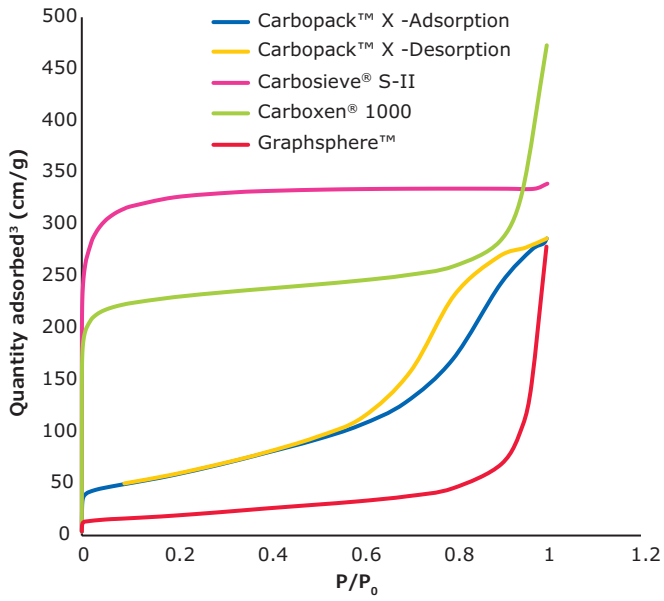
Pore Size Distribution

The pore structure of all our carbon adsorbents can be modified to possess ultramicropores (pore diameter less than 7 Å) to macroporous with pore diameters larger than 500 Å. The plots in **Figure 1** show the N_2 adsorption isotherms at 77 K for different adsorbents. Type I isotherms as seen with Carbosieve® S-II are characteristic of microporous materials, while mesoporous materials such as Carbopack™ X show a hysteresis loop characteristic of a mesoporous material.

Non-porous materials, such as Graphsphere™, show very low adsorption values.

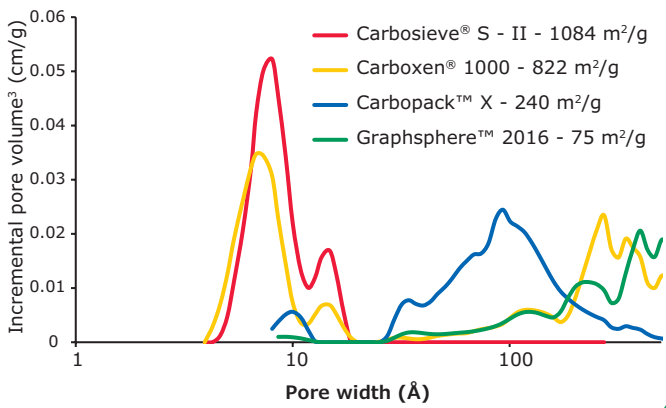
Multiporous carbons are also available; these carbons contain different pores with various sizes. An overlay

Figure 1. N_2 Adsorption isotherms for Supelco® carbons



of representative examples of four families of carbons which have different pore size distributions is shown in **Figure 2**. On the y-axis of this figure, the specific volume of nitrogen gas fitted at a pore size interval is plotted against the pore size/width in the x-axis. Micropores are defined as pores below 20 Å, mesopores are those pores between 20-500 Å, and pores larger than 500 Å are considered macroporous. **Figure 2** shows the pore size distributions of various carbons containing either micro, meso and/or macro pores. Carbosieve® S-II is a microporous only carbon with an apex at 8 Å, while Carboxen® 1000 is a multiporous carbon that contains both micro and macropores. Carbopack™ X is a mesoporous only carbon while Graphsphere™ has pores mostly in the macropore region. Microporous carbons such as the Carbosieves and Carboxens have larger surface areas and provide larger capacities compared to macroporous carbons like Carbopack™ and Graphsphere™.

Figure 2. Pore size distributions (PSD) and surface areas (m^2/g) for representative Supelco® carbons



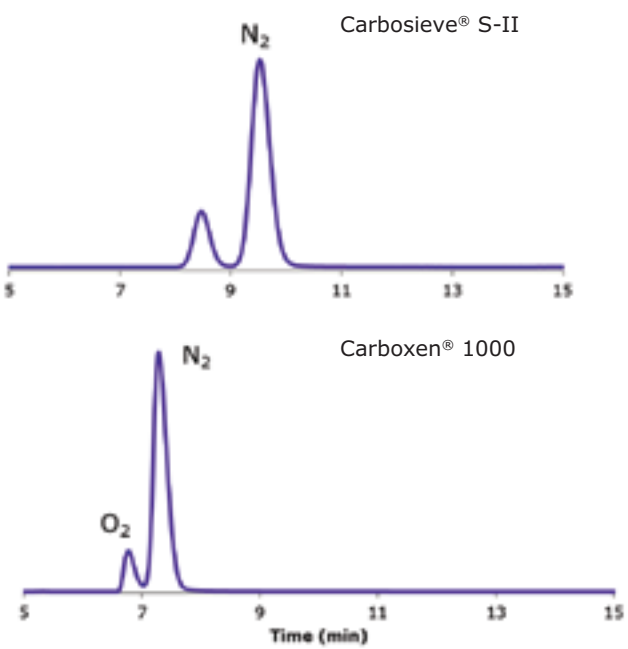
Example Applications of Supelco® Carbons

Gas Chromatography

Supelco® microporous carbons can be used in packed columns for the separation of permanent gases such as N_2 and O_2. Carbosieve® S-II and Carboxen® 1000 effectively separate air in stainless steel packed columns with 6 ft x 1/8 in dimensions (see **Figure 3**). Lateral diffusion of gas molecules is significant in packed columns, and because of this phenomenon, the diameter of the column can affect the separation efficiency and is balanced by optimizing the particle size.

The use of 180-250 µm particles in a 4.8 m x 3.18 mm packed column has the same retention time as a 30 m x 0.53 mm ID PLOT column with 2 µm particles adhered to the side walls [i.e., porous layer open tubular (PLOT)] (see **Figure 4**), although the peak width will be wider. In **Figure 4**, the red chromatogram represents the signal from a thermal conductivity detector and the black chromatogram represents the signal from a methanizer/flame ionization detector (FID).

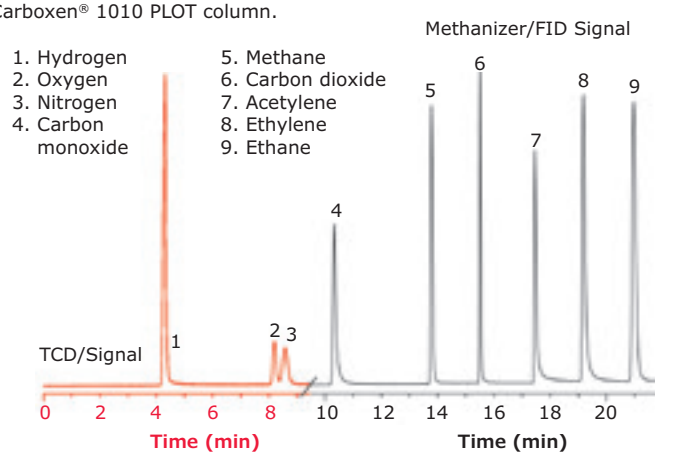
Figure 3. Separation of air using chromatographic columns packed with Carbosieve® S-II and Carboxen® 1000



(continued on next page)

Figure 4. Separation of permanent gases and light hydrocarbons with a Carboxen® 1010 PLOT column.

column:	Carboxen® 1010 PLOT, 30 m x 0.53 mm I.D., 30.0 µm (25467)
oven:	35 °C (7.5 minutes) to 250 °C at 24 °C/minute
injector:	200 °C
detector:	TCD/methanizer-FID, 230 °C
flow:	Argon, 3.0 mL/min
valve:	150 °C
injection:	10.0 µL valve injection
sample:	transformer gas for ASTM method D3612-96

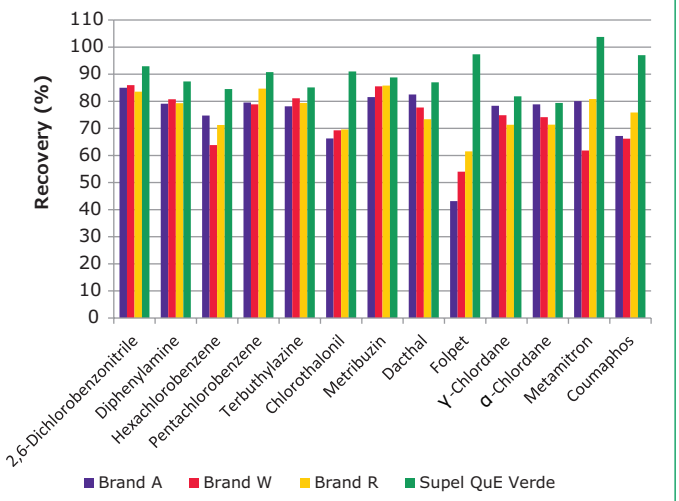


Sample Preparation

SPE

These carbon adsorbents are also be widely used in sample preparation techniques. Carbon removes matrix interferences in SPE cartridge applications, so a clean sample can be injected and precisely analyzed by HPLC or GC. For this application, carbons with larger particle and pore sizes like Carbopack™ and Graphsphere™ are effective for cleaning samples without retaining the molecules of interest. ENVI-Carb™ and ENVI-Carb™ Y are carbons from the family of Carbopacks that are used in SPE products such as ENVI-Carb™ and Supel™ QuE Verde, respectively. ENVI-Carb™ is highly effective in removing chlorophyll and carotenoids; similarly, the product Supel™ QuE Verde removes chlorophyll and gives a high recovery of planar pesticides (see **Figure 5**).

Figure 5. Recoveries of planar pesticides for different QuEChERS brands compare to Supel™ QuE Verde

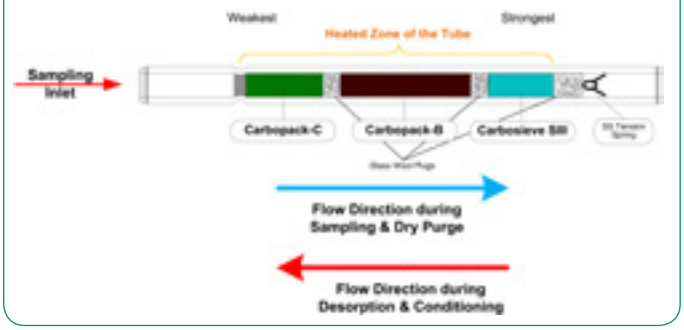


Recoveries between 70% and 120% are typically considered to be acceptable.

Air Sampling

Single bed and multi-bed carbon adsorbent tubes have become significant tools for air sampling analysts. One example is the Carbotrap® 300 3-bed tube (i.e. Carbotrap® C, Carbotrap® B and Carbosieve® S-III) which was the first tube developed for the US EPA for monitoring toxic, volatile, and semi-volatile organic compounds (see **Figure 6**). The development of a 2-bed tube containing Carbopack™ B and Carboxen® 1000 was key for the 61 compounds list of airborne contaminants established later by the EPA.

Figure 6. Multi-bed tube for air sampling (Carbotrap® 300)



Additional efforts with the EPA focused on the development of a single bed tube containing a mesoporous graphitized carbon black, Carbopack™ X, for 72-hour passive sampling of 1,3-butadiene and various other airborne organic compounds (see **Figure 7**).

Solid Phase Microextraction (SPME)

Carbons with particle sizes of 2.0 µm have been adhered, using a Merck patented adhesive, to SPME fibers for the extraction of organic compounds from aqueous and atmospheric environments (see **Figure 8**).

Figure 7. Carbopack™ X passive sampling tube data

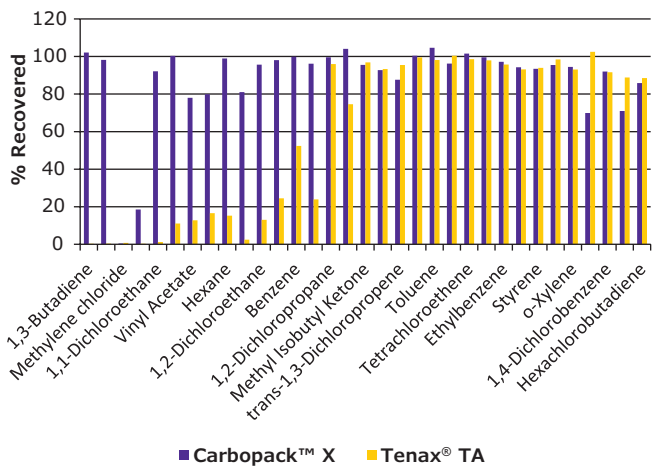
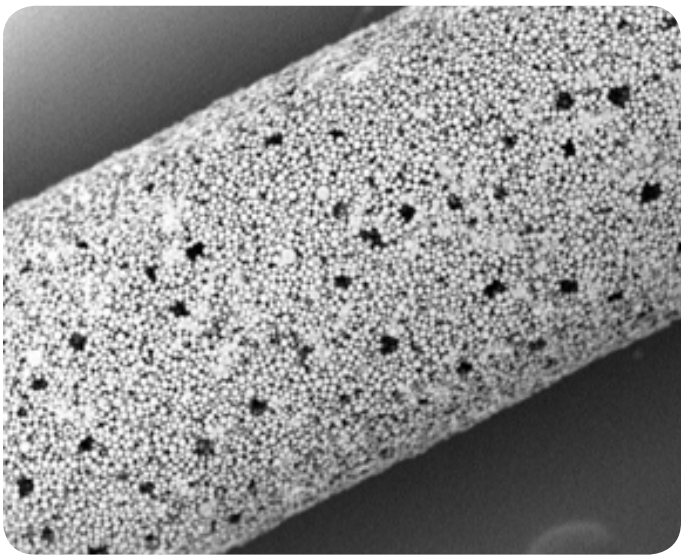


Figure 8. SPME fiber with Carboxen® 1006



Conclusion

Supelco has a 40+ year commitment to carbon adsorbent research and product development. Evidence of this can be seen in our high purity, specialty carbon adsorbents, which are currently used for:

- Collection media in air sampling devices
- Packings in SPE hardware, purge traps, and GC columns
- Purification of gas or liquid streams
- Recovery of synthesized compounds from reaction mixtures
- And many more exciting applications

If you are interested in a new adsorbent and know the target physical specifications (surface area, porosity, pore diameter, particle size range, etc.), let us know and we can investigate the possibility of manufacturing it. You can also try one of our ready-made sample kits, which you can find at **SigmaAldrich.com/carbon**

However, most requests require a specialty carbon adsorbent that can perform a specific task. In that case, tell us the type of sample (gas, liquid, or paste) you are working with, what analytes you want to adsorb and analyze, and if there is a need to recover the analytes after adsorption. Our R&D group will investigate whether an existing adsorbent is appropriate, or if a new adsorbent needs to be developed.

To learn more about our portfolio of specialized carbon adsorbents and/or download the "Supelco® Specialty Carbon Adsorbent" brochure, visit us at **SigmaAldrich.com/carbon**

To request a quote, contact Supelco_Quotes@sial.com

SCIENCE & TECHNOLOGY INNOVATIONS

New Certified Reference Materials for Refractive Index and Density

Matthias Nold, Global Product Manager Reference Materials, **matthias.nold@merckgroup.com**

As part of our comprehensive offering of Reference Materials for Physical Property Testing, we are proud to offer products from Paragon Scientific, a recognized expert in Certified Reference Materials. Paragon Scientific is UKAS accredited for ISO/IEC 17025 and recently updated its ISO Guide 34 accreditation to the new standard ISO 17034:2016 (general requirements for the competence of reference material producers).

The portfolio of Paragon Scientific products, which contains reference materials for a large variety of physical properties such as viscosity, density, cloud point, pour point, flash point, freezing point and many more, can be viewed and easily ordered at **SigmaAldrich.com/paragon**.

Recently a range of Multi-Parameter Certified Reference Materials (CRMs) for refractive index and density measurements has been launched (see **Table 1**). The combined Refractive Index and Density CRM is available in four different materials, with each material including certified data for both refractive index and density at 15 °C, 20 °C and 25 °C.

These products provide traceability of measurement to recognized national standards, and to units of measurement realized at the National Physical Laboratory (NPL) or other recognized national standards laboratories. Both density values and refractive index values are traceable to National Institute Standards and Technology (NIST). Additional product features include:

- Density certified in accordance with primary level ASTM D1480 methodology
- Ensures full compliance to ASTM and IP test method protocols
- Low levels of uncertainty, ensuring maximum accuracy of data and dependable results
- Supplied in 30 mL volume sealed glass vials for ample measurement readings

Table 1. Multi-Parameter Refractive Index & Density Certified Reference Material, at 15 °C, 20 °C and 25 °C

Nominal RI Value at 20 °C	Qty.	Cat.No.
1.333	30 mL	**PSRVD01**
1.4217	30 mL	**PSRVD02**
1.5463	30 mL	**PSRVD03**
1.6579	30 mL	**PSRVD04**

We also offer **Refractive Index Certified Reference Materials** (CRMs) solely for refractive index calibration and verification. These products are manufactured by Paragon Scientific, too, and are ideal for the verification and calibration of temperature controlled refractometers, with each CRM offering certified values for Refractive Index measurements at 20 °C, 25 °C and 30 °C. Additional features are:

- All measurements are fully traceable to NIST and international protocols
- Low levels of uncertainty, ensuring maximum accuracy of data at hand and dependable results
- Available in single 10 mL volume tamper-evident glass bottles or as a multi-pack of 5 per material (includes set of disposable pipettes)

Table 2. Refractive Index Certified Reference Materials at 20 °C, 25 °C and 30 °C, in Tamper-Evident Glass Vials

Nominal RI Value at 25 °C	Qty.	Cat.No.
1.3325	10 mL	**PSRI01**
1.3325	5 x 10 mL	**PSRI01K**
1.3891	10 mL	**PSRI02**
1.3891	5 x 10 mL	**PSRI02K**
1.4023	10 mL	**PSRI03**
1.4023	5 x 10 mL	**PSRI03K**
1.4196	10 mL	**PSRI04**
1.4196	5 x 10 mL	**PSRI04K**
1.4206	10 mL	**PSRI05**
1.4206	5 x 10 mL	**PSRI05K**
1.4573	10 mL	**PSRI06**
1.4573	5 x 10 mL	**PSRI06K**
1.4941	10 mL	**PSRI07**
1.4941	5 x 10 mL	**PSRI07K**
1.5349	10 mL	**PSRI08**
1.5349	5 x 10 mL	**PSRI08K**
1.544	10 mL	**PSRI09**
1.544	5 x 10 mL	**PSRI09K**
1.6556	10 mL	**PSRI10**
1.6556	5 x 10 mL	**PSRI10K**

Find a complete list of our refractive index reference materials at **SigmaAldrich.com/refractiveindex**

Mobile Environmental Analysis Methods – What to Expect from New Smartphone Technology

Saskia Schröter, Product Manager Mobile and Analytical Workflows, **saskia.schroeter@merckgroup.com**

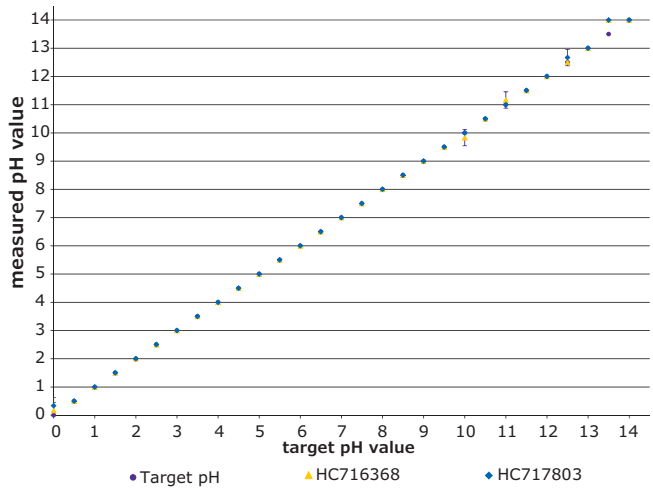
Regular soil testing is important to ensure quality and continued optimal performance of agricultural areas managed under modern agricultural conditions. Parameters such as nitrate, phosphate, and potassium, as well as pH or ammonium, are tested.

For on-site analysis, sensors, mobile photometers (i.e., Spectroquant® Move) or reflectometers (i.e., Reflectoquant® RQflex 20), as well as liquid colorimetric tests or strip-based colorimetric tests (i.e., MQuant® test strips), are available. A recent addition is the option to use modern smartphone technology for strip readout, such as with the new app reader MQuant® StripScan. The app* guides the user through the measuring process, reading MQuant® test strips using the phone camera and a credit card-sized color reference as external standard. The benefits include higher resolution readings (**Figure 1**) and automatic value recording, as well as additional share, export and visualization options for documentation and reporting. The app is complemented by the web platform **mquant-stripscan.com** for convenient result monitoring, management and transfer. With this concept, the new app reader combines the ease of use, intuitive handling, and affordability of test strips with the increased accuracy and data management options of instrumental readout.

How to use smartphone technology for soil analysis – application example

To measure pH in soil using MQuant® StripScan, 10 g of the homogenized sample is mixed with 25 mL 0.0125 M $CaCl_2$ solution, incubated for 15 min, then analyzed using MQuant® pH 0-14 strips: A test strip is immersed in the soil slurry for 10 min, then measured by following the instructions on the StripScan app (ensuring that no residual particles rest on the reaction zones). Automatic camera acquisition provides a pH measurement with values in 0.5 increments (vs. 1.0 visual only) within seconds, and the data is automatically stored and graphed.

Figure 1. pH measurements of Certipur® buffer solutions (Merck KGaA, Darmstadt) (triplicate values ± SD) as compared to pH target values. Measurements were made using the smartphone app reader MQuant® StripScan according to instructions, along with pH test strips (Cat. No. 109535, batches HC17803, HC716368), and color reference card (Cat. No. 103736).



Featured Products

Description	Qty.	Cat. No.
MQuant® StripScan Reference Card for pH	1	**1.03736.0001**
pH-indicator strips pH 0 - 14 Universal indicator	100	**1.09535.0001**

Related Products

Description	Qty.	Cat. No.
MQuant® StripScan Reference Card for Nitrate	1	**1.03733.0001**
MQuant® Nitrate test strips 0 - 500 mg/L NO_3^-	100	**1.10020.0001**
	25	**1.10020.0002**
MQuant® Nitrate test strips 0 - 500 mg/L NO_3^- (individually sealed)	1000	**1.10092.0021**

The smartphone app (currently for iPhone only) can be downloaded for free from the AppStore:

Download on the App Store

Discover our StripScan Website here: **mquant-stripscan.com**

For more information, visit **SigmaAldrich.com/mquant-stripscan**

IN ESSENCE

American Oil Chemists' Society (AOCS):

Ongoing Sponsorship of the Supelco AOCS Research Award and Election of Len Sidisky to Presidency

Since 1909, the aim of the AOCS is to advance the science and technology of oils, fats, proteins, surfactants, and related materials.[1] Employees of our organization have been involved in the leadership and activities of the AOCS for decades. Two significant recent events are reported here.

AOCS Supelco Award in Lipid Chemistry

The genesis of the AOCS Supelco Award in Lipid Chemistry goes back to 1964, when it was created as a way to recognize lipid chemists who were doing important work. Later, in 1982, Mr. Nicholas Pelick, Supelco's then president, suggested that the company assume sponsorship of the award. The award recognizes an individual with outstanding original research in fats, oils, lipid chemistry, or biochemistry. Winners represent the very best and brightest minds in research areas of prime concern to the AOCS: organic chemistry, physical chemistry, biochemistry, and nutrition (**Table 1**). Indeed, among its ranks are three Nobel laureates.

The 2018 winner, Dr. Alice H. Lichtenstein, continues the tradition of high quality lipid research. She is the Stanley N. Gershoff Professor of Nutrition Science and Policy at the Friedman School, director and senior scientist at both the Cardiovascular Nutrition Laboratory at Tufts University and the Jean Mayer USDA Human Nutrition Research Center on Aging. Dr. Lichtenstein's research group focuses on assessing the interplay between diet and heart disease risk factors. Past and current work includes addressing issues related to trans fatty acids, soy protein and isoflavones, sterol/stanol esters, novel vegetable oils differing in fatty acid profile, and glycemic index. She also serves as an associate editor of the *Journal of Lipid Research* and executive editor of the Tufts Health and Nutrition Letter. She has received numerous awards in several scientific societies, including the Ralph Holman Lifetime Achievement Award at AOCS in 2017.

Dr. Alice Lichtenstein receiving the 2018 AOCS Supelco Award in Lipid Chemistry from AOCS President Len Sidisky

Len Sidisky Becomes AOCS President

The responsibilities of the AOCS president include supervision and direction of all of the Society's business affairs, subject to the governing board's direction and control. The president is also in charge of the Strategic Planning Working Group for the Society. Len Sidisky, R&D Manager for Gas Separations at MilliporeSigma, the life science business of Merck, was installed as president at the May AOCS Annual Meeting. Len received a B.S.

Handing off the AOCS Presidency to Len Sidisky (right) is Neil Widlak (left), past president of AOCS.

degree in Biology and an M.S. in Food Science from The Pennsylvania State University. He joined Supelco in 1982 and has been responsible for new technologies in the fields of gas chromatography, sample preparation, air sampling, solid phase microextraction (SPME), and high performance carbon adsorbents. Prior to the AOCS presidency, Len served the AOCS's Northeast Section (President, 2000-2001 and Vice President 1998-1999), Chromatography Committee Chairperson (2000-2009), Governing Board Technical Steering Committee Chairperson (2010-2017), Vice President (2017-present), National Secretary (2016-2017), AOCS Fellow 2016, NE-AOCS Section President (2000-2001) as well as Vice President (1998-1999).

Congratulations to Len and Dr. Lichtenstein!

References

1. The AOCS website https://www.aocs.org, accessed September 26, 2018.
2. The AOCS Lipid Library http://lipidlibrary.aocs.org/History/content.cfm?ItemNumber=39252, accessed September 26, 2018.

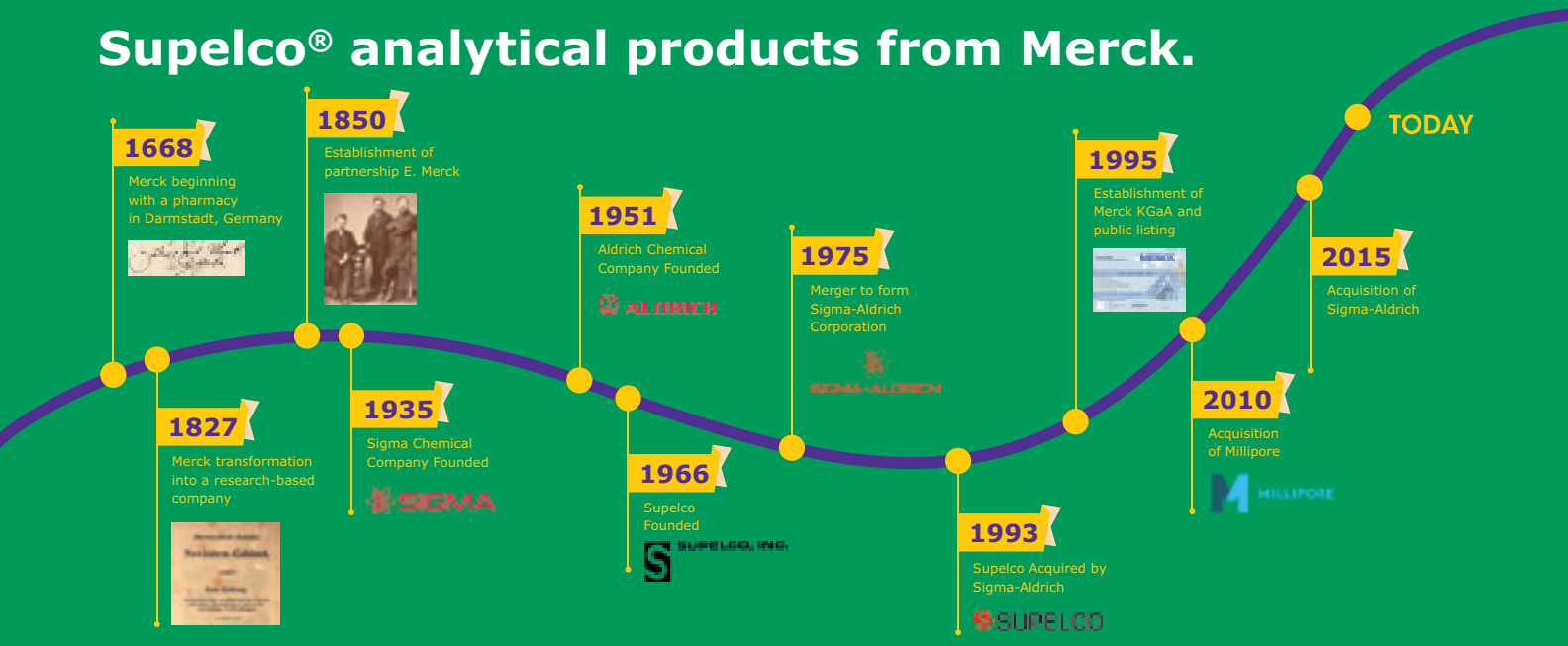
Table 1. Supelco AOCS Research Award Winners

2018	Alice Lichtenstein	1991	Fred Mattson
2017	Fereidoon Shahidi	1990	Rodolfo Brenner
2016	Julian McClements	1989	Mats Hamberg
2015	Gary R. List	1988	Konrad E. Bloch[b]
2014	Alejandro Marangoni	1987	Andrew A. Bensen
2013	Nissim Garti	1986	Robert R. Allen
2012	Casimir C. Akoh	1985	Bengt Samuelsson[b]
2011	John Harwood	1984	Morris Kates
2010	William W. Christie	1983	David A. van Dorp
2009	Thomas A. Foglia	1982[a]	Rex Malcolm Chaplin Dawson
2008	Edward Emken	1981	Laurens van Deenen
2007	Edwin Frankel	1980	James F. Mead
2006	Earl G. Hammond	1979	Stephen S. Chang
2005	Marcel S.F. Lie Ken Jie	1978	Ralph Holman
2004	George M. Carman	1977	George Popjak
2003	Milton Rosen	1975	W. O. Lundberg
2002	Norman Salem, Jr.	1974	P. K. Stumpf
2001	Ching-Hsein Huang	1973	F. D. Gunstone
2000	Howard Sprecher	1972	A. T. James
1999	Andrew J. Sinclair	1971	E. S. Lutton
1998	Robert G. Jensen	1970	E. P. Kennedy
1997	William E. R. Lands	1969	H. J. Dutton
1996	David Kritchevsky	1968	Daniel Swern
1995	John S. O'Brien	1967	Sune Bergstrom[b]
1994	Robert G. Ackman	1966	H. E. Carter
1993	Salih J. Wakil	1965	Ernest Klenk
1992	Aloys L. Tappel	1964	Erich Baer

(a) Supelco began sponsorship of the award
(b) Also Nobel Prize Winners

IMAGINE THE NEXT 350 YEARS

Supelco® analytical products from Merck.



Footprint of Supelco

1966: Enters GC business with adsorbents and packed GC columns
1971: Enters carbon adsorbent business with Carbosieve®
1979: Enters HPLC with SUPELCOSIL™ line of stable 5 µm spherical particles with true monolayer bonding
1983: Enters the air sampling market by introducing a line of solvent desorption tubes for industrial hygienists to help protect workers from being exposed to toxic chemicals
1985: Enters sample preparation business with Supelclean™ SPE tubes
1993: Launches SPME fibers
1994: ISO 9001 – Quality Management System
2006: Sigma-Aldrich acquires Astec®: leader in chiral chromatography
2007: First to market globally Fused-Core® particles jointly with Advanced Materials Technology, introduced Ascentis® Express
2018: Supelco® expanded to include all analytical products from Merck KGaA for a comprehensive range of analytical techniques

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Technical Service: **SigmaAldrich.com/techservice**
Safety-related Information: **SigmaAldrich.com/safetycenter**

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