ISSUE 7 | 2020

Analytix Reporter

Container Materials and Light Induced Off-Flavors in Milk, a Study using SPME-GC-MS

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ISSUE 7 | 2020

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Dear Reader,

As we all know, sample preparation is a very important step in most analytical workflows. The ability to isolate analytes of interest before the subsequent analysis by gas chromatography (GC), liquid chromatography (LC) or mass spectrometry (MS) is essential to achieving accurate, precise and reliable results.

Solid phase microextraction (SPME), introduced by Professor Janusz Pawliszyn in 1990, streamlined the analytical process by integrating several operations such as sample collection, extraction, analyte enrichment and isolation from sample matrices. The technology has been widely accepted in the analytical community due to its applicability without major GC instrument modifications and its amenability to efficient automation. After the technology's commercialization by Supelco Inc. in 1993, it has been widely utilized as a sensitive, accurate, and precise sample preparation technique across many industries including environmental, flavor/fragrance, pharma, food/beverage and clinical/forensic.

As the SPME market leader, our company has focused on continuous innovation of the SPME technology. Among other innovations, like dual coated DVB/Carboxen®/PDMS fibers and overcoated fibers for direct immersion into complex matrices (see SigmaAldrich.com/SPME-OCF), specific focus has also been placed upon the development of more rugged and robust fiber cores. When the technology was first introduced, SPME fibers were based on a pure fused silica (FS) core accommodating the extraction phase/coatings. As fused silica is known to be prone to breakage, we sought out to improve upon the initial technology and introduced Stableflex™ fibers (coated FS) to provide a more rugged and robust product. As these are still fused silica based, we continued our research for an even more rugged core that could be easily substituted into current methods.

In 2019, we launched the new nitinol-core type fibers, which offer extended fiber lifetime while maintaining extraction selectivity as compared to the pure fused-silica fibers. The new nitinol-core fibers are more robust with simple method transfer. The nitinol-core type offers improved lot-to-lot and fiber-to-fiber reproducibility, thus lending to improved overall reproducibility of your analytical results.

In this current issue, we explore the use of nitinol-core SPME fibers for the analysis of light induced off-flavors (LIOFs) in milk. Headspace SPME with a Carboxen®/PDMS (CAR/PDMS) coated nitinol-core fiber followed by GC-MS analysis was used to analyze the formation of low molecular weight flavors and off-flavors in milk stored in glass and plastic containers.

Sincerely yours,

Stacy Shollenberger

Stacy Shollenberger
Senior Product Manager
Food and Environmental Sample Preparation

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FOOD & BEVERAGE

The Effect of Container Materials on Production of Light Induced Off-Flavors in Milk - A Study using SPME-GC-MS

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Introduction

Light induced off-flavors (LIOFs) in milk became an issue when dairies began to package milk in high density polyethylene (HDPE) plastic jugs instead of glass bottles. There are several types of LIOFs with the most common coming from oxidation of lipids and degradation of sulfur containing amino acids. Light induced lipid oxidation occurs when free radicals react with the unsaturated fatty acids in milk. The free radical reaction cleaves the double bond and forms hydroperoxides that degrade predominately to aldehydes and to a lesser degree, ketones and alcohols. The most common light activated analytes in this class are hexanal and pentanal, primarily induced from linoleic acid.[1]

The mechanism for the breakdown of sulfur containing amino acids in whey protein is not fully understood. The most common breakdown products in this class are dimethyl sulfide (DMS), methanethiol (MT) and dimethyl disulfide (DMDS). Due to the high volatility of DMS and MT, this study focused primarily on DMDS.

It is well documented that UV rays do not easily penetrate glass, but have been known to penetrate various types of plastic materials. In the US milk is predominately sold in HDPE jugs. Some of these jugs contain white or colored pigments to increase the effectiveness of the plastic to serve as a barrier to UV light. The goal of this study was to evaluate various types of plastics to determine which type provides the best barrier for preserving the integrity of the milk.

Several analytical methods have been used for the analysis of LIOFs in milk. In this study we chose solid-phase microextraction (SPME) to analyze the various milk samples, because of its automation capabilities. Furthermore this technique is sensitive, easy to automate, and is accurate with good precision.

Materials & Methods

Milk containing 2% fat was purchased from a local dairy farm and was stored in ½ gallon glass jugs with a wall thickness of approximately 5 mm. The plastic sealing cap was immediately covered with aluminum foil upon purchase and the milk was stored at 4 °C in the dark.

Different types of plastic containers were obtained from various sources throughout the lab. Each of the plastic containers contained a symbol indicating the type of plastic. Effort was taken to find containers with similar surface areas and volumes. The wall thickness of each container was measured with calipers. The containers were filled to 93% ±1% of the internal volume. The purpose was to keep the void volume of the containers consistent since the shape of the containers varied. The caps and container necks were wrapped with aluminum foil to prevent UV permeation through the cap. The container materials and dimensions are shown in **Table 1**.

Table 1. Container Materials and Dimensions Used in Milk Light Exposure Study

Container Material	Wall Thickness (mm)	Base shape	Total surface area (mm^2)	Volume of milk in container (mL)	Internal volume of container (mL)	Percent of fluid volume
PETE[1]	0.60	Circular	10241	55	59	93%
HDPE[2]	0.80	Circular	9864	65	71	92%
PP[3]	1.32	Circular	9694	50	54	93%
White HDPE[4]	1.50	Rectangular	10400	67	72	93%
Glass bottle	2.00	Circular	11327	75	80	94%

[1]PETE - polyethylene terephthalate ether

[2]HDPE – high density polyethylene

[3]PP-Polypropylene

[4]White HDPE – HDPE impregnated with white opaque pigment

A 500 mL volumetric flask was filled with cold milk and spiked with an internal standard, hexanal-d_{12}, at 5 µg/L. The milk was immediately dispensed into containers at the volume levels listed in **Table 1** and into two glass vials sealed and placed in the refrigerator at 4 °C. Caps were covered with aluminum foil to reduce UV permeation. The containers were placed in a foil-lined tray about 10 cm beneath Sylvania Octron 32 W fluorescent lights, which were used as a UV light source. The exposure time was 2 hours.

After the milk was exposed, the containers were placed in the refrigerator at 4 °C for 1 h to cool the milk and prevent rancidity. During the time the milk samples were being cooled, ten empty 10 mL vials were placed in a Peltier-cooled vial tray holder set at 4 °C on a Gerstel MPS II multi-purpose sampler. The sampler was also equipped with a needle conditioner to clean the fiber, and an agitator for sample mixing.

Five mL of milk was transferred in duplicate into ten cooled vials. The two vials containing the spiked fresh milk in the refrigerator were added to the tray. A Supelco® CAR/PDMS fiber on a Nitinol core was used to extract the samples. The extraction conditions used in the study are shown in **Table 2**.

Table 2. SPME Sampling Conditions

auto sampler:	Gerstel MPS II
sample:	5 mL cooled milk
fiber:	Carboxen®/PDMS (CAR/PDMS) on Nitinol core (**57907-U**)
incubation:	50 °C for 1 min with agitation at 255 rpm
extraction:	headspace, 15 min, 50 °C, with agitation at 250 rpm
desorption:	3 min, 300 °C
post desorption:	2 min, 280 °C, in needle cleaner

The samples were analyzed with an Agilent 7890B GC connected to a 5977 A MSD. The conditions used to analyze the desorbed analytes are shown in Table 3.

Table 3. GC/MS Analysis Conditions

GC:	Agilent® 7890
column:	VOCOL®, 30 m x 0.25 mm I.D., 1.5 µm df (**24205-U**)
oven program:	45 °C (2 min) to 100 °C at 8 °C/min to 140 °C at 12 °C/min to 180 °C at 16 °C min (0.2 min)
carrier gas:	helium at 1 mL/min constant flow rate
inlet:	300 °C with 0.75 mm ID liner (**2637501**)
injection port:	splitless for 0.75 min then vent at 20 mL/min
transfer line:	250 °C
detector:	MSD quadrupole, m/z 40-150
quantitation ions:	pentanal-44; hexanal-56; dimethyl disulfide-94; hexanal-d_{12} -64

Results

The CAR/PDMS fiber on the Nitinol core is an excellent choice for this application due to the small micropores of CAR/PDMS. These pores are ideal for extracting small and midsized analytes. The Nitinol core is very durable and inert. The coating process is produced with state-of-the-art coating equipment that assures good reproducibility by constant monitoring of the coating thickness.

The addition of sodium chloride does increase recovery of these analytes in water, but not in milk containing fat. The responses in milk samples were higher with better precision without added salt; therefore, salt was not added to the samples. Various extraction times were evaluated, but it was determined that 15 minutes enabled samples to be quantified below µg/L concentration levels. The other SPME parameters were optimized to provide good extraction and desorption efficiencies without compromising sample integrity.

A calibration curve was generated by spiking seven fresh milk samples with a standard of the LIOFs analytes from 1-10 µg/L sample concentration and with hexanal-d_{12} at 5 µg/L. Another vial of fresh milk was only spiked with hexanal-d_{12} at 5 µg/L. The samples were extracted and analyzed according to the methods

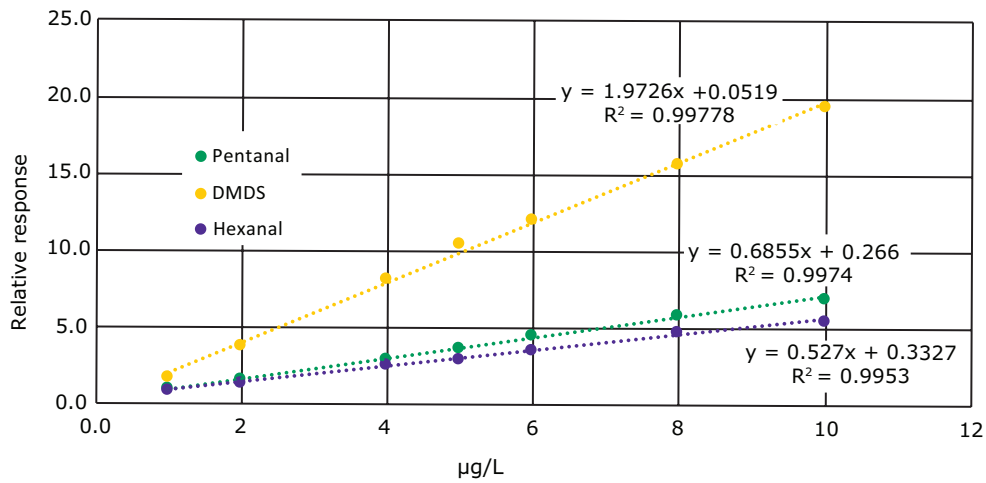

Figure 1. Calibration Curve of Relative Responses of LIOFs with Background Subtraction

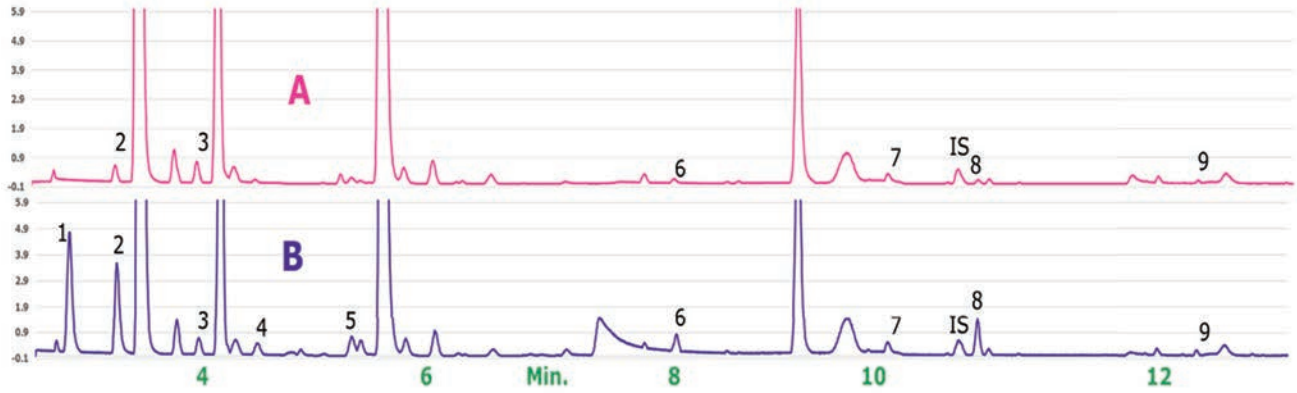

Figure 2. Chromatograms of Milk Spiked with IS not Exposed to Light (A); Milk Spiked with IS Exposed to Light Stored in a Polypropylene Container (B)

Peak IDs: 1. Pentane, 2. Isopropanol, 3. Dimethyl sulfide, 4. n-Hexane, 5. 2-Butanol, 6. Pentanal, 7. Dimethyl disulfide, IS. Hexanal-d_{12}, 8. Hexanal, 9. Heptanal. (See **Table 3** for Run Conditions)

listed in **Tables 2** and **3**. The relative responses of each analyte were calculated and the relative responses from the sample not spiked with the LIOF standard were subtracted from the seven LIOF spiked samples.

Relative responses of the three analytes over the 1-10 µg/L spiking range had regression coefficient values > 0.99, and low Y-intercept values (**Figure 1**). These results were obtained in full scan mode, so greater sensitivity could be obtained using SIM mode if needed.

Chromatograms of milk not exposed to light spiked with the IS (A) and milk exposed to light in a polypropylene container (B) are shown in **Figure 2**.

The comparison of the chromatograms shows that light exposure in the PP container increased the response of many of the analytes. Both chromatograms are at the same scale, and the response of the internal standard (hexanal-d_{12}) is similar in both plots. Even though this study focused on three analytes, other analytes are generated from the light exposure or some other mechanism. Two small volatile analytes, pentane and isopropanol, have much larger responses on the light exposed samples. Note that the samples were run in duplicate (data not shown here) and the responses of duplicate samples were similar.

To calculate the concentration level of the selected LIOFs obtained from milk exposed in the various containers, the average relative responses from duplicate runs were calculated. The average relative responses are shown in **Table 4**.

Table 4. Relative Responses of LIOFs in Milk after Exposure to Light in Various Containers

	No Light	PP	HDPE	PETE	HDPE White	Glass
Pentanal	0.206	0.826	0.572	1.142	0.470	0.282
DMDS	0.000	0.172	0.170	0.196	0.000	0.000
Hexanal	0.122	1.454	1.027	1.826	0.551	0.438

The average relative responses for each analyte obtained from the no light exposed milk samples were subtracted from the average relative responses obtained from the various containers. The background subtracted relative responses were divided by the slope of the line as listed in **Figure 1**. **Figure 3** shows the calculated results.

The results show that two of the plastics, PETE and PP, were the least efficient barrier to UV light. The PETE container had the thinnest wall of the containers, which may have contributed to the barrier properties. PP had the thickest wall of any plastic but the formation of LIOFs was quite high. The addition of white pigment to the HPDE plastic made it a much better barrier to UV light. Its properties were similar to glass. The thickness of the glass does affect the barrier properties as we demonstrated in an additional study.

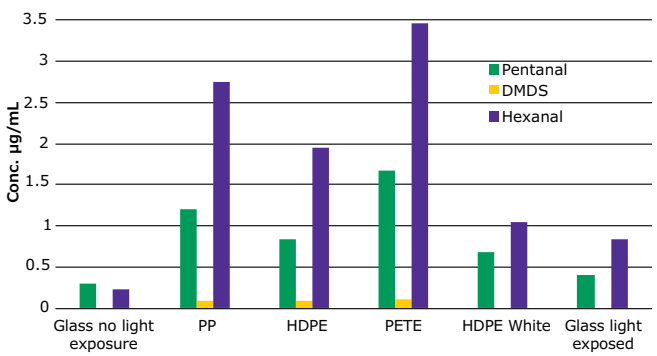

Figure 3. Concentration in µg/L of LIOFs in Milk with Background Subtraction

Conclusions

The type of material used to store milk can be critical in the prevention of lipid oxidation. This study shows that glass is still the best barrier to UV light, but HDPE impregnated with a pigment is a good option. In this case white pigmentation helped to reduce LIOF formation, but studies have shown that yellow or pink pigments may be even better.

The CAR/PDMS fiber on the Nitinol core was able to retain the small flavoring analytes. The micropores retain and release these analytes efficiently. In addition, the Nitinol core is highly inert and extremely durable. This fiber is a viable alternative to this coating on a fused silica core.

Reference:

1. Marsili, R. T., Journal of Chromatogrphic Science 37 (1999) 17–23

Featured Products

Description	Cat. No.
SPME Fiber Assembly Carboxen®/PDMS (CAR/PDMS) on Nitinol Core (NIT), Pk.3	**57907-U**
VOCOL®, 30 m x 0.25 mm I.D., 1.5 µm df	**24205-U**
Inlet Liner, Direct (SPME) Type, Straight Design (unpacked), for Agilent®, Pk.1	**2637501**
Reference Materials	
Dimethyl disulfide (DMDS), analytical standard, neat, 1 mL	**68986**
Hexanal, analytical standard, neat, 1 mL	**18109**
Hexanal-d_{12}, ≥98 atom % D, ≥96% (CP), neat, 250 mg	**732338**
Valeraldehyde (Pentanal), analytical standard, neat, 1 mL	**42272**

To read more about the new Nitinol-core SPME Fibers or to request an evaluation fiber, visit

SigmaAldrich.com/NITSPME

For more information on SPME in general and to download the "*SPME for GC - Setting Started with Solid Phase Microextraction*" brochure, visit

SigmaAldrich.com/spme

FOOD & BEVERAGE

Determination of Dehydroacetic Acid in Bread Using HPLC with UV Detection Following the Chinese National Standard (GB) Method

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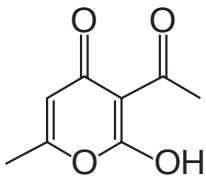
Introduction

Dehydroacetic acid is an organic compound which has several industrial applications. It can be used as a plasticizer in synthetic resins; as a fungicide or bactericide; but also as a food preservative (European food additive number E265).

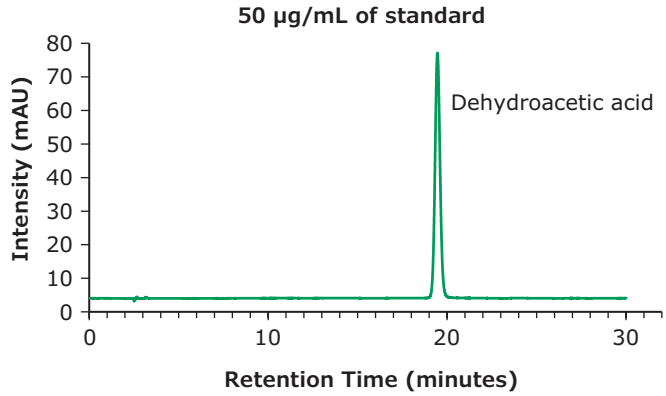


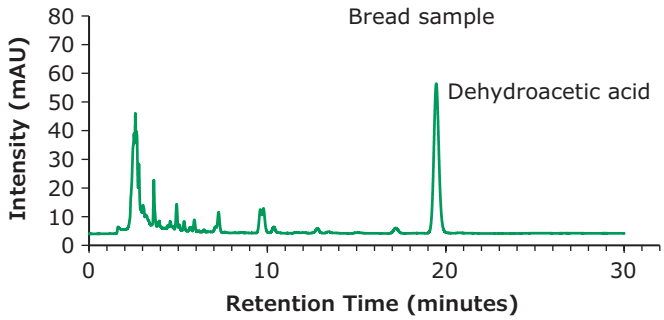
Dehydroacetic acid

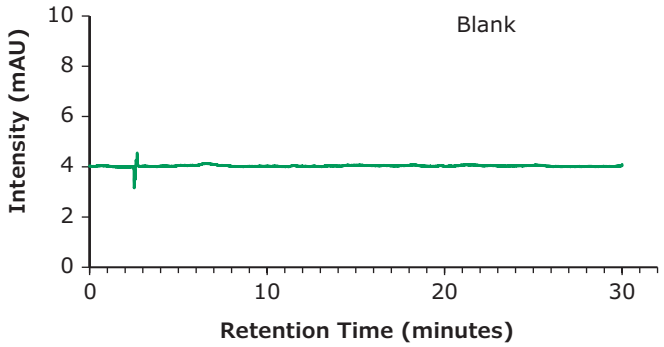
This application focuses on testing dehydroacetic acid in bread using a Discovery® HS C18 HPLC column, following the current Chinese national standard method (GB 5009.121-2016).

Obtained results show satisfactory chromatographic resolution of dehydroacetic acid from the bread sample matrix, and the method linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ) meet set testing requirements.

Experimental Conditions	
column:	Discovery® HS C18, 25 cm x 4.6 mm I.D., 5 µm (**568523-U**)
mobile phase:	[A] 20 mM ammonium acetate, pH 3.5 with acetic acid; [B] methanol; (70:30, A:B)
flow rate:	1.0 mL/min
column temp:	30 °C
detector:	UV, 293 nm
injection:	10 µL
sample:	standard stock solution: Weigh accurately 0.1 g of dehydroacetic acid into a 100 mL volumetric flask, add into 10 mL of 20 g/L of sodium hydroxide solution, add water to volume to obtain a 1.0 mg/mL of stock solution. Standard solution: Dilute the stock solution with water to obtain a 50 µg/mL of solution.
	sample preparation: Homogenize bread sample, weigh accurately 2–5 g of homogenized sample to a 25 mL centrifuge tube. Add into 10 mL of water, 5 mL of 120 g/L of $ZnSO_4$ aqueous solution, adjust pH value to pH 7.0 with 20 g/L of NaOH aqueous solution. Add water to volume, shake well. sonicate for 10 min. Transfer the supernatant over the bread crumb suspension to separating funnel, add into 10 mL of hexane for protein and fat removal, shake for 1 minute, stand for separating, remove the hexane layer, repeat the procedure twice, pool the aqueous layer to centrifuge, centrifuge at 4000 r/min for 10 minutes. Filter the supernatant with 0.45 µm filter membrane for HPLC analysis.







Chromatographic Data

	Compound	RT (min)	Resolution	Plates (USP)	Tailing Factor
1	Dehydroacetic acid	19.5	--	22832	1.0

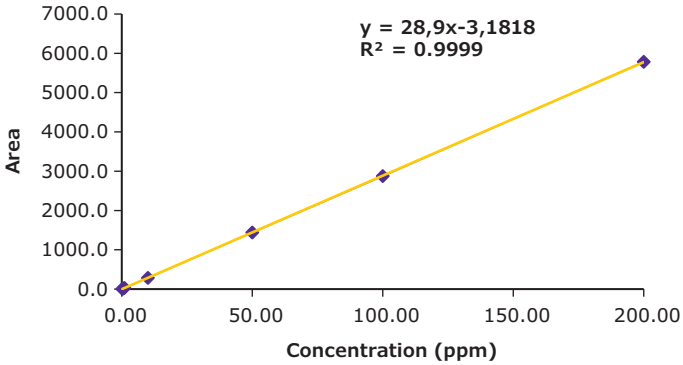
Specificity and Repeatability

1. Specificity: Inject Standard Solution and Determine the Retention Time and Monitor the Peak Purity

No.	Compound	RT (min)	Plates (USP)	Tailing Factor	Peak Purity
1	Dehydroacetic acid	19.5	22832	1.0	1.0000

2. Standard Repeatability (Dehydroacetic acid, 10 ppm)

Measurements	Mean Area
STD 1	283.7
STD 2	284.8
STD 3	284.2
STD 4	284.8
STD 5	283.2
Mean	284.1
Standard Deviation	0.7
RSD (%)	0.2



3. Linearity

Concentration (µg/mL)	Mean Area
0.20	5.7
0.50	14.8
1.00	28.8
10.0	284
50.0	1439
100.0	2875
200.0	5783

4. LOD & LOQ

Concentration (µg/mL)	Mean Area
0.20	5.73
0.50	14.79
1.00	28.76
STEYEX	0.35
Slope	28.70
LOD (ppm)	0.04
LOQ (ppm)	0.12

Featured Products

Description	Cat. No.
Discovery® HS C18 HPLC Column, 25 cm × 4.6 mm I.D., 5 µm particle size	**568523-U**
Syringe Filter Millex-HV Durapore® (PVDF), hydrophilic, non-sterile 0.45 µm pore size, 13 mm diameter, PK.100	**SLHVX13NK**
Dehydroacetic acid Pharmaceutical Secondary Standard; Certified Reference Material, 1 g	**PHR1582**
Solvents & Reagents	
n-Hexane for liquid chromatography LiChrosolv®	**1.04391**
Methanol gradient grade for liquid chromatography LiChrosolv®	**1.06007**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
Ammonium acetate for analysis EMSURE® ACS,Reag. Ph Eur	**1.01116**
Formic acid 98-100% for HPLC LiChropur®	**5.43804**
Sodium hydroxide pellets EMPLURA®	**1.06462**
Zinc sulfate heptahydrate for analysis EMSURE®ACS,ISO,Reag. Ph Eur	**1.08883**

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FOOD & BEVERAGE

Vanilla - Natural or Out of the Reaction Flask?

HPLC Fingerprint Method and Reference Materials Help to Distinguish Natural from Synthetic or Adulterated Vanilla

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Stephan Altmaier, Principal Scientist Advanced Analytical R&D
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Introduction

Vanilla is one of the most popular flavors in food and beverage products. The demand far exceeds the global supply of naturally grown vanilla; therefore, in addition to natural vanilla, artificial vanilla flavors are used in the food industry. Natural vanilla is commonly substituted for synthetically produced vanillin or by other compounds with a similar flavor such as ethyl vanillin.

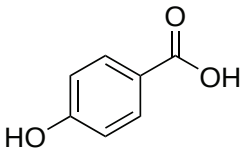
And because of the large price difference between natural and synthetic vanilla, this is a very attractive target for food criminals and frauds.

Analysis of the chromatographic fingerprint of a vanilla flavor represents an efficient method to detect these types of adulteration and mislabeling.[1] Characteristic markers for natural vanilla are vanillic acid, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde and vanillin (**Figure 1**).

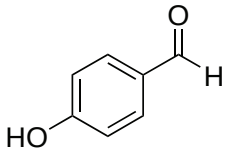
For artificially produced vanilla, cheap chemicals such as guaiacol or eugenol are typically used as starting materials. The presence of traces of these compounds are indicators of synthetically produced vanilla. Ethyl vanillin or coumarin are also often added to enhance the flavor (**Figure 2**).

We recently launched a set of two reference materials for natural and synthetic vanilla extracts for the testing of vanilla authenticity by chromatographical fingerprint. These two reference extracts are also available individually (**Table 1**).

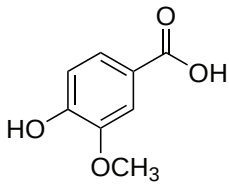
Figure 1. Compounds Found in Natural Vanilla



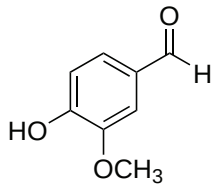
4-Hydroxybenzoic acid
(Cat.No. **92596**)



4-Hydroxybenzaldehyde
(Cat.No. **91554**)

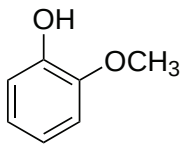


Vanillic acid
(Cat.No. **68654**)

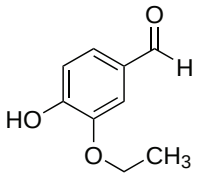


Vanillin
(Cat.No. **30304**)

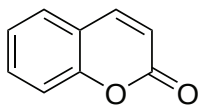
Figure 2. Markers for Artificial Vanilla



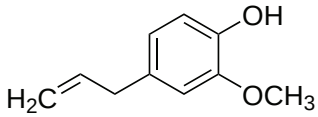
Guaiacol
(Cat.No. **PHR1136**)



Ethyl vanillin
(Cat.No. **75042**)



Coumarin
(Cat.No. **72609**)



Eugenol
(Cat.No. **79891**)

Table 1. Vanilla Extract Reference Materials (Natural and Synthetic)

Description	Quantified Components	Qualitatively Confirmed Components	Package Size	Cat. No.
Vanilla extract, natural	Vanillin, Vanillic acid	Vanillin, Vanillic acid, Ethyl Vanillin (absence)	1 mL	**06261501**
Vanilla extract, synthetic	Vanillin, Vanillic acid	Vanillin, Vanillic acid, Ethyl Vanillin	1 mL	**06271501**
Vanilla extract set, natural and synthetic	Vanillin, Vanillic acid	Vanillin, Vanillic acid, Ethyl Vanillin (absence / presence)	2 x 1 mL	**06281501**

The products are developed and manufactured by HWI pharma services GmbH in Rülzheim, Germany, and are qualified as secondary standards, traceable to HWI primary reference standards quantified by qNMR. These products add to a range of plant extract reference materials (**SigmaAldrich.com/plantextracts**) designed for rapid identification and quantification of typical constituents of plants used as food additives or as herbal medicinal products.

HPLC fingerprint method

In the following we present an HPLC method to detect natural and synthetic vanilla markers using a Chromolith® Performance RP-18 endcapped 100x2 mm column (**Table 2**). Results for both the synthetic and the natural vanilla reference material extract are shown. In addition, samples of food and beverage products containing vanilla flavor, such as bourbon vanilla, ice cream and Rooibos tea, were also tested (see **Table 2**. for sample preparation details)

For all the standards, extensive studies were made to determine LOD, LOQ, linearity, repeatability and standard deviation. The complete dataset incl. the data for Rooibos tea and validation data for the method can be viewed online in the full version of this article at **SigmaAldrich.com/Analytix**, Issue 7.

Table 2. Experimental Conditions & Sample Preparation

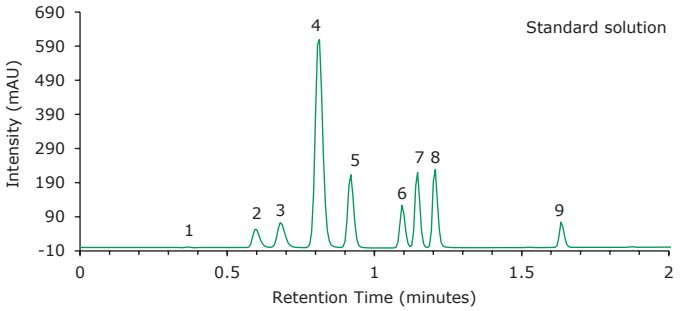
column:	Chromolith® Performance RP-18 endcapped 100x2 mm (**1.52006**)
mobile phases:	[A] 0.05% TFA in water, [B] acetonitrile
injection volume:	0.5 µL
gradient:	**Time** / **%A** / **%B** 0 / 90 / 10 0.5 / 65 / 35 1.3 / 25 / 75 1.5 / 0 / 100 2 / 0 / 100
flow rate:	0.8 mL/min
pressure:	75-118 bar (1088-1711 psi)
column temp.:	40 °C
detector:	Dionex Ultimate 3000 VWD-3400 @ UV = 280 nm (micro flow cell; 1.4 µL/7 mm)
Standard & Sample Preparation:	
standard solution :	the standards 4-hydroxybenzoic acid (c = 0.1 mg/mL), vanillic acid (0.1 mg/mL), 4-hydroxybenzaldehyde (0.2 mg/mL), vanillin (0.1 mg/mL), guaiacol (0.2 mg/mL), ethyl vanillin (0.1 mg/mL), coumarin (0.1 mg/mL), and eugenol (0.2 mg/mL) were dissolved in mobile phases A/B 90/10 (v/v).
matrix standard solution natural:	transfer approximately 100 mg of vanilla extract natural into a 5 mL volumetric flask, dissolve in mobile phases A/B 95/5 (v/v) and fill up to mark with mobile phases A/B 95/5 (v/v).
matrix standard solution synthetic:	transfer approximately 25 mg of vanilla extract synthetic into a 25 mL volumetric flask, dissolve in mobile phases A/B 95/5 (v/v) and fill up to mark with mobile phases A/B 95/5 (v/v)
sample solution Bourbon vanilla:	one piece of Bourbon vanilla was cut into small pieces and placed in a 50 mL volumetric flask. The flask was filled to the mark with ethanol and after ultrasonic extraction at room temperature for 30 min the sample mixture was filtered through a 0.45 µm membrane filter.
sample solution ice cream:	500 mg vanilla ice cream was placed in a 50 mL volumetric flask and filled up with ethanol to the mark. After ultrasonic extraction at room temperature for 30 min the sample mixture was filtered through a 0.45 µm syringe filter.
sample solution Rooibos tea:	one pad of vanilla flavored Rooibos tea was placed in a 50 mL volumetric flask and filled up with ethanol to the mark. After ultrasonic extraction at room temperature for 30 min the sample mixture was filtered through a 0.45 µm membrane filter.

Results & Discussion

The prepared standard solution was used for method development and validation. The chromatogram and retention data is shown in **Figure 3**. Vanillin is the main component of the natural vanilla extract reference material (cat. no. 06261501), in addition traces of 4-hydroxybenzoic acid, vanillic acid and 4-hydroxybenzaldehyde could be detected. No ethyl vanillin, guaiacol, coumarin or eugenol were present (**Figure 4**). For comparison, the chromatogram of a commercial Bourbon vanilla sample (**Figure 5**) is very similar to the natural vanilla extract reference material.

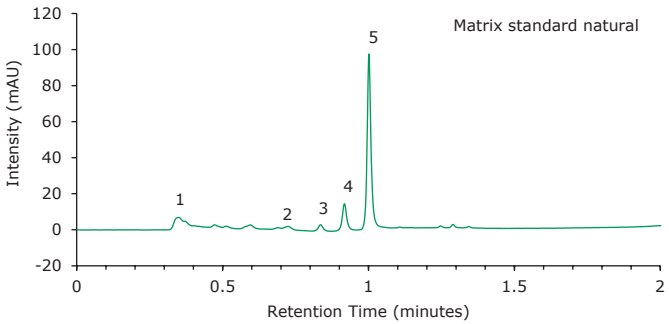
In contrast to natural vanilla, the synthetic vanilla extract reference material (cat.no. 06271501) shows, besides vanillin as the major peak, ethyl vanillin, coumarin and traces of eugenol (**Figure 6**). In the ice cream sample, guaiacol is the major peak (**Figure 7**). In addition, traces of ethyl vanillin, coumarin and eugenol were detected, indicating the synthetic nature of the material.

Figure 3. HPLC-UV Analysis of the Standard Solution



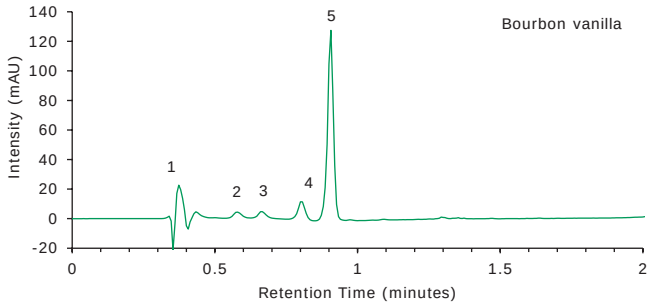
No.	Compound	Retention Time (min)	RRT	Area (mAU* min)	Tailing Factor
1	t_0 void volume	0.37			
2	4-Hydroxybenzoic acid	0.59	0.64	1.537	1.5
3	Vanillic acid	0.68	0.74	2.256	1.28
4	4-Hydroxybenzaldehyde	0.81	0.88	18.34	1.03
5	Vanillin	0.92	0.00	5.06	1.05
6	Guaiacol	1.09	1.18	2.391	1.16
7	Ethyl vanillin	1.15	1.25	4.243	1.02
8	Coumarin	1.21	1.32	4.384	1.05
9	Eugenol	1.63	1.77	1.416	1.32

Figure 4.HPLC-UV Analysis of the Matrix Standard Solution Natural (Vanilla Extract, Natural, Cat.No. **06261501**)



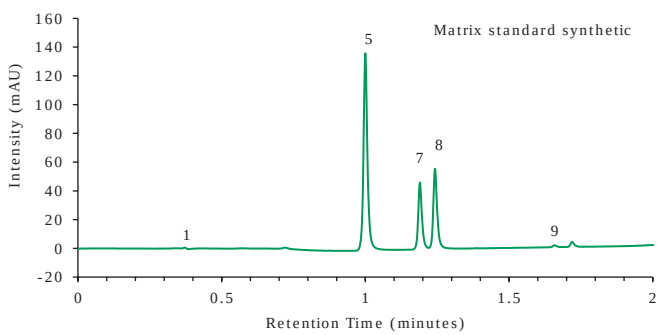
No.	Compound	Retention Time (min)	RRT	Area (mAU* min)	Tailing Factor
1	t_0 void volume	0.35			
2	4-Hydroxybenzoic acid	0.72	0.72	0.0317	1.13
3	Vanillic acid	0.84	0.84	0.0621	1.12
4	4-Hydroxybenzaldehyde	0.92	0.92	0.2579	1.03
5	Vanillin	1.00	0.00	1.5233	1.16
6	Guaiacol				
7	Ethyl vanillin				
8	Coumarin				
9	Eugenol				

Figure 5. HPLC-UV Analysis of a Commercial Bourbon Vanilla Sample



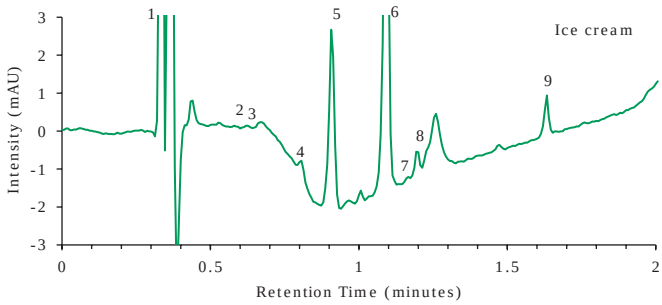
No.	Compound	Retention Time (min)	RRT	Area (mAU* min)	Tailing Factor
1	t_0 void volume	0.37			
2	4-Hydroxybenzoic acid	0.58	0.64	0.127	1.05
3	Vanillic acid	0.67	0.74	0.153	1.15
4	4-Hydroxybenzaldehyde	0.80	0.87	0.340	1.08
5	Vanillin	0.91	0.00	3.094	0.89
6	Guaiacol				
7	Ethyl vanillin				
8	Coumarin				
9	Eugenol				

Figure 6. HPLC-UV Analysis of the Matrix Standard Solution Synthetic (Vanilla Extract, Synthetic, Cat.No. **06271501**)



No.	Compound	Retention Time (min)	RRT	Area (mAU* min)	Tailing Factor
1	t_0 void volume	0.37			
2	4-Hydroxybenzoic acid				
3	Vanillic acid				
4	4-Hydroxybenzaldehyde				
5	Vanillin	1.00	0.00	2.2404	1.16
6	Guaiacol				
7	Ethyl vanillin	1.19	1.19	0.7156	1.22
8	Coumarin	1.24	1.24	0.9187	1.33
9	Eugenol	1.66	1.66	0.0216	1.27

Figure 7. HPLC-UV Analysis of an Ice Cream Sample



No.	Compound	Retention Time (min)	RRT	Area (mAU* min)	Tailing Factor
1	t_0 void volume	0.37			
2	4-Hydroxybenzoic acid	0.58	0.64	0.001	1.29
3	Vanillic acid	0.67	0.74	0.004	1.22
4	4-Hydroxybenzaldehyde	0.81	0.89	0.008	1.06
5	Vanillin	0.91	0.00	0.099	0.96
6	Guaiacol	1.09	1.20	0.244	0.85
7	Ethyl vanillin	1.14	1.25	0.001	
8	Coumarin	1.19	1.31	0.009	1.25
9	Eugenol	1.63	1.79	0.008	0.88

Conclusion

The examples shown demonstrate the applicability and value of matrix reference materials to help detect food adulterations and mislabeling.

Reference:

1. Cicchetti, Chaintreau J. Sep. Sci. 2009, 32, 3043 – 3052.

Featured Products

Description	Cat. No.
Vanilla extract reference materials (natural and synthetic)	
Vanilla extract, natural, secondary reference standard, 1 mL	**06261501**
Vanilla extract, synthetic, secondary reference standard, 1 mL	**06271501**
Vanilla extract set, natural and synthetic secondary reference standard, 2x1 mL	**06281501**
Reference Materials for ingredients of natural vanilla and markers for synthetic vanilla	
Coumarin, Certified Reference Material*, 100 mg	**72609**
Ethyl vanillin, Certified Reference Material*, 100 mg	**75042**
Eugenol, Certified Reference Material*, 100 mg	**79891**
Guaiacol, Certified Reference Material*, 1.5 g	**PHR1136**
4-Hydroxybenzaldehyde, Analytical Standard, 250 mg	**91554**
4-Hydroxybenzoic acid, Certified Reference Material*, 50 mg	**92596**
Vanillic acid, Certified Reference Material*, 50 mg	**68654**
Vanillin, Certified Reference Material*, 50 mg	**30304**
Sample Prep, HPLC Column, Solvents & Reagents	
Chromolith® Performance RP-18 endcapped 100-2 (100x2 mm)	**1.52006**
Millex® syringe filter units, disposable, Durapore® PVDF, pore size 0.45 µm, non-sterile, Pk.1000	**SLHVX13NK**
Ethanol gradient grade for liquid chromatography LiChrosolv®	**1.11727**
Acetonitrile gradient grade for liquid chromatography LiChrosolv®	**1.00030**
Water for chromatography (LC-MS grade) LiChrosolv®	**1.15333**
Trifluoracetic acid for spectroscopy Uvasol®	**1.08262**

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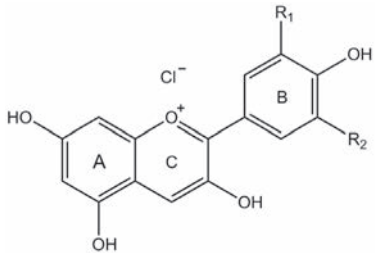
Reference Materials for Accurate Quantification of Anthocyanins & Anthocyanidins

New products from PhytoLab now available

Matthias Nold, Product Manager Reference Materials, Analytix@milliporesigma.com

Figure 1. Chemical Structure of Anthocyanidins



Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Pelargonidin	H	H
Peonidin	OCH_3	H
Petunidin	OH	OCH_3

In a series of articles, we have highlighted specific product groups of phytochemical standards from PhytoLab. These products represent the extensive portfolio of more than 1400 extensively documented herbal reference substances of all classes of natural compounds. The initial article in Analytix Reporter, Issue 5 dealt with Pyrrolizidine alkaloids and Issue 6 highlighted Glucosinolates. We will now focus on the product group of Anthocyanins & Anthocyanidins.

Anthocyanins are water-soluble secondary plant metabolites that can occur in all parts of higher plants, including leaves, stems, roots, flowers and fruits.

They are responsible for making bright-colored flowers and fruits attractive to pollinators or animals. Anthocyanins also act as a “sunscreen” and protect cells from damage due to exposure to UV-light. They may also act as antioxidants in the cell vacuoles.

Plants rich in anthocyanins include blueberries, cranberries, raspberries, blackberries, strawberries, cherries and grapes, among many other species.

Anthocyanins belong to the class of natural compounds known as flavonoids. Their 15-carbon skeleton consists of two phenyl rings and one heterocyclic ring containing a positively charged oxygen atom. In nature, usually carboxylate anions of water-soluble acids would act as counter ions, while the pure compounds are most frequently isolated as chloride salts. The most common anthocyanins exhibit hydroxyl functions in positions 3, 5, 7 and 4’. The anthocyanidins are the aglycones of the anthocyanins, which most often bear a sugar moiety bound to position 3. Structural variation is usually achieved by the substitution pattern in the B-ring and differences in the glycosidic profile as shown in **Figure 1**.

In the European Pharmacopoeia, a specification for total content of anthocyanins, calculated as cyanidin 3-glucoside, is given in the monographs for fresh bilberry fruit and fresh bilberry fruit dry extract, refined and standardized. The latter monograph also specifies a maximum limit for anthocyanidins, calculated as cyanidin, describes a certain chromatographic profile of 15 anthocyanins and 5 anthocyanidins to confirm identity. A minimum content of procyanidins, expressed as cyanidin, is given in the monograph on hawthorn berries.

In the United States Pharmacopoeia, the dietary supplements monographs on powdered bilberry extract and European elder berry extract specify a minimum content of anthocyanins, calculated as cyanidin 3-glucoside, and a maximum limit for anthocyanidins, calculated as cyanidin. Requirements on chromatographic profiles, including peak intensities of various anthocyanins, are given.

For a reliable quantitative analysis of anthocyanins & anthocyanidins, well characterized reference substances are essential.

Due to the positive charge of the molecule, the counter ion has to be taken into account. For all anthocyanins and anthocyanidins characterized as primary reference substances, chloride was determined quantitatively

and considered as an impurity in the calculation of the absolute content, which therefore refers to the pure anthocyanin or anthocyanidin only. Another very useful feature of the phyproof® standards is that the exact weight of each package is printed on the label of the product vial, which offers the convenience of dissolving the analyte directly in the vial.

Available Anthocyanins & Anthocyanidins Reference Materials

Description	Package Size	Cat. No.
Cyanidin chloride	20 mg	**PHL80022**
Cyanidin 3-arabinoside	10 mg	**PHL89614**
Cyanidin 3,5-diglucoside	10 mg	**PHL89615**
Cyanidin 3-galactoside	10 mg	**PHL89463**
Cyanidin 3-glucoside	10 mg	**PHL89616**
Cyanidin-3-(6''-malonylglucosid)	5 mg	**PHL85728***
Cyanidin 3-rutinoside	10 mg	**PHL80577**
Cyanidin 3-sambubioside	5 mg	**PHL89617**
Cyanidin 3-sophoroside	5 mg	**PHL80579**
Delphinidin chloride	10 mg	**PHL89625**
Delphinidin 3,5-diglucoside	5 mg	**PHL89626**
Delphinidin 3-galactoside	5 mg	**PHL89506**
Delphinidin 3-glucoside	10 mg	**PHL89627**
Delphinidin 3-rutinoside	10 mg	**PHL80735**
Delphinidin 3-sambubioside	5 mg	**PHL82249**
Malvidin chloride	10 mg	**PHL80083**

Description	Package Size	Cat. No.
Malvidin 3,5-diglucoside	10 mg	**PHL89727**
Malvidin 3-galactoside	10 mg	**PHL80600**
Malvidin 3-glucoside	10 mg	**PHL89728**
Pelargonidin chloride	10 mg	**PHL80084**
Pelargonidin 3,5-diglucoside	10 mg	**PHL80334**
Pelargonidin 3-glucoside	10 mg	**PHL89753**
Peonidin chloride	5 mg	**PHL80085**
Peonidin 3,5-diglucoside	10 mg	**PHL80335**
Peonidin 3-glucoside	5 mg	**PHL89754**
Petunidin chloride	5 mg	**PHL80225***
Petunidin 3-glucoside	5 mg	**PHL89755**

* = coming soon

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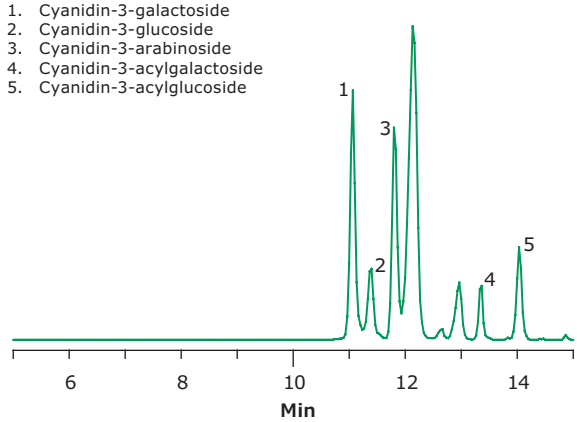
LC/MS (TOF) Analysis of Cyanidin Glycosides from High Bush Blueberries

1. Cyanidin-3-galactoside
2. Cyanidin-3-glucoside
3. Cyanidin-3-arabinoside
4. Cyanidin-3-acylgalactoside
5. Cyanidin-3-acylglucoside



Analytical Conditions	
column:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (**53823-U**)
mobile phase:	[A] 0.1% (v/v) formic acid in water; [B] 0.1% (v/v) formic acid in 75:25 (v/v) acetonitrile:water
gradient:	2% B for 2 min, to 100% B in 38 min
flow rate:	0.2 mL/min
column temp.:	35 °C
injection:	1 µL
detector:	ESI(+) TOF, extracted ions m/z 449.1100, 419.0979, 491.1213
sample preparation:	berries (1.0 g) were added to 1.0 mL of 1% (v/v) formic acid in methanol. Samples were crushed in the solvent mixture and extracted (refrigerated) for 2 hours. A portion of the extracted sample was removed, centrifuged and the supernatant was collected for HPLC analysis.

To see more applications for Anthocyanins, **visit us at SigmaAldrich.com/applications** and search for "**Anthocyanins**"

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Analysis of Pesticide Residues in Pistachios

Using QuEChERS Extraction and Cleanup with Supel™ QuE Z-Sep+

Kathy Stenerson, Principle R&D Scientist, Megan Wesley, 2016 R&D Summer Intern, Analytix@milliporesigma.com

Introduction

Pistachios are popular and enjoyed for both taste and health benefits such as decreased cholesterol, weight management, protection against diabetes and hypertension, and improved digestion.[1] These nuts are grown in the United States (specifically, California), Italy, and countries in Central Asia like Iran, Turkey, Afghanistan and Syria. Pesticide tolerances set by the US EPA for pistachios range from 0.01 - 0.7 µg/g before harvest to 3 - 200 µg/g after harvest, depending on the pesticide.[2] Testing for pesticide residues then requires a method which will allow for low level and accurate determination. The "quick, easy, cheap, effective, rugged and safe" (QuEChERS) approach has been used to analyze multiple pesticide residues found in pistachios.[3] Pistachios contain approximately 45% fat, which can result in a significant amount of co-extracted matrix in the acetonitrile extract generated using the QuEChERS procedure. The use of a cleanup sorbent which can reduce this fat is essential to prevent fouling of LC-MS/MS and GC-MS/MS systems, and minimize ion suppression, thus allowing low level detection. In this application, Supel™ QuE Z-Sep+ sorbent was used as part of the QuEChERS method in the analysis of pesticide residues in pistachios. Z-Sep+ is a zirconia and C18 functionalized silica sorbent which acts to retain fatty constituents through both Lewis acid/base and hydrophobic interactions. The selectivity of the zirconia present in Z-Sep+ offers retention of a wider range of fats than C18 alone. In this application, QuEChERS extraction and cleanup using Z-Sep+ sorbent were used before the LC-MS/MS and GC-MS/MS analysis of pesticide residues in pistachios. The targeted analyte list included pesticides relevant to pistachios.[4,5]

Experimental

Pistachios were purchased from a local grocery store. They were frozen with liquid nitrogen (shells on), ground, and spiked at 10 ng/g with the pesticides listed in **Tables 2** and **4**, and allowed to equilibrate for 1 hour. Samples were then subjected to QuEChERS extraction and cleanup with Z-Sep+ following the procedure in **Figure 1**. A 100 µL aliquot of the final extract was diluted to 1 mL with 5 mM ammonium formate/0.1% formic acid in water, and analyzed by LC-MS/MS using the conditions shown in **Table 1**. The remaining acetonitrile extract was analyzed directly by GC-MS/MS using the conditions shown in **Table 3**. Spiked samples were quantitated against 5-point matrix-matched calibration curves prepared in unspiked pistachio matrix blanks (after cleanup). No internal standard was used.

Figure 1. QuEChERS Extraction and Cleanup Procedure Used for Pistachios.

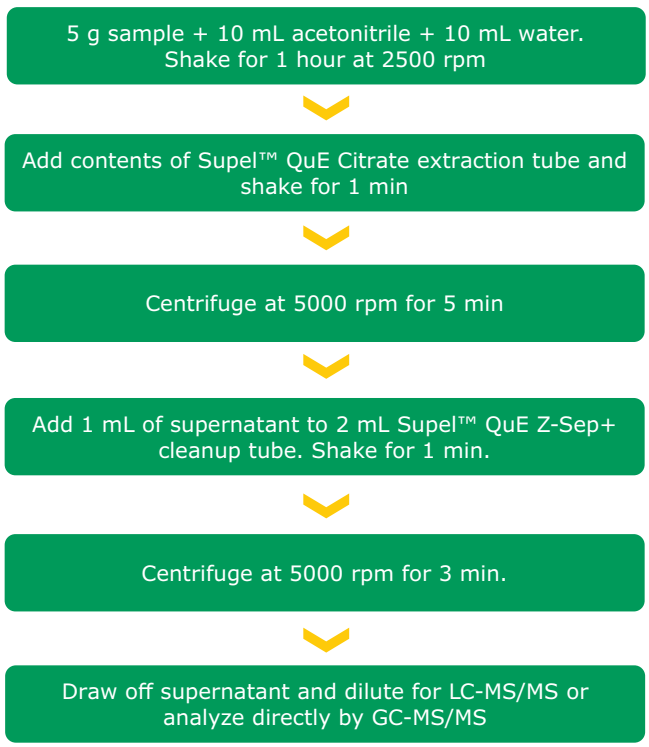

Table 1. LC-MS/MS Analysis Conditions

column:	Ascentis® Express RP-Amide, 10 cm × 2.1 mm I.D., 2 µm (51576-U)
mobile phase:	[A] 5 mM ammonium formate, 0.1% formic acid in water; [B] 5 mM ammonium formate, 0.1% formic acid in 95:5 acetonitrile:water
gradient:	5% B held for 1 min; 5 to 100% B in 12 min; held at 100% B for 1.5 min; 100 to 5% B in 0.5 min; held at 5% B for 1.5 min
flow rate:	0.4 mL/min
column temp.:	30 °C
detector:	MS, ESI (+), MRM (see Table 2)
injection:	5 µL

Table 2. MRMs Used for Quantitation, LC-MS/MS

Compound	CAS No.	MRM	Frag (V)	CE
Aclonifen	74070-46-5	265/182.1	115	28
Aldicarb	116-06-3	208.1/89.1	70	12
Aldicarb-sulfone	1646-88-4	223.1/86.1	80	8
Bifenazate	149877-41-8	301.1/170.1	95	16
Butocarboximsulfoxide	34681-24-8	207.1/132	65	0
Carbendazim	10605-21-7	192.1/160.1	105	16
Carbofuran	1563-66-2	222.1/165.1	80	20
Chlorantraniliprole	500008-45-7	483.9/452.9	105	16
Etrimfos	38260-54-7	293.1/125	120	28
Flufenoxuron	101463-69-8	489.1/158	100	20
Isoxathion	18854-01-8	314.1/105	135	12
Malathion	121-75-5	331/126.9	80	5
Methabenzthiazuron	18691-97-9	222.1/165.1	90	12
Methomyl	16752-77-5	163.1/106	50	4
Neburon	555-37-3	275.07/57.1	100	20
Omethoate	1113-02-6	214/109	80	24
Pyraflufen-ethyl	129630-19-9	413/339	120	25
Quinalphos	13593-03-8	299/163	90	20
Rotenone	83-79-4	395/213.1	145	20
Spinetoram	187166-40-1	748.5/142.2	206	32
Spiromesifen	283594-90-1	388/273	110	10
Thiacloprid	111988-49-9	253/126	100	16
Thiophanate-methyl	23564-05-8	343/151	90	20
Triazophos	24017-47-8	314.1/162.1	110	16
Trichlorfon	52-68-6	256.9/109	80	12

Table 3. GC-MS/MS Analysis Conditions

column:	SLB®-5ms, 20 m × 0.18 mm I.D., 0.18 µm (**28564-U**)
oven:	50 °C (2 min), 15 °C/min to 320 °C (5 min)
inj. temp.:	250 °C
carrier gas:	helium, 1.2 mL/min constant flow
detector:	MSD, scan and MRM (see Table 4)
MSD interface:	325 °C
injection:	1 µL, splitless (0.75 min)
liner:	4 mm I.D. FocusLiner™ with taper

Table 4. MRMs Used for Quantitation; GC-MS/MS

Compound	CAS #	MRM	CE
Chlorpyrifos-methyl	5598-13-0	286/93	20
Tolclofos-methyl	57018-04-9	265/250	15
Fenthion	55-38-9	278/169	15
MGK-264	18691-97-9	164/98	10
Endosulfan sulfate	1031-07-8	274/239	15
Etoxazole	153233-91-1	141/63	30

Results and Discussion

Background

Initially, cleanup using Z-Sep+ sorbent was compared to PSA/C18, a common QuEChERS cleanup sorbent for fat-rich samples. A visual comparison of the QuEChERS extracts (in acetonitrile) is shown in **Figure 2**. Both cleanups removed some green color, resulting in similar light yellow extracts. GC-MS-scan comparisons (**Figure 3**) show lower background after Z-Sep+ cleanup compared to PSA/C18. The predominant peaks present in the uncleaned extract are fatty acids and monoglycerides. While PSA/C18 only reduced the levels of these compounds, almost none were detected after Z-Sep+ cleanup.

Pesticide Recovery

Table 5 shows the average %Recovery and %RSD for n=3 replicates of spiked pistachio samples. The majority of the pesticides were analyzed by LC-MS/MS; and those without sufficient response were analyzed by GC-MS/MS. Out of the 30 pesticides analyzed, 22 had recoveries within the generally accepted range of 70-120 %. Reproducibility was good, with RSD values < 20% for all 30 pesticides, and < 10% for many. Two pesticides, etoxazole and trichlorfon, had recoveries < 50%. Trichlorfon was most likely retained by the Z-Sep+ sorbent during the cleanup step. This could be due to the Lewis base character of the phosphate group present in its structure. Etoxazole, on the other hand, does not contain a phosphate group. It is a very lipophilic pesticide, indicated by its log P value of 5.6. Extraction efficiency of this compound from the fatty pistachio matrix was probably very poor using acetonitrile. Spinetoram, with a log P of 6.3, also showed lower recovery (56%) than a majority of the pesticides studied. This trend of decreased recovery for high log P pesticides has been observed by others for high fat matrices.[6] Recovery of both of these compounds may be increased by addition of a less polar solvent such as ethyl acetate for the extraction; however, an increase in the level of co-extracted background can be expected.

Figure 2. Comparison of Pistachio Extracts; Before and After Cleanup.

No cleanup PSA/C18 Z-Sep +

Figure 3. GC-MS-Scan Comparison of Pistachio Extracts With (a) No Cleanup, (b) PSA/C18 Cleanup, and (c) Z-Sep+ Cleanup; All the Same Y-scale

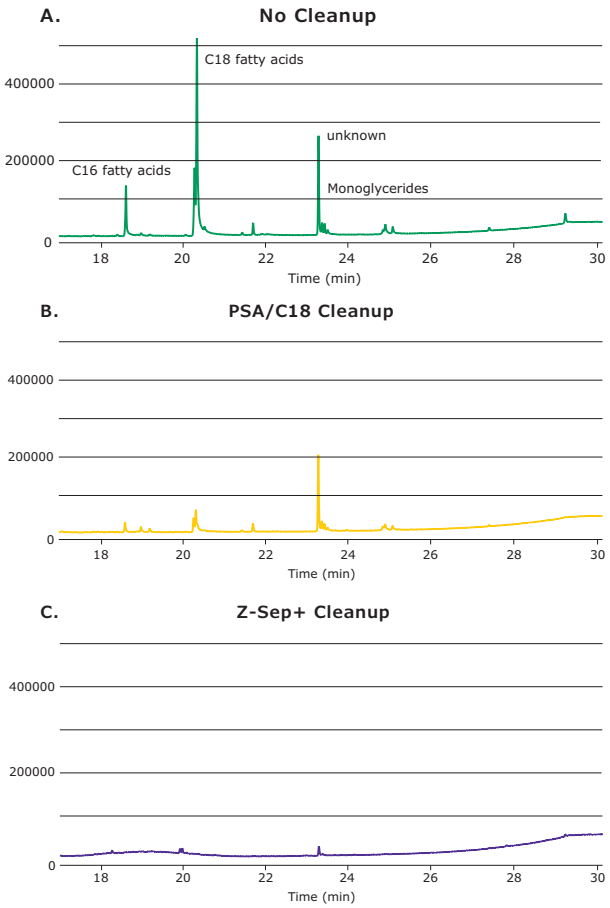

Table 5. Pesticide Recoveries from Pistachios Using Z-Sep+ Cleanup, Spike Level of 10 ng/g

Pesticide	Avg. % Recovery (n=3)	% RSD	Analysis
Aldicarb	102%	3%	LC-MS/MS
Aldicarb-sulfone	108%	1%	LC-MS/MS
Bifenazate	88%	4%	LC-MS/MS
Butocarboximsulfoxide	83%	5%	LC-MS/MS
Carbendazim	71%	4%	LC-MS/MS
Carbofuran	104%	4%	LC-MS/MS
Chlorantraniliprole	90%	5%	LC-MS/MS
Chlorpyrifos-methyl	66%	10%	GC-MS/MS
Endosulfan sulfate	58%	6%	GC-MS/MS
Etoxazole	45%	9%	GC-MS/MS
Etrimfos	90%	7%	LC-MS/MS
Fenthion	72%	9%	GC-MS/MS
Flufenoxuron	62%	15%	LC-MS/MS
Isoxathion	92%	3%	LC-MS/MS
Malathion	102%	4%	LC-MS/MS
Methabenzthiazuron	84%	3%	LC-MS/MS
Methomyl	106%	5%	LC-MS/MS
MGK-264 (avg. 2 isomers)	57%	17%	GC-MS/MS
Neburon	92%	7%	LC-MS/MS
Omethoate	66%	2%	LC-MS/MS
Pyraflufen-ethyl	97%	18%	LC-MS/MS
Quinalphos	104%	7%	LC-MS/MS
Rotenone	100%	3%	LC-MS/MS
Spinetoram	56%	10%	LC-MS/MS
Spiromesifen	83%	4%	LC-MS/MS
Thiacloprid	100%	2%	LC-MS/MS
Thiophanate-methyl	100%	3%	LC-MS/MS
Tolclofos-methyl	71%	10%	GC-MS/MS
Triazophos (avg. 2 isomers)	89%	3%	LC-MS/MS
Trichlorfon	14%	13%	LC-MS/MS

Conclusions

Pistachios, which contain 45% fat, present a challenging matrix when doing pesticide residue analysis. If using QuEChERS extraction, some fat will be co-extracted with the analytes of interest. Thus, the cleanup step must be able to reduce this background. In this application, the use of Supel™ QuE Z-Sep+ was demonstrated for the effective cleanup of these

extracts prior to LC-MS/MS and GC-MS/MS analysis. Fatty acid and monoglyceride background were significantly reduced using Z-Sep+, and compared to PSA/C18 cleanup, the resulting extract had lower background; as evidenced by GC-MS-scan data. Pesticide recovery was within the acceptable range of 70-120% for 22 out of 30 targeted pesticides, with excellent reproducibility demonstrated for spiked replicates.

References

1. Organic Facts. Health Benefits of Pistachios. www.organicfacts.net/health-benefits/seed-and-nut/health-benefits-of-pistachio.html (accessed June 2016)
2. Index to Pesticide Chemical Names, Part 180 Tolerance Information, and Food and Feed Commodities (by Commodity); US Environmental Protection Agency Office of Pesticide Programs, U.S. Government Publishing Office: Washington, DC, 2012.
3. Emami, A.; Rastegar, H.; Amirahmadi, M.; Shoeibi, S.; Mousavi, Z. Multi-Residue analysis of pesticides in pistachio using gas chromatography-mass sSpectrometry (GC/MS). Iranian J. of Tox. 2015, 8 (27), 1174-1181.
4. California Pistachio Research Board. Pistachio Pesticides (as of May 2012). calpistachioresarch.org, (accessed May 2016)
5. PAN Pesticide Database. Pesticide use in California, Pesticide Use on Pistachios 2012. www.pesticideinfo.org (accessed May 2016)
6. Rajski, L.; Lozano, A.; Uclés, A.; Ferrer, C.; Fernández-Alba, A.R. Determination of pesticide residues in high oil vegetal commodities by using various multi-residue methods and clean-ups followed by liquid chromatography tandem mass spectrometry. J. Chrom. A, 2013, 1304, 109-120.

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Featured Products

Description	Cat. No.
Supel™ QuE QuEChERS Products	
Empty Centrifuge Tube, 50 mL, pk of 50	**55248-U**
Supel™ QuE Citrate Extraction Tube, 12 mL, pk of 50	**55227-U**
Supel™ QuE Z-Sep+, 2 mL, pk of 100	**55414-U**
Supel™ QuE Z-Sep+, 15 mL, pk of 50	**55486-U**
Capillary GC Column	
SLB®-5ms, 20 m × 0.18 mm I.D., 0.18 µm	**28564-U**
HPLC Column	
Ascentis® Express RP-Amide, 10 cm × 2.1 mm I.D., 2 µm	**53913-U**
Accessories	
QuEChERS Shaker and Rack Starter Kit, USA compatible plug	**55278-U**
QuEChERS Shaker and Rack Starter Kit, EU Schuko plug	**55438-U**

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Related Products

Description	Cat. No.
Accessories	
Inlet Liner, Split/Splitless Type	
Single Taper FocusLiner™ Design (wool packed)	**2879901-U**
Molded Thermogreen® LB-2 Septa, solid discs, pk of 50	**28676-U**
Thermo-O-Ring™ Inlet Liner O-Ring, pk of 10	**21003-U**
Gold-Plated Inlet Seal (Straight Design), pk of 2	**23318-U**
Capillary Column Nut for Agilent®MS, pk of 5	**28034-U**
Vials	
Certified Vial Kit, Low Adsorption (LA), 2 mL, amber, w/slit caps pk of 100	**29654-U**
Certified Vial Kit, Low Adsorption (LA), 2 mL, clear with marking spot, PTFE-silicone septa w/slit, pk of 100	**29652-U**

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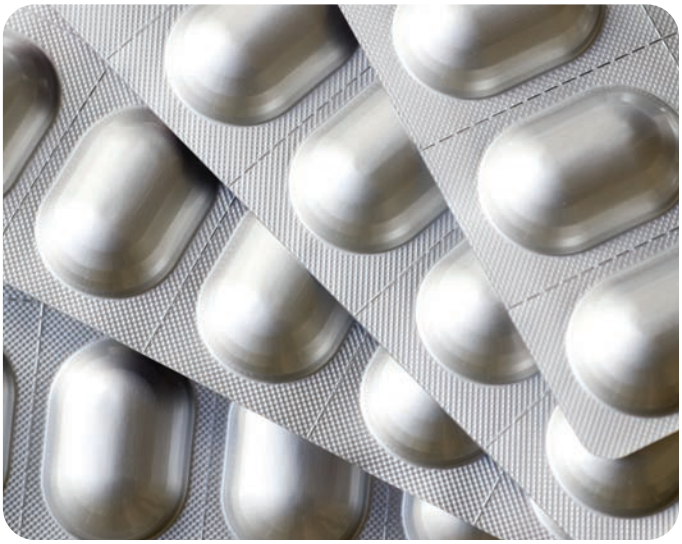
Reference Materials for Extractables and Leachables Testing

New certified reference materials solution mixes

Matthias Nold, Product Manager Reference Materials, Analytix@milliporesigma.com



Extractables and leachables (E&L) are chemical compounds with the potential to migrate into pharmaceutical or clinical products from packaging materials, tubing, or medical devices. This can lead to patient exposure to these compounds.

Extensive E&L studies to identify compounds that might leach into the product are obligatory for pharmaceutical products and medical devices.

Since it is never entirely predictable which chemicals could migrate, it is crucial that no potential extractables and leachables are overlooked in the analysis. Depending on the nature of the packaging material, the product, and the applied conditions, new unexpected or unknown compounds may be found. There is, therefore, no finite list of analytes for which products should be tested. However, there are certain monomers or additives that are more commonly detected in studies examining extractables and leachables.

In Issue 6 of Analytix Reporter we presented a new series of neat reference materials for some of the most frequently found extractables and leachables. To facilitate your identification and quantification of these extractables and leachables, we developed two certified calibration mixes for extractables and leachables. One mix is designed for LC (21 components) and another one for GC detection (14 components). These two products are Certified Reference Materials (CRM) produced under ISO/IEC 17025 and ISO 17034 double accreditation:

- Certification of each individual component by qNMR (following ISO/IEC 17025 accreditation)
- Mixes produced following the ISO 17034 workflow
- Tested for homogeneity and long-term stability using GC-MS
- Traceability to NIST SRMs
- Supplied with a comprehensive certificate including the overall uncertainty

The components were chosen to reflect a broad spectrum of typical extractables and leachables compound classes, taking into account the toxicity and also how frequently they are typically found in E&L tests.

LC Mix - 21 Components

Catalog Number:	**95636**
Product Name:	Extractables and Leachables Screening Standard for LC
Type:	Certified Reference Material, TraceCERT®
Concentrations:	50 µg/mL per component in acetonitrile
Package Size:	1 mL

Compound	CAS
Irganox 1010 (Ir 1010)	**6683-19-8**
Irganox 1076 (Ir1076)	**2082-79-3**
Dometrizol (Dome) / Tinuvin P/2-(2H-Benzotriazol-2-yl)-p-cresol	**2440-22-4**
ε-Caprolactam (CAP)	**105-60-2**
Dibenzylamine (DBA)	**103-49-1**
Benzoic acid (BA)	**65-85-0**
2-Mercaptobenzothiazole (2-MBT)	**149-30-4**
Bisphenol A (BPA)	**80-05-7**
2-Ethylhexanoic acid (EHA)	**149-57-5**
Bis(4-chlorophenyl)sulfone (CPS)	**80-07-9**
2,6-Di-tert-butyl-4-hydroxymethyl-phenol (DBOHP)	**88-26-6**
Butylhydroxytoluene (BHT)	**128-37-0**
1,3-Di-tert-butyl-benzene (DBB)	**1014-60-4**
Oleamide (Ole)	**301-02-0**
Bis(2-ethylhexyl) phthalate (DEHP)	**117-81-7**
Stearic acid (SA)	**57-11-4**
Erucamide (Eruca)	**112-84-5**
Irganox 3114 (Ir3114)	**27676-62-6**
Irgafos 168-oxide	**95906-11-9**
2,4-di-tert-Butylphenol	**96-76-4**
Palmitic acid	**57-10-3**

GC Mix - 14 Components

Catalog Number:	01829
Product Name:	Extractables and Leachables Screening Standard for GC
Type:	TraceCERT® Certified Reference Material
Concentrations:	50 µg/mL per component in TBME
Package Size:	1 mL

Compound	CAS
Irganox 1076 (Ir1076)	**2082-79-3**
ε-Caprolactam (CAP)	**105-60-2**
2-Mercaptobenzothiazole (2-MBT)	**149-30-4**
Bisphenol A (BPA)	**80-05-7**
Butylhydroxytoluene (BHT)	**128-37-0**
1,3-Di-tert-butyl-benzene (DBB)	**1014-60-4**
Oleamide (Ole)	**301-02-0**
Bis(2-ethylhexyl) phthalate (DEHP)	**117-81-7**
Stearic acid (SA)	**57-11-4**
Erucamide (Eruca)	**112-84-5**
Irgafos 168-oxide	**95906-11-9**
2,4-di-tert-Butylphenol	**96-76-4**
2,6-di-tert-Butylphenol	**128-39-2**
Palmitic acid	**57-10-3**

Featured Products

Description	Cat.No.
Extractables and Leachables Screening Standard for LC, Certified Reference Material *Trace*CERT®, 1 mL	**95636**
Extractables and Leachables Screening Standard for GC, Certified Reference Material *Trace*CERT®, 1 mL	**01829**

In addition to certified reference materials (CRM) mixes for LC and GC we have also developed individual compounds found in extractables and leachables studies. Check our website regularly for the most recent product additions.

SigmaAldrich.com/extractablesandleachables

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PHARMA & BIOPHARMA

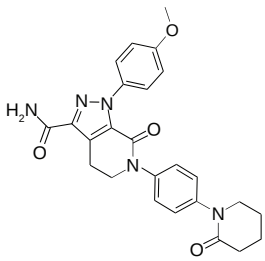
Assay and Organic Impurity Profiling of Apixaban Using an Ascentis® Express C18 Column and UV Detection

Sonal Shinde, Application Scientist, Analytix@milliporesigma.com

Introduction

Apixaban is a selective, orally bioavailable, and reversible inhibitor of free and clot-bound factor Xa (which catalyzes the conversion of prothrombin to thrombin). Apixaban is sold under the trade name Eliquis®, and is typically prescribed to lower the risk of stroke and embolism in people with nonvalvular atrial fibrillation. It is also used to prevent deep vein thrombosis (DVT) which may lead to pulmonary embolism (PE) in knee or hip replacement surgery patients. Apixaban was developed in a joint venture by Pfizer and Bristol-Myers Squibb, and it was approved in 2012 in Europe, and in 2014 in the U.S.

At present, there are no pharmacopoeial monographs available with either an assay and/or organic impurity profiling method for Apixaban. We have therefore developed and validated a new HPLC method for said purposes using a column with C18 Fused-Core® particles and UV detection at 235 nm. The limit of detection (LOD) is 0.33 ppm and the limit of quantitation (LOQ) is 1.0 ppm at 235 nm for Apixaban using HPLC-UV detection.



Apixaban

Experimental Conditions			
column:	Ascentis® Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (**53829-U**)		
buffer:	dissolve 2.04 g of potassium dihydrogen phosphate in 1500 mL Milli-Q® water (10mM). Adjust to pH 5.0 with triethylamine.		
mobile phase:	[A] buffer:methanol (90:10) (v/v) [B] buffer:acetonitrile:methanol (20:20:60) (v/v/v)		
gradient:	**Time (min)**	**A(%)**	**B(%)**
	0	65	35
	18	55	45
	20	55	45
	22	50	50
	25	40	60
	30	30	70
	35	30	70
	40	65	35
	50	65	35
flow rate:	1 mL/min		
pressure:	132-147 bar (1914-2131 psi)		
column temp.:	40 °C		
detector:	UV @ 235 nm (analytical flow cell; 10 µL)		
injection:	3 µL		
diluent:	mix water and acetonitrile 50:50 (v/v)		
stock standard solution:	0.5 mg/mL of Apixaban standard in diluent.		
standard solution:	dilute 5 mL of stock standard solution to 100 mL to obtain 25 ppm standard solution. (**Figure 1**)		
SST solution:	weigh 2.5 mg of each impurity in 20 mL volumetric flask. Dilute to volume with diluent. Add 1 mL of this solution into a 50 mL volumetric flask and dilute to volume with standard stock solution. (**Figure 2**)		
sample solution:	0.5 mg/mL of Apixaban sample in diluent (**Figure 3**)		

Figure 1. Chromatographic Data - Blank & Standard Solution

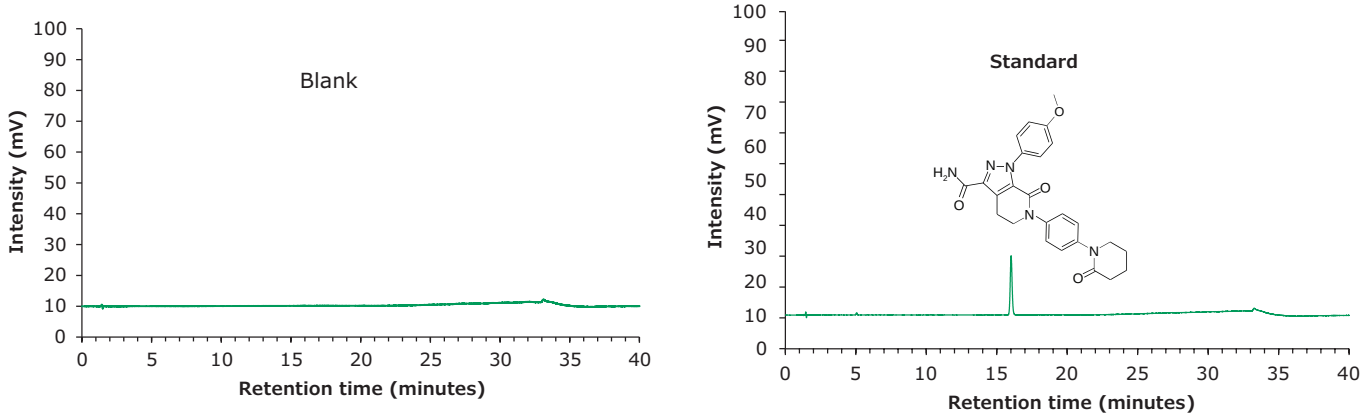
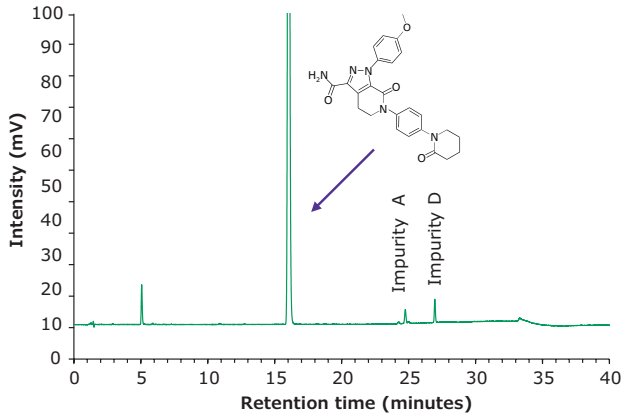
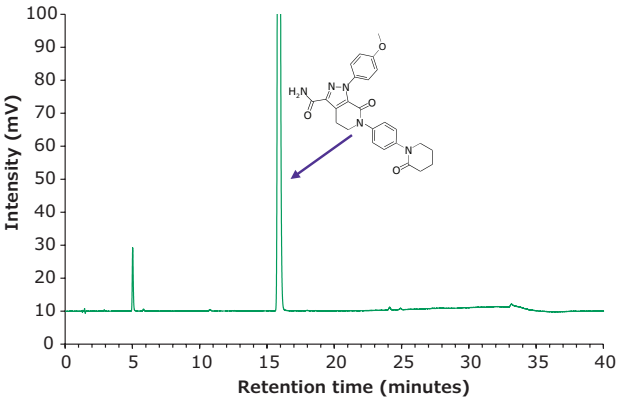


Figure 2. Chromatographic Data - System Suitability Test (SST) Solution



Peak	Compound	Retention Time (min)	RRT	Resolution	Tailing Factor
1	Apixaban	16.0	1.0	-	1.2
2	Impurity A	24.8	1.5	40.3	1.0
3	Impurity D	27.0	1.7	13.6	1.1

Figure 3. Chromatographic Data - Sample Solution



Peak	Compound	Retention Time (min)	Resolution	Tailing Factor
1	Apixaban	16.0	50327	1.2

Validation and Verification

1. Specificity

Inject standard solution and determine the retention time of desired analyte in the presence of other components like impurities and excipient.

	Compound	RT (min)
1	Apixaban	16.0
2	Impurity A	24.8
3	Impurity D	27.0

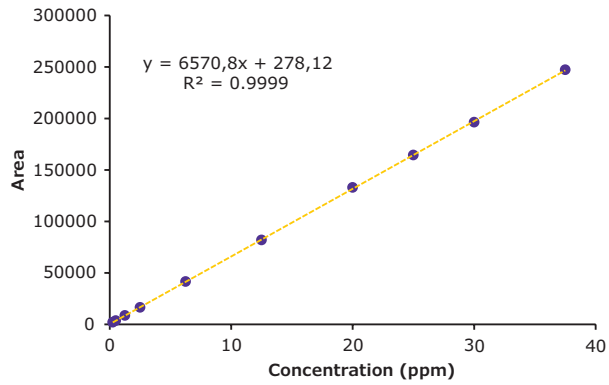
2. Standard Repeatability (25 ppm)

Sample	Apixaban (Area)
STD 1	165029
STD 2	165045
STD 3	165194
STD 4	165159
STD 5	165032
Mean	165091.8
Standard Deviation	78.5
RSD (%)	0.1

3. Linearity, LOD & LOQ

Concentration (µg/mL)	Apixaban (Area)
0.25	2015
0.5	3527
1.25	8605
2.5	16392
6.25	41517
12.5	81937
20	132946
25	164362
30	196291
37.5	247176
LOQ (S/N ratio 10)	1.0 ppm
LOD (S/N ratio 3)	0.33 ppm

Figure 4. Apixaban Calibration Curve Data (0-37.5 ppm)



Featured Products

Description	Cat. No.
Ascentis® Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles	**53829-U**
Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur.	**1.00030**
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur.	**1.06007**
Potassium dihydrogen phosphate for analysis EMSURE® ISO	**1.04873**
Triethylamine for HPLC, LiChropur™, ≥99.5% (GC)	**81101**

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Qualification of Sunscreens Using Certified Reference Materials

Nick Hauser, Product Manager Reference Materials, Analytix@milliporesigma.com

Absorbing the sun's rays helps the body create an important nutrient, Vitamin D, for immune system function and proper bone density. Exposing oneself to sunlight can have positive effects - setting the body's internal clock and thus affecting sleep cycles and emotional well-being. In some cases, however, too much exposure to sunlight can be harmful. The ultraviolet (UV) radiation that the skin is exposed to while soaking in the sun's rays may lead to skin damage. Sunburn may be an immediate result of exposure to UV radiation, while over time, there is even the potential for developing skin cancer. Worldwide, nearly three million new diagnoses of skin cancer are observed every year.

UV radiation from the sun that reaches the earth is composed of two types of light, ultraviolet A (UVA) and ultraviolet B (UVB). Both UVA and UVB rays can cause cancer; however, roughly 95% of the sun's rays are made up of UVA rays. UVA rays are considered to be long wave light and play a major part in skin aging and wrinkling. Even though UVB rays are much less prominent, they are potentially more harmful in the short-term as they are often identified as the cause of sunburns.

One way that people help protect themselves from the harmful effects of the sun is through the use of sunscreens. The use of sunscreen can help to prevent skin from aging and wrinkling as well as prevent sunburns. The risk of skin cancer can also be decreased with the use of sunscreens.

Sunscreens are widely available in various types and forms such as lotions, gels, sprays, and creams. Many of the active ingredients found in sunscreen will absorb the UV radiation from the sun, thereby protecting the skin. These are typically organic compounds and can include several different individual compounds which may specifically target the absorption of UVA or UVB rays depending on their structure. Several other inactive compounds to comprise the remaining portion of the formulation. These inactive compounds will vary depending on the specific form of the sunscreen itself and can include preservatives and excipients.

Due to the inherent absorption of sunscreens directly into a person's skin, several regulatory bodies across the world, such as the U.S. Food and Drug Administration, regulate sunscreen products as over-the-counter drugs. Manufacturers of sunscreens are required to provide information regarding the safety of the ingredients that are included in their particular formulation. The use of Reference Materials is imperative to prove the quality and safety of those ingredients.

Supelco® Certified Reference Materials (CRMs) are available for several ingredients found in sunscreens as listed below. These CRMs are Secondary Pharmaceutical Reference Standards and are traceable to and qualified against the corresponding pharmacopoeia primary standards, eliminating the time and effort involved with preparing and validating in-house working standards. A comprehensive Certificate of Analysis offers details of the methodology and results of the characterization. For more information on sunscreen related CRMs, or to view a complete product listing of our pharmaceutical secondary standards, please visit our website at **SigmaAldrich.com/secondarystandards**

Description	UV Absorbent	Preservative	Excipient	Pack Size	Cat.No.
Avobenzone	x			1 g	**PHR1073**
Ensulizole	x			1 g	**PHR1301**
Homosalate	x			1 g	**PHR1085**
Octinoxate	x			1 g	**PHR1080**
Octisalate	x			1 g	**PHR1081**
Octocrylene	x			1 g	**PHR1083**
Oxybenzone	x			1 g	**PHR1074**
Padimate O	x			10 g	**PHR1355**
Butylparaben		x		1 g	**PHR1022**
Ethylparaben		x		1 g	**PHR1011**
Methylparaben		x		1 g	**PHR1012**
Propylparaben		x		1 g	**PHR1010**
Cetyl Alcohol			x	1 g	**PHR1133**
D4 Cyclomethicone			x	500 mg	**PHR1565**
D5 Cyclomethicone			x	500 mg	**PHR1566**
Dibutyl Phthalate			x	1 g	**PHR1366**
Diethyl Phthalate			x	1 g	**PHR1476**
Disodium EDTA			x	1 g	**PHR1068**
DMDM Hydantoin			x	1 mL	**PHR1358**
Phenoxyethanol			x	1.5 g	**PHR1121**
Propylene Glycol			x	1.5 g	**PHR1051**
Retinyl Palmitate			x	1 g	**PHR1235**
Sodium Lauryl Sulfate			x	2 g	**PHR1949**
Tocopheryl Acetate			x	500 mg	**PHR1030**

For our complete offering of reference materials, visit **SigmaAldrich.com/standards**

An overview of our offering for the Cosmetic & Personal Care R&D Workflow can be found at **SigmaAldrich.com/cosmetics**

Authenticity of Essential Oils: A HPTLC Fingerprint Method

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Melanie Broszat, Scientific Business Development Manager (CAMAG)
Monika Bäumle Product Manager TLC, Matthias Nold Product Manager Reference Materials, Analytix@milliporesigma.com

Due to the increasing popularity of natural essential oils as products for personal care, pharmaceuticals, or for aromatherapy, the global market for essential oils is growing rapidly.[1] Unfortunately, this increases the likelihood of adulteration by the addition of cheaper oils or oil constituents to maximize profits. Since there are so many possible ways adulteration can occur, it is a challenge to analyze these products. An efficient method for the detection of such adulteration is the use of chromatographic fingerprinting methods, in particular, gas chromatography or as shown in this article, High-Performance Thin-Layer Chromatography (HPTLC).

To help analytical labs test for essential oil adulteration, we offer reference materials from HWI pharma services for twenty-five commonly used essential oils. **Table 1** shows the ten most recent additions that were subjects of this study. These products are secondary reference materials, traceable to HWI primary reference materials quantified by qNMR (quantitative NMR) or mass balance. Similar to the vanilla extract reference materials shown in the article on page 9, for each product, a quantitative value is provided for at least one of the major components, while several other key components are qualitatively verified by GC-FID. You will find the complete range on our webpage at SigmaAldrich.com/essentialoils.

As mentioned, HPTLC is a fast and efficient fingerprinting method and is therefore the perfect alternative or complement to GC for authenticity testing of essential oils. Fingerprints for ten essential oil secondary reference standards from HWI were created using the standard HPTLC method for identification of essential oils (submitted to Ph. Eur. for evaluation) by comparison of R_F values and colors of reference substances and matching zones in the oils. On the next page the fingerprints for cinnamon, lemon and pine needle oil are shown as examples (**Figures 1-3**). The results for all 10 essential oils can be seen in the on-line version of this article at **SigmaAldrich.com/analytix**, issue 7.

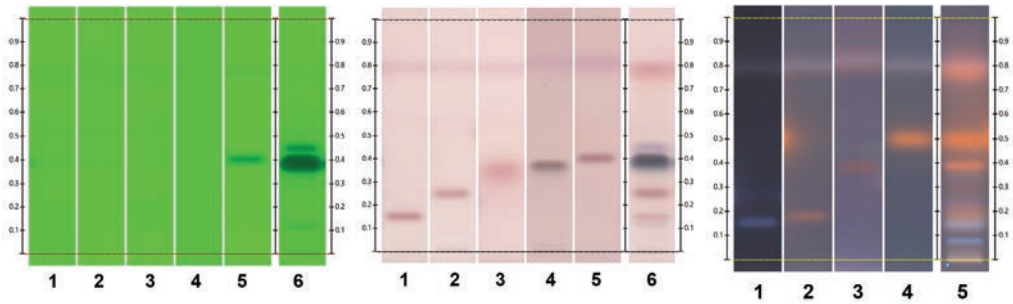
While not all potential components of essential oils are detectable with this method (e.g., camphene, menthofurane, α-pinene, β-pinene or γ-terpinene), the variety of detection methods (UV and chemical derivatization) increases the chance to detect adulteration.

Chromatographic Conditions

instrumentation	automatic TLC Sampler (ATS 4), Automatic Developing Chamber (ADC 2), Derivatizer, TLC Plate Heater 3, TLC Visualizer 2, *visionCATS* 2.5
samples (see Table 1)	essential oils are prepared in toluene as follows: • Cinnamon oil at 30 µL/mL • Tea tree oil and Ylang-Ylang at 15 µL/mL • Bergamot oil, mint oil, patchouli oil, peppermint oil, and sage oil at 20 µL/mL • Lemon oil at 80 µL/mL • Dwarf pine oil, pine oil, and spruce needle oil at 100 µL/mL • Frankincense oil at 200 µL/mL

Standards used for this Study (see Table 2)	
Solutions were prepared in toluene at the following conc.:	
Bornyl acetate, eugenol and geranyl acetate	0.50 µL/mL
Linalool and terpinen-4-ol	1.00 µL/mL
Bornyl acetate	1.50 µL/mL
Linalyl acetate and terpinen-4-ol	2.00 µL/mL
Bornyl acetate	3.00 µL/mL
1.8-Cineole	10.00 µL/mL
Menthone	20.00 µL/mL
(R)-(+)-limonene	120.00 µL/mL
(+)-Borneol	0.25 mg/mL
(-)-Carvone	0.35 mg/mL
(+)-Borneol	0.50 mg/mL
Borneol, isoeugenyl acetate, and menthol	1.00 mg/mL
Menthol	1.50 mg/mL
Menthol	3.00 mg/mL
Patchoulol	4.00 mg/mL
(+)-(α)-Terpineol in methanol	0.50 mg/mL
(+)-(α)-Terpineol in methanol	1.00 mg/mL

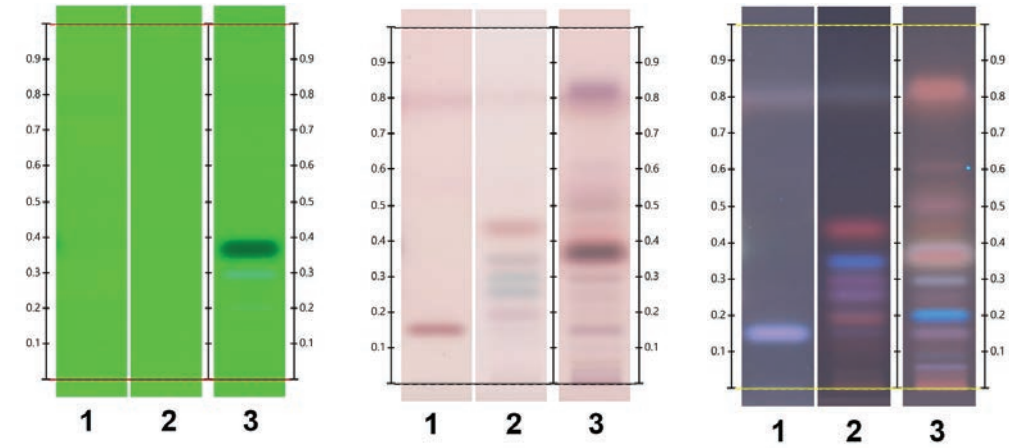
Figure 1 Individual Report for Cinnamon Oil (Cat. No. 06031501, batch HWI01937)



From left to right: HPTLC chromatograms under UV 254 prior to derivatization, under white light and UV 366 nm after derivatization with anisaldehyde reagent.

Track 1: (+)-α-terpineol, 1 mg/mL (R_F 0.15) (cat. no. 83073, batch: BCBS7535V); **2:** linalool 1 µL/mL (R_F 0.25) (cat. no. 00350190, batch: HWI01135-1); **3:** 1.8-cineole, 10 µL/mL (R_F 0.35) (cat. no. 00020590, batch: HWI00654-2); **4:** eugenol, 0.5µL/mL (R_F 0.37) (cat. no. 01050595, batch: HWI00301-1), **5:** isoeugenyl acetate, 1 mg/mL (R_F 0.40) (cat. no. 0.7055, batch: BCBV6080); **6:** cinnamon oil, 30 µL/mL (cat. no. 06031501, batch: HWI01937)

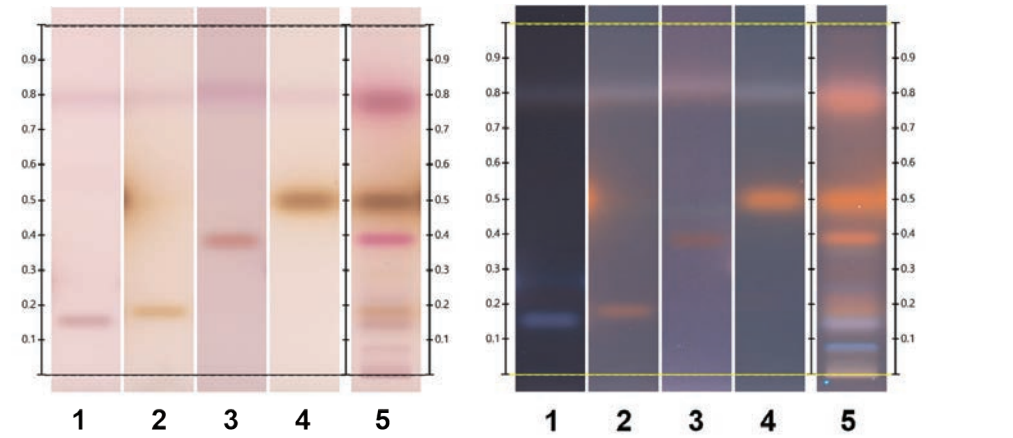
Figure 2. Individual Report for Lemon Oil (Cat. no. 05981501, batch HWI01936)



From left to right: HPTLC chromatograms under UV 254 nm prior to derivatization, under white light and UV 366 nm after derivatization.

Track 1: (+)-α-terpineol, 0.5 mg/mL (R_F 0.15) (cat. no. 83073, batch: BCBS7535V); **2:** (R)-(+)-limonene, 120 µL/mL (main zone at R_F 0.44; zones below due to impurities) (cat. no. 00590590, batch: HWI01323-2); **3:** lemon oil, 80 µL/mL (cat. no. 05981501, batch: HWI01936)

Figure 3. Individual Report for Pine Needle Oil (Cat. No. 05991501, batch HWI01932)



From left to right: HPTLC chromatograms under white light and UV 366 nm after derivatization.

Track 1: (+)-α-terpineol, 0.5 mg/mL (R_F 0.15) (cat. no. 83073 batch: BCBS7535V); **2:** (+)-borneol, 0.5 mg/mL (R_F 0.18) (cat. no. 68878, batch: 14627); **3:** (-)-carvone, 0.35 mg/mL (R_F 0.38) (cat. no. 00290595, batch: HWI00236-1); **4:** bornyl acetate, 1.5 µL/mL (R_F 0.50) (cat. no. 00400585, batch: HWI01713); **5:** pine oil, 100 µL/mL (cat. no. 05991501, batch: HWI01932)

See the Chromatograms of all 10 essential oils in the online version of this article at **SigmaAldrich.com/Analytix**, Issue 7

Chromatography according to standard HPTLC method for identification of essential oils (submitted to Ph. Eur. for evaluation)	
stationary phase:	HPTLC Silica gel 60 F_{254}, 20 x 10 cm (**1.05642**)
sample application:	bandwise application with ATS 4, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2 µL for test solutions and standards solutions
developing solvents:	ethyl acetate – toluene (5:95; v/v)
development:	in the ADC 2 with chamber saturation (with filter paper) for 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride $MgCl_2$
developing distance:	70 mm (from the lower edge)
plate drying:	drying 5 min in the ADC 2
documentation:	with the TLC Visualizer 2 under white light and under UV 366 nm after derivatization
derivatization:	the plate is sprayed with the Anisaldehyde reagent using the Derivatizer (nozzle: blue, spraying level: 1-3*; spraying volume: 3 mL) and heated at 100 °C for 3 min
	anisaldehyde reagent: mixture of 0.5 mL of p-anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of sulfuric acid

*based on the nozzle series

Table 1. Essential Oil Reference Materals Recently Added

Description	Package Size	Cat. No.
Bergamot oil	1 mL	**05941501**
Cinnamon oil	1 mL	**06031501**
Dwarf pine oil	1 mL	**06001501**
Frankincense oil	1 mL	**05971501**
Lemon oil	1 mL	**05981501**
Mint oil	1 mL	**06011501**
Pine needle oil	1 mL	**05991501**
Sage oil	1 mL	**06021501**
Spruce needle oil	1 mL	**05961501**
Ylang-ylang oil	1 mL	**05951501**

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Table 2. Reference Standards

Description	Package Size	Cat. No.
(+)-Borneol	10 mg	**68878**
Bornyl acetate*	50 mg	**00400585**
(-)-Carvone*	100 mg	**00290595**
1.8-Cineole*	100 mg	**00020590**
Eugenol*	100 mg	**01050595**
Isoeugenyl acetate	100 mg	**07055**
(R)-(+)-Limonene*	100 mg	**00590590**
Linalool*	100 mg	**00350190**
Linalyl acetate	100 mg	**49599**
(-)-Menthol*	100 mg	**00580590**
(-)-Menthone*	100 mg	**04660585**
Patchoulol*	10 mg	**05690595**
Terpinen-4-ol*	50 mg	**03900590**
(+)-α-Terpineol	5 mL	**83073**

* HWI primary reference material

Reference:

1. Boren KE, Young DG, Woolley CL, Smith BL, Carlson RE, J Environ Anal Chem 2015, 2:2

Featured Products

Description	Cat. No.
HPTLC Silica gel 60 F_{254}, 20 x 10 cm, 50 ea.	**1.05642**
Reference Materials see **Tables 1 & 2**	

Related Products

Description	Cat. No.
Solvents & Reagents	
Acetic acid, for luminescence, BioUltra, ≥99.5% (GC)	**45726**
p-Anisaldehyde, 98%	**A88107**
Ethyl acetate, for HPLC, ≥99.7%	**34858**
Magnesium chloride hexahydrate for analysis EMSURE® ACS,ISO,Reag. Ph. Eur.	**1.05833**
Methanol, for liquid chromatography LiChrosolv®	**1.06018**
Toluene, for liquid chromatography LiChrosolv®	**1.08327**
Sulfuric acid, puriss., meets analytical specification of Ph. Eur.	**07208-M**

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ENVIRONMENTAL

Screening for Estrogen Active Nonylphenols in Water by Planar Solid Phase Extraction – Planar Yeast Estrogen Screen

Dinah Schick, Claudia Oellig, Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany, claudia.oellig@uni-hohenheim.de

Introduction

Nonylphenols (NP) are ubiquitous substances that have been detected in highly diverse foodstuff and that *inter alia* show estrogenic activity.[1,2] NP can presumably end up in the aquatic environment as a result of breakdown of nonylphenol ethoxylates (NPE), which are used as non-ionic surfactants.[3] According to the water framework directive regarding water quality of the European Union, NP are classified as priority hazardous substances, and thus "*present a significant risk to or via the aquatic environment*".[4–7] Different isomers of NP are collectively classified as one group, and the maximum acceptable concentration in inland and other surface waters is stated at 2 µg/L.

Because of the high risk emanating from NP, and due to existing regulations, the determination of their total concentration is very important. For this purpose, **planar solid phase extraction (pSPE)**[8] is perfectly suited; and the estrogenic properties of NP can be used for their selective detection by **planar yeast estrogen screen (pYES)**[9]. Thus, the combined approach pSPE–pYES was developed.[10]

The determination of NP as a group is realized by pSPE - a method based on high-performance thin-layer chromatography (HPTLC) - that enables the analysis of matrix-rich extracts on planar thin-layers by separating matrix compounds, while simultaneously focusing the analytes of interest in a common target zone. The highly selective detection and quantitation of NP is eventually performed by means of pYES. As a screening tool for estrogen active compounds (EAC), pYES - also based on HPTLC - uses genetically modified yeast cells and water-wettable reversed phase HPTLC (RP-18 W) plates. The human estrogen receptor and a reporter gene encoding for β-galactosidase, respectively, are integrated in the yeast cells, leading to the production of the enzyme in the presence of estrogenic substances.[11,12] The enzyme produced subsequently cleaves the suitable substrate resorufin-β-D-galactopyranoside, releasing orange fluorescing resorufin as a positive signal of estrogenicity.

pSPE–pYES

The combination of pSPE and pYES on HPTLC RP-18 W plates was designed for the detection of EAC as a group, and was successfully applied to screen for estrogen active NP in surface waters. Prior to the analysis of environmental samples, the response of the pSPE–pYES for the detection of different commercially available NP mixtures was investigated. Additionally, its suitability for the analysis of NP aside from other possible EAC was proven.

By pSPE–pYES, NP were successfully separated from other EAC and simultaneously focused in a common zone. An example is indicated for the synthetic hormone EE2 and the natural hormone E2 (see **Figure 2**). The experiments revealed similar responses of different purchased NP, except for NP3 that has a linear nonyl side chain. The NP with the highest response, NP4, that has a branched side chain, was used as the representative reference substance for future experiments.

Figure 1. Workflow of Planar Solid Phase Extraction–Planar Yeast Estrogen Screen (pSPE–pYES). After Application and pSPE, pYES was Performed on the Same Plate by Means of Yeast and Substrate Incubations and Subsequent Detection of Released Orange Fluorescing Resorufin.*

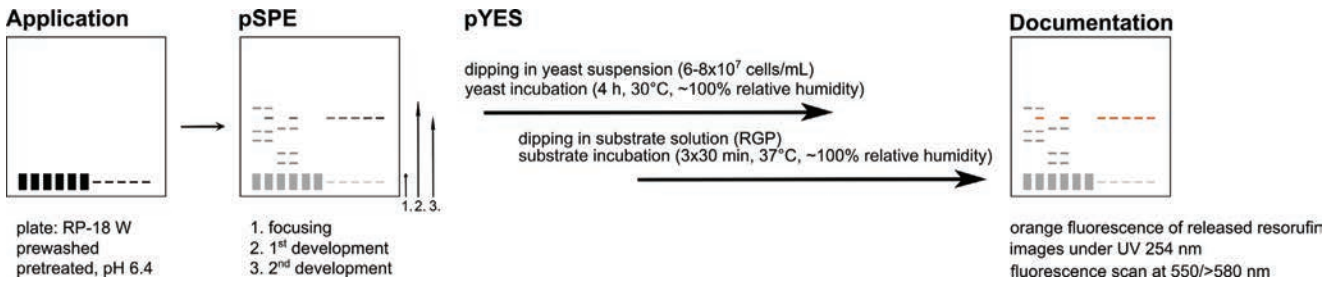
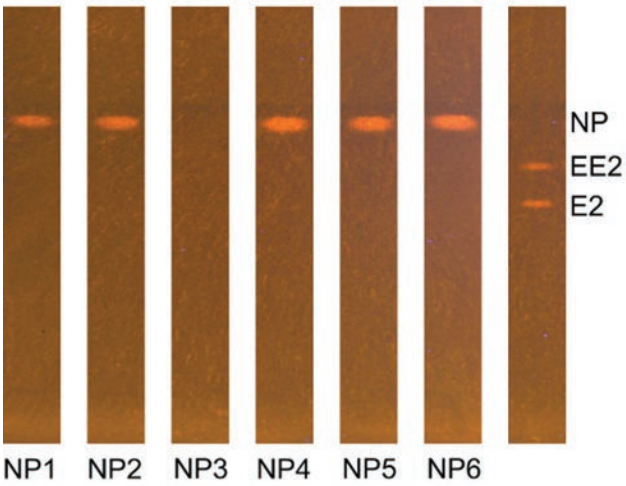


Table 1. HPTLC Conditions

HPTLC plate:	**HPTLC silica gel 60 RP-18 W glass plates**	
pretreatment:	pre-washed (acetone/water, 9:1, *v/v*), dried at 120 °C for 30 min	
	pH adjusment to pH 6.5 with $NaHCO_3$ (25 g/L, pH 6.4)[9]	
application:	standard solution as 5-mm bands	
	extracts as 5 mm x 10 mm areas (15 µL)	
pSPE		
focusing:	acetonitrile	15 mm
1st development:	*n*-hexane/acetonitrile/toluene (4:2:1.5, *v/v/v*)	60 mm
2nd development:	*n*-hexane/ethyl acetate/toluene (2.5:4:0.5, *v/v/v*)	50 mm
Detection after pYES		
documentation:	under UV 254 nm illumination	
densitometry:	fluorescence scan at 550/>580 nm	

Figure 2. Track Images of Six Different Nonylphenols (NP, each 200 ng/zone) and a Track with 17α-Ethinylestradiol (EE2, 200 pg/Zone) and 17β-Estradiol (E2, 200 pg/Zone) under UV 254 nm Illumination after pSPE–pYES. NP1, NP2, NP4, NP5 and NP6 are Technical Mixtures of NP; NP3 is 4-n-NP.



Sensitivity of pSPE–pYES and the **recovery rate** of the entire method (extraction of 200 mL water with 20 mL of dichloromethane by stirring for 10 min at 1000 rpm, separating and removing of the organic phase, and dissolving of the residue in 200 µL of ethanol)[10] were determined to evaluate the performance of the screening. Limits of detection and quantitation (LOD and LOQ) were 14 ±4 and 26 ±4 ng NP/zone (each *n=8*), corresponding to ~1 µg/L LOD taking the extraction and application volume into account.[10] Recovery of NP was 95 ±17% (*n=12*, *n=4* extracts on *n=3* days, see **Table 2**).[10]

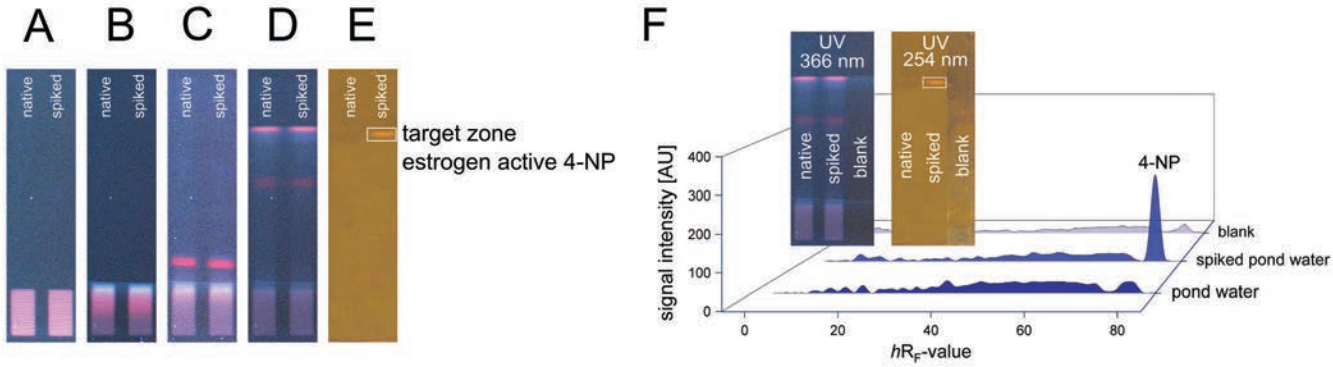
Table 2. Recovery Rates for NP4 from Spiked Water Samples (each *n=4*).*

Day	Mean recovery ±RSD [%]	Coefficient of determination of calibration (R^2)
1	115 ±7	0.9749
2	96 ±21	0.9898
3	74 ±6	0.9959

Repetitions were performed on 3 days
RSD relative standard deviation.

Screening of surface waters for NP was performed after extraction of samples by application of the extracts and standard solutions on HPTLC plates, followed by a quick focusing step, whereupon pSPE–pYES was performed. Water samples (*n=7*) were taken from different sites such as lakes, ponds, and streams, and were each analyzed as native samples and after spiking with NP at a concentration of 2 µg/L. Example images of the single steps of pSPE–pYES for extracts of pond water are shown in **Figure 3**. Estrogen active NP were not detected in any of the investigated water samples by the developed screening; however, the analysis of the spiked samples showed the applicability of the screening for matrix-containing environmental samples. The applied extraction method was also shown to be suitable as the recovery rate from spiked environmental samples (95 ±17%, *n=7*) was very similar to the recovery rate determined from spiked ultrapure water.[10]

Figure 3. Track Images of Extracts of a Native and Spiked (2 µg NP4/L) Sample of Surface Water from a Pond under UV 366 nm Illumination after (A) Application, (B) Focusing Step, (C) 1st Development and (D) 2nd Development, and (E) under UV 254 nm Illumination after pYES. (F) Track Images after pSPE and pYES, Respectively, of the Same Extracts and a Blank Extract and the Respective 3D Densitogram of the Fluorescence Scan at 550/>580 nm. (Partly modified and reprinted by permission from Springer*).



The advantage of pSPE—pYES was especially evident after analysis of environmental samples. Since no further cleanup of the extracts was necessary, all matrix compounds were applied onto the HPTLC plate, partly visible by native blue and red fluorescence (see **Figure 3**). Despite the fact that the complete matrix was subjected to the analysis, estrogen active NP were clearly detectable, quantifiable, and differentiable from co-extracted substances.[10] Since pSPE is based on HPTLC, it serves as planar cleanup, separating substances of interest from matrix compounds while the analytes are simultaneously focused in a common target zone.[8] The high selectivity of the applied pYES is caused by the screening itself. Positive signals, shown by orange fluorescence of released resorufin, are based only on the activity of the yeasts and the reporter gene after estrogenic compounds have been bound by the receptor. This is why false positive signals or interferences are not to be expected.[9,10]

Conclusion

By combining pSPE and pYES on HPTLC RP-18 W plates, a meaningful and straightforward approach was developed for the rapid screening and detection of estrogen active NP in surface waters. By use of pSPE, complex sample treatments are redundant and matrix-rich extracts can directly be applied to analysis. At the same time, pSPE focuses the analytes of interest in a common zone, enabling the detection and quantitation as a whole. By combination with pYES, i.e., the detection of the analytes on the basis of their estrogenicity, pSPE–pYES represents a highly selective and innovative approach for the analysis of EAC.

* Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer, Schick, D.; Oellig, C., Screening for estrogen active nonylphenols in surface waters by planar solid phase extraction–planar yeast estrogen screen, *Analytical and Bioanalytical Chemistry*. **2019**, 411, 6767-6775. (https://doi.org/10.1007/s00216-019-02053-0)

References

1. Günther, K; Räcker, T; Böhme, R., An Isomer-Specific Approach to Endocrine-Disrupting Nonylphenol in Infant Food. *J. Agric. Food Chem.* **2017**, 65, 1247–1254.
2. Guenther, K; Heinke, V; Thiele, B; Kleist, E; Prast, H; Raecker, T., Endocrine Disrupting Nonylphenols Are Ubiquitous in Food. *Environ. Sci. Technol.* **2002**, 36, 1676–1680.
3. Guenther, K; Kleist, E; Thiele, B., Estrogen-active nonylphenols from an isomer-specific viewpoint: A systematic numbering system and future trends. *Anal. Bioanal. Chem.* **2006**, 384, 542–546.
4. The European Parliament and the Council of the European Union, Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Official Journal of the European Union*, L 226 (**2013**).
5. The European Parliament and the Council of the European Union, Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. *Official Journal of the European Union*, L 348 (**2008**).
6. The European Parliament and the Council of the European Union, Decision No. 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. *Official Journal of the European Communities*, L 331 (**2001**).
7. The European Parliament and the Council of the European Union, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities*, L 327 (**2000**).
8. Oellig, C; Schwack, W., Planar solid phase extraction—a new clean-up concept in multi-residue analysis of pesticides by liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2011**, 1218, 6540–6547.
9. Schick, D; Schwack, W., Planar yeast estrogen screen with resorufin-beta-d-galactopyranoside as substrate. *J. Chromatogr. A* **2017**, 1497, 155–163.
10. Schick, D; Oellig, C., Screening for estrogen active nonylphenols in surface waters by planar solid phase extraction-planar yeast estrogen screen. *Anal. Bioanal. Chem.* **2019**, 411, 6767–6775.
11. McDonnell, D.P; Nawaz, Z; O'Malley, B.W., In situ distinction between steroid receptor binding and transactivation at a target gene. *Mol. Cell. Biol.* **1991**, 11, 4350–4355.
12. McDonnell, D.P; Nawaz, Z; Densmore, C; Weigel, N.L; Pham, T.A; Clark, J.H; O'Malley, B.W., High level expression of biologically active estrogen receptor in Saccharomyces cerevisiae. *J. Steroid Biochem. Mol. Biol.* **1991**, 39, 291–297.

Featured Products

Description	Cat. No.
HPTLC silica gel 60 RP-18 W glass plates, Pk.25	**1.14296**
Solvents & Reagents	
Acetone for HPLC, ≥99.8%	**34850-M**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Toluene for HPLC, 99.9%	**34866-M**
n-Hexane hypergrade for LC-MS LiChrosolv®	**1.03701**
Water for UHPLC, for mass spectrometry	**900682**
Sodium bicarbonate ($NaHCO_3$), powder, BioReagent	**S5761**

Related Products

Description	Cat. No.
Reference Materials	
4-Nonylphenol, PESTANAL®, analytical standard, 100 mg	**46405**
4-Nonylphenol, certified reference material, TraceCERT®, 50 mg	**04029**
Nonylphenol solution, 1000 µg/mL in acetone, analytical standard, 1 mL	**32468**
Nonylphenol, PESTANAL®, analytical standard, technical mixture, 1 g	**46018**

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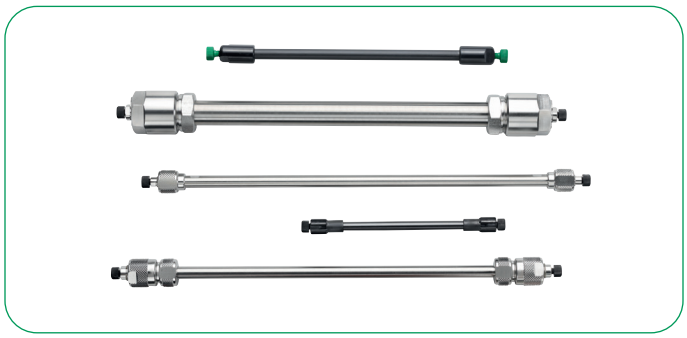
How to Extend Liquid Chromatography Column Life: Regenerate or Use Guard Columns

Egidijus Machtejevas, Lead Expert, Chromatography Product & Portfolio Management, Analytix@milliporesigma.com



Any HPLC or UHPLC column can typically be used for only a limited number of injections. Correct usage and maintenance of the column extends its lifetime and benefits the economy of the method. It is important to follow generally accepted chromatography practices, like adequate sample preparation (sample should be filtered through 0.45 µm membrane filter for HPLC and 0.22 µm filter for UHPLC); suitable quality/purity of the mobile phase (for example, isocratic grade solvents for isocratic mode, gradient grade solvents for gradient elution); filtered mobile phase, well developed method with sufficiently strong elution to minimize unspecific sorption; temperature, pH, and maximum backpressure ranges compatible with selected column. Also important to mention is that the total number of injections is inversely proportional to the volume of the sample injected, e.g., if 10 µL sample is applied per injection and this results in a column lifetime of 5000 injections, then an increased sample load of 100 µL per injection would typically shorten column lifetime to approximately 500 injections. Even if all the above listed points are carefully followed, users sometimes feel that column lifetime should be longer. In order to find out how to extend the lifetime of the column, it is first necessary to understand what causes the aging of the column.

1. **Column contamination/clogging by particles.** Keep in mind that your column is a very efficient filter. Any particulate material coming from the mobile phase or sample will contaminate frits, and some particles might also migrate into the column packing. This will result in an increase in column backpressure. This creates more stress for the pump and can cause a column to settle, creating a void which result in peak splitting.
2. **Unspecific sorption.** An HPLC column can encounter many different substances during its use, such as salts from buffers or impurities from the mobile phase and sample. These materials can have lesser or greater retention than the analytes separated. These undesired interferences, if observed by the detector, appear as ghost peaks, blobs, baseline upsets or even negative peaks. Absorbed impurities might negatively contribute to retention mechanism (shorten retention) or begin to act as a new stationary phase (increased retention).
3. **Column repacking.** Every particle packed column contains a range of particle sizes. Particle size distribution might be wider or narrower. During chromatographic analysis, columns will experience pressure stresses when a non-pressurized injection loop is connected to a pressurized column. During these pressure stresses, the column packing is getting shocked, then particles might swap positions, causing the generally bigger particle to go up (towards column inlet), and the smaller down (towards column outlet). After a number of injections, smaller particles will accumulate at the bottom, and bigger at the top. The smaller particle zone then has a higher packing density backpressure, which users will observe due to the steady increase in backpressure. This again creates more pressure for the instrument and can cause a column to settle, creating a void and consequently peak splitting.

Many customers are asking for procedures which could regenerate a column to the initial performance level in order to extend column lifetime. Therefore, the importance of very carefully reading the instruction sheet which comes with every column cannot be overstressed. The sheet also contains information about suggested column care and regeneration procedures. This advice must be followed and can be different from column/phase type to column/phase type. However, we can theoretically analyze how efficient column regeneration might be. Column contamination at the inlet by particles should generally be easily removed by washing the column for about 5 to 20 column volumes in a backflush mode, but only if the column manufacturer allows it. It is important to make sure that both column frits (inlet and outlet) have the same porosity. Please keep in mind that small

impurity particles trapped deeper in the column packing are very unlikely to be removed. As a result of this washing, the column backpressure should be somewhat decreased. To remove unspecifically bound material from the column is often more difficult and the outcome is usually unpredictable, because typically we do not know what compounds these contaminants contain. The success ratio might be from 0 to 100%. To fully restore column packing uniformity for columns packed with smaller than 5 µm particles is very unlikely. Normally column backflush only helps for a short time.

So, what is the most reliable and certain solution offered for this topic? The use of guard columns! Guard columns will filter all particles, accumulate unspecific adsorbed materials, and will also extend the lifetime of the analytical main columns used at marginally alkaline pH. Typically, when the guard column is replaced, a majority of the problems will be eliminated, and even more importantly, it will occur without any lengthy time and solvent consuming washing procedure with questionable outcome. Important note: the Guard column must be the same particle size as the analytical column, the same modification and the same material type.

But how will you know when to replace the guard column? General advice: Replace the guard column when the nature of the sample is changing, when backpressure is increased about 10-15%, or by schedule/time basis (for example, every 200 to 500 injections – depending on the injection volume and sample purity).

If you routinely have any problems with the lifetime of your main column, think early enough about guard columns to save yourself analytical headaches!

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