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Analytix Reporter

Analysis of Pesticides in Turmeric Powder

by LC/MS/MS and GC/MS/MS after Cleanup with a Novel Dual-Layer SPE Cartridge

Analytical Products

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analytix reporter

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The acquisition of Sigma-Aldrich by Merck KGaA, Darmstadt, Germany brought together two strong analytical product portfolios. We have taken this opportunity to create a unified approach to our literature and support materials covering the integrated range. This first combined newsletter of 2018 is an opportunity to present the fruits of our labors to you.

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Regarding newsletters, we learned that our readers want them to be technical. The content of this inaugural edition of AnalytixReporter reflects the preferences expressed: new scientific research, advice on experiments, and product innovations.

Scientists are tasked with solving ever tougher problems, and we aspire to provide solutions to them. I am excited by what we have to offer to the Analytical community, now and in our innovation pipeline.

I welcome your impressions of our new newsletter and your experiences with us and our analytical products.

I hope you like it.

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FOOD & BEVERAGE

Analysis of Pesticides in Turmeric Powder by LC/MS/MS and GC/MS/MS

After Cleanup with a Novel Dual-Layer SPE Cartridge

Katherine K. Stenerson, Principal R&D Scientist, **katherine.stenerson@sial.com**

Introduction

Turmeric is a plant indigenous to south Asia, with a majority of its production coming from India. The rhizome of the plant is used to produce powdered turmeric, which is used in foods, cosmetics, and some medicines. It is also an essential constituent of curry, which is a mixture of spices used extensively in Indian cooking. Turmeric has also been used in traditional medicines for thousands of years, and recently has garnered attention for studies showing its potential antioxidant, anti-inflammatory, antimutagenic, antimicrobial and anticancer properties.[1]

Pesticide residue testing of turmeric and other spices is required by many countries. For example, Canada has set maximum residue limits for 42 different pesticides in turmeric root.[2] The US EPA has set tolerance limits for a variety of pesticides in root and tuberous vegetables, of which turmeric is included.[3]

Turmeric contains more than 100 different components, with two of the main constituents being curcumin and volatile oils. Curcumin gives turmeric its distinctive yellow/orange color, while the volatile oils consist primarily of terpenes. Turmeric also contains some fats; specifically, sterols and fatty acids.[4] This complex composition makes extracts produced from turmeric a challenge in the chromatographic analysis of pesticides, as residual pigments and oils can contaminate both GC/MS and LC/MS systems.

When dealing with very high background samples such as turmeric, standard QuEChERS cleanup may not offer enough capacity. For better cleanup, solid phase extraction (SPE), including dual-layer cartridges, can be used. These cartridges often contain graphitized carbon black (GCB) in the top bed and primary-secondary amine (PSA) in the bottom bed. PSA retains acidic interferences such as fatty acids. GCB removes planar molecules such as pigments and sterols. Common GCBs, however, will retain all molecules with planar structures, including some pesticide analytes such as hexachlorobenzene. To increase recoveries of these pesticides, toluene is normally added to the elution solvent. However, there are issues associated with the use of toluene. It can affect the ability of the PSA to retain fatty acids, and its presence in the final extract is problematic for HPLC analysis.[5]

In this application, a different dual-layer SPE cartridge was used in the cleanup of extracts of turmeric powder prior to pesticide analysis by GC/MS/MS and LC/MS/MS. This cartridge, the Supelclean™ Ultra 2400, was designed for the cleanup of acetonitrile extracts made from difficult matrices such as dry commodities (spices, tea, etc.) prior to pesticide residue analysis. The top bed consists of a mixture of PSA, C18 and a graphitized, spherical carbon known as Graphsphere™ 2031. This carbon was engineered to remove sufficient pigmentation while allowing for better recoveries of planar compounds, without the need for toluene in the elution solvent. The bottom layer of the cartridge contains Z-Sep, a zirconia-coated silica. Z-Sep removes oily residues and provides additional retention of some pigments. The combination of these sorbents in an SPE format offers more capacity than QuEChERS cleanup, and compared to traditional GCB/PSA dual layer cartridges, does not require the use of toluene in the elution solvent to recover planar pesticides.

Experimental

Turmeric powder was obtained from a local grocery store. Samples were spiked at 100 ng/g with the pesticides listed in **Tables 1** and **2**. Sample extracts were prepared and cleaned following the procedures in **Figure 1**. A set of 3 spiked samples and 1 unspiked (blank) were prepared and analyzed for each set of pesticides. Analysis was done by GC/MS/MS and LC/MS/MS using the conditions listed in **Tables 3** and **4** (with MS/MS transitions shown in **Tables 1** and **2**). Quantitation was performed against multi-point calibration curves prepared in unspiked turmeric extract (after cleanup). Recoveries were calculated as the average of the three spiked replicates, less anything found in the unspiked extract. No internal standards were used, thus the values reported represent absolute recoveries.

Results and Discussion

Background

Prior to cleanup, the extract appeared orange-brown in color with a yellow oily residue (**Figure 2**). After cleanup for both LC and GC, the extracts appeared

(continued on next page)

substantially lighter and clearer. **Figures 3** and **4** show a comparison between extracts at the same level of dilution with and without cleanup. The LC extract (in 80% aqueous) was almost devoid of color, with very little cloudiness. The extract for GC analysis was a pale yellow color, with substantially less oily residue. Full scan GC/MS analyses of GC extracts are shown in **Figure 5** as total ion chromatograms (TICs). The peak pattern is similar between the two, with the main peaks consisting primarily of terpenes. These compounds are easily volatilized in the GC inlet, and do not pose issues with system contamination, however they can interfere with mass spectral detection, requiring the use of MS/MS for selectivity. The overall amplitude of the peaks was less after cleanup, as is shown by a 21% reduction in the peak area sums for each in **Figure 5**.

Table 1. Pesticides Studied in Turmeric Powder by GC/MS/MS Analysis

	MRM 1	CE	MRM 2	CE
Alachlor	188/160	10	188/130	40
Aldrin	263/193	35	263/191	35
γ-BHC	183/147	15	181/145	5
Azinphos-methyl	160/77	15	132/77	15
Chloropyrifos	314/286	5	314/258	15
Chloropyrifos-methyl	286/93	20	288/93	20
Cypermthrins	165/91	10	163/91	10
4,4'-DDT	235/199	15	235/165	25
Diazinon	199/135	15	137/84	10
Dichlorvos	185/93	25	145/109	25
Dimethoate	125/79	20	93/63	10
Disulfoton	88/60	5	88/59	15
Endosulfan β	241/206	15	241/170	30
Endosulfan-α	241/206	15	241/170	30
Ethion	231/129	20	121/65	10
Fenitrothion	277/125	20	277/109	20
Heptachlor	274/239	15	272/237	15
Hexachlorobenzene	284/249	20	284/214	35
Iprodione I	314/56	35	187/124	25
Iprodione II	316/56	35	187/124	25
Malathion	173/99	15	158/125	5
Metalaxyl	234/174	10	234/146	20
Methoxychlor	227/169	30	227/141	30
Mevinphos	192/127	25	192/109	25
Parathion-methyl	233/109	10	124/47	10
Permethrins	183/168	10	183/165	10
Phenthoate	274/125	15	274/121	10
Phorate	260/75	5	231/129	25
Phosalone	182/102	15	182/75	30
Pirimiphos-methyl	290/151	20	290/125	25
Profenophos	339/269	15	339/188	15
Quintozene	295/237	20	237/143	30
Vinclozolin	212/145	30	187/124	20

Table 2. Pesticides Studied in Turmeric Powder by LC/MS/MS Analysis

	MRM	Frag (V)	CE (V)	Cell Acc (V)
Acephate	184/143	70	0	5
Acetamiprid	223.1/126	80	27	2
Boscalid (Nicobifen)	343/307.1	145	16	6
Carbendazim (Azole)	192.1/160.1	105	16	2
Chlorbufam	224/172.02	120	5	3
Cycluron	199.2/72	120	20	2
Diflubenzuron	311/158	80	8	2
Fenoxanil	329.08/189	80	30	3
Fosthiazate	284/61	90	60	2
Methabenzthiazuron	222.1/165.1	90	12	2
Methamidophos	142/125	85	10	2
Methomyl	163.1/106	50	4	2
Monocrotophos (Azodrin)	224.1/193	65	0	5
Nitralin	346.11/304	100	10	3
Oxamyl	237.1/72	60	12	2
Pirimicarb	239.15/72.1	100	20	2
Procymidon	301/284*	70	8	2
Propaquizafop	444.12/100.1	125	16	2
Tetraconazole	372/159	130	36	2
Uniconazole-P	292.1/125	135	40	2

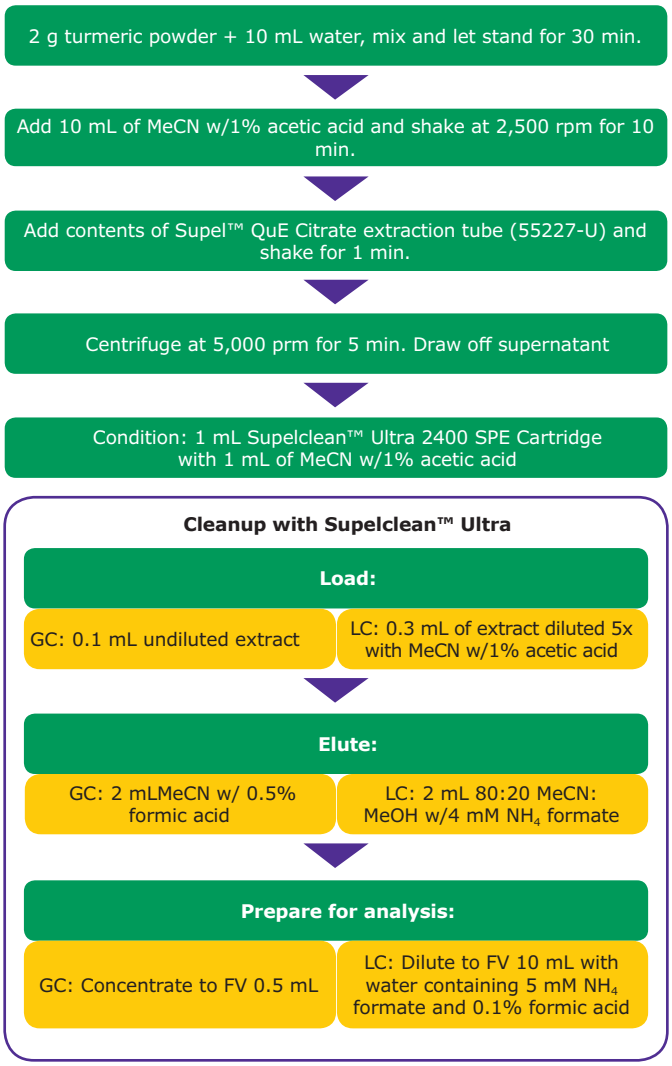
Table 3. GC/MS/MS Run Conditions for the Analysis of Pesticides in Turmeric

column:	SLB®-5ms, 30 m × 0.25 mm ID, 0.25 µm (28471-U)
oven:	50°C (2 min), 8°C/min to 320°C (5 min)
inj. temp.:	250°C
carrier gas:	helium, 1.4 mL/min, constant
detector:	MRM (see table 1)
MSD interface:	320°C
injection :	1µL, splitless (splitter open at 0.75 min)
liner:	4 mm I.D., split/splitless type, single taper wool packed FocusLiner™ design (2879901-U)

Table 4. LC/MS/MS Run Conditions for the Analysis of Pesticides in Turmeric

column:	Ascentis® Express C18, 10 cm × 2.1 mm ID, 2 µm (50813-U)
mobile phase:	[A] 5 mM ammonium formate, 0.1% formic acid in water; [B] 5 mM ammonium formate, 0.1% formic acid in methanol
gradient:	95% A, 5% B held for 1 min; to 50% A in 3 min; to 100% B in 8 min; held for 1 min;, to 95% A in 1.5 min; held at 95% A for 1.5 min
flow rate:	0.4 mL/min
detector:	MRM (see table 2)
injection:	5 µL

Figure 1. Extraction and Cleanup Procedure Used for Turmeric Powder, GC and LC



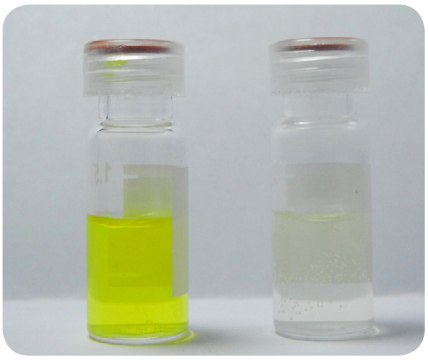
Pesticide Recovery and Reproducibility

The average recoveries obtained from spiked turmeric samples (n=3) are presented in **Table 5**. Of the 51 pesticides spiked, all except hexachlorobenzene had recovery of greater than 70%. Hexachlorobenzene, a pesticide with a planar structure, was recovered at 67% after cleanup. It should be noted that this was without using toluene in the elution solvent, as is necessary to obtain good recoveries from dual-layer cartridges containing graphitized carbon black.[5] Although not shown here, higher recovery of hexachlorobenzene has been obtained by loading more turmeric extract (300 µL) on the Supelclean™ Ultra 2400 cartridge. This indicates that the presence of more matrix displaced the hexachlorobenzene, thus reducing its retention on the carbon. However the higher sample loading produced an extract with more color, a sign that the cleanup capacity of the cartridge had been reached or exceeded for this matrix.

Reproducibility, calculated as %RSD for the sets of spiked replicates, was less than 20% for 44 of the 51 pesticides. As is indicated in **Figure 6**, many compounds had RSD values of less than 10%. Pesticides with RSD values greater than 20% were attributed to those showing low response in the MS/MS method.

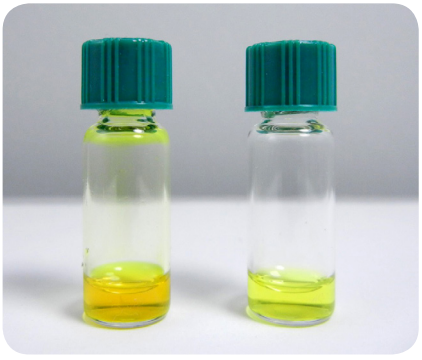
Figure 2. Undiluted Acetonitrile Extract of Turmeric Powder Before Cleanup

Figure 3. Turmeric Extracts at the Same Dilution (167X total); Without Cleanup, and After Cleanup for LC/MS/MS Analysis



Without Cleanup After Cleanup

Figure 4. Turmeric Extracts at the Same Dilution (5x); Without Cleanup, and After Cleanup for GC/MS/MS Analysis



Without Cleanup After Cleanup

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Table 5. Pesticide Recoveries and % RSD Values (*n*=3) for Spiked Replicates; Turmeric Spiked at 100 ng/g

Pesticide	Avg. Recovery	RSD	Analysis
Alachlor	99%	23%	GC/MS/MS
Aldrin	85%	10%	GC/MS/MS
Azinphos-methyl	89%	11%	GC/MS/MS
γ-BHC	83%	8%	GC/MS/MS
Chloropyrifos	96%	12%	GC/MS/MS
Chloropyrifos-Methyl	113%	6%	GC/MS/MS
Cypermthrin (isomer 1)	99%	15%	GC/MS/MS
4,4'-DDT	95%	8%	GC/MS/MS
Diazinon	92%	14%	GC/MS/MS
Dichlorvos	78%	31%	GC/MS/MS
DIsulfoton	86%	7%	GC/MS/MS
Endosulfan β	86%	35%	GC/MS/MS
Endosulfan-α	92%	23%	GC/MS/MS
Ethion	97%	7%	GC/MS/MS
Fenitrothion	63%	5%	GC/MS/MS
Heptachlor	81%	7%	GC/MS/MS
Hexachlorobenzene	67%	9%	GC/MS/MS
Iprodione (isomer 1)	103%	5%	GC/MS/MS
Malathion	90%	10%	GC/MS/MS
Metalaxyl	86%	21%	GC/MS/MS
Methoxychlor	78%	12%	GC/MS/MS
Mevinphos	73%	7%	GC/MS/MS
Parathion-Methyl	88%	8%	GC/MS/MS
Permethrin (isomer 1)	104%	24%	GC/MS/MS
Phenthoate	89%	7%	GC/MS/MS

Pesticide	Avg. Recovery	RSD	Analysis
Phorate	82%	10%	GC/MS/MS
Phosalone	90%	7%	GC/MS/MS
Pirimiphos-methyl	74%	3%	GC/MS/MS
Profenophos	88%	7%	GC/MS/MS
Quintozene	75%	8%	GC/MS/MS
Vinclozolin	90%	6%	GC/MS/MS
Acephate	89%	6%	LC/MS/MS
Acetamiprid	102%	4%	LC/MS/MS
Boscalid (Nicobifen)	86%	7%	LC/MS/MS
Carbendazim (Azole)	106%	7%	LC/MS/MS
Chlorbufam	92%	18%	LC/MS/MS
Cycluron	103%	5%	LC/MS/MS
Diflubenzuron	101%	5%	LC/MS/MS
Fenoxanil	91%	10%	LC/MS/MS
Fosthiazate	95%	4%	LC/MS/MS
Methabenzthiazuron	96%	4%	LC/MS/MS
Methamidophos	85%	5%	LC/MS/MS
Methomyl	106%	6%	LC/MS/MS
Monocrotophos (Azodrin)	97%	3%	LC/MS/MS
Nitralin	124%	55%	LC/MS/MS
Oxamyl	104%	3%	LC/MS/MS
Pirimicarb	97%	3%	LC/MS/MS
Procymidon	91%	13%	LC/MS/MS
Propaquizafop	97%	1%	LC/MS/MS
Tetraconazole	98%	2%	LC/MS/MS
Uniconazole-P	103%	19%	LC/MS/MS

Figure 5. GC/MS Scan Analyses of Turmeric Extracts Before and After Cleanup with Supelclean™ Ultra 2400 cartirgde Shown with same Y-scale. Sum of area counts for all peaks is indicated with each.

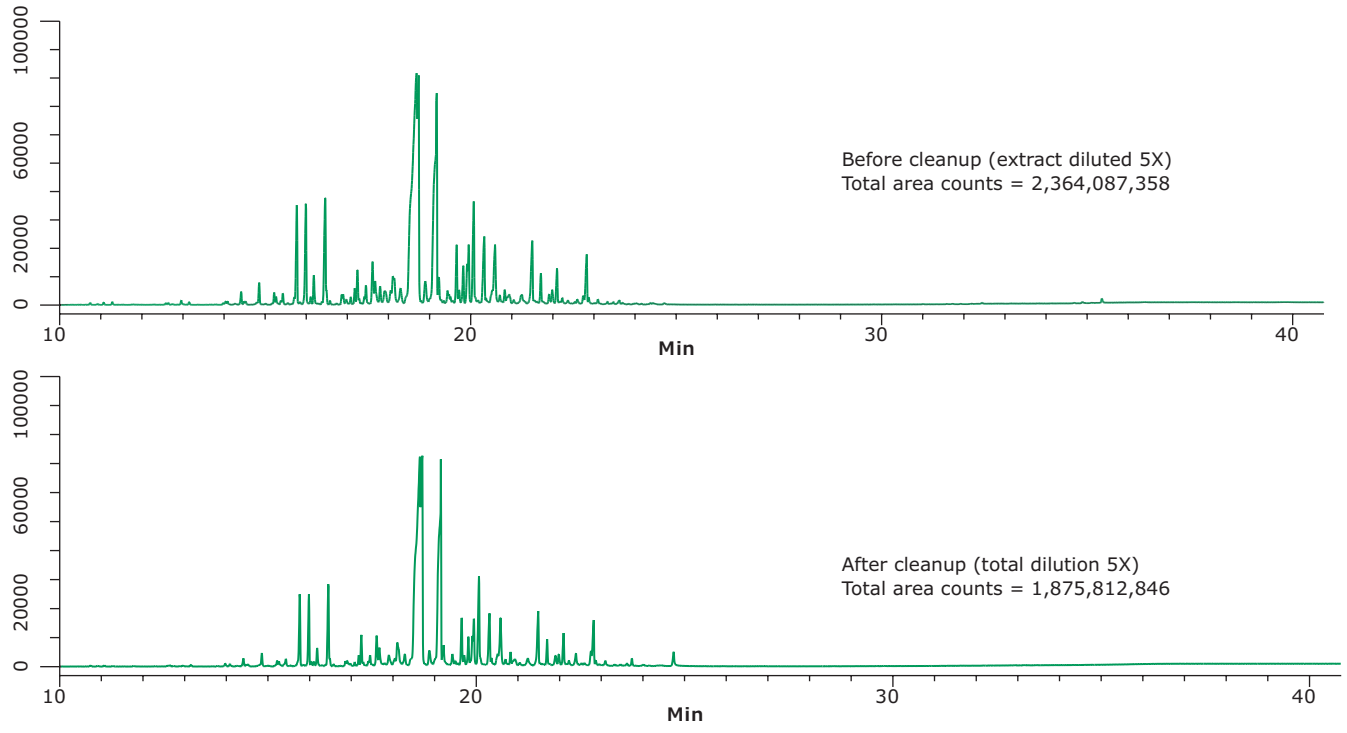
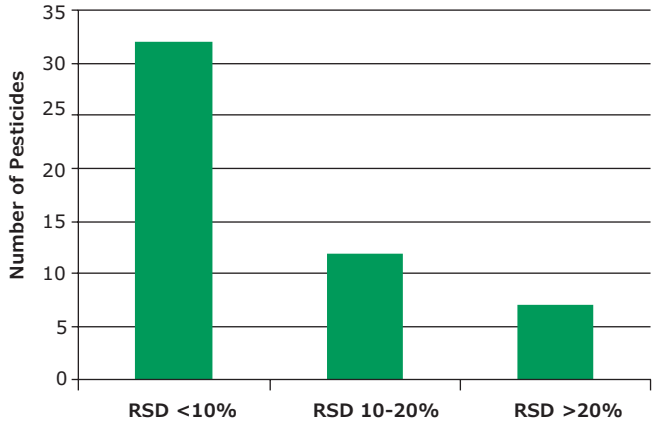


Figure 6. Number of Pesticides with Average Recoveries Within Indicated Percent Relative Standard Deviation (%RSD) Ranges After Cleanup with Supelclean™ Ultra 2400

(Recoveries from turmeric powder spiked at 100 ng/g.)



Conclusion

A new cleanup method has been developed using the Supelclean™ Ultra 2400 dual-layer SPE cartridge. The selection of sorbents in this cartridge allows for cleanup of acetonitrile extracts of very difficult samples such as spices and other dry commodities. The Graphsphere™ 2031 carbon used in the upper layer removes/reduces pigmentation while still allowing for recovery of planar pesticides without the use of toluene in the elution solvent. Z-Sep sorbent in the bottom layer of the cartridge removes oils and some pigments, as was indicated in the cleanup of turmeric extracts for both GC and HPLC analysis. Suitable recoveries for a wide range of pesticides of different polarities and classes were obtained from turmeric extract, and minimal background interference was noted. In this work, a 1 mL Supelclean™ Ultra 2400 cartridge was used. A larger 3 mL version of the cartridge is also available which can accommodate a higher sample loading.

Acknowledgements

The author would like to thank Richard Schriner and Bruce Morris of R.J. Hill Laboratories for their helpful discussions and input.

Did you know?

We also provide phytochemical reference materials for the natural constituents of turmeric.

To learn more, visit
SigmaAldrich.com/turmeric

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Featured Products

Description	Cat. No.
Supelclean™ Ultra 2400 SPE Cartridges	
1 mL, pk of 108	**52779-U**
3 mL, pk of 54	**54281-U**
Supel™ QuE QuEChERS Products	
Citrate Extraction Tube, 12 mL, pk of 50	**55227-U**
Empty Centrifuge Tube, 50 mL, pk of 50	**55248-U**
Columns	
SLB®-5ms Capillary GC Column, 30 m × 0.25 mm I.D., 0.25 µm	**28471-U**
Ascentis® Express C18 HPLC Column, 10 cm × 2.1 mm I.D., 2 µm particle size	**50813-U**
Accessories	
QuEChERS Shaker and Rack Starter Kit, USA compatible plug, AC input 115 V	**55278-U**
QuEChERS Shaker and Rack Starter Kit, Schuko plug, AC input 230 V	**55438-U**
Visiprep™ DL 12-port Solid Phase Extraction Manifold	**57044**
Disposable valve liners, PTFE, 100 ea.	**57059**

Related Products

Description	Cat. No.
Solvents and Reagents	
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Acetic acid 100% for LC-MS LiChropur®	**5.33001**
Formic acid 98% - 100% for LC-MS LiChropur®	**5.33002**
Ammonium formate for mass spectrometry, ≥99.0%	**70221**
Acetonitrile for GC-MS SupraSolv®	**1.00665**
Accessories	
Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100	**29653-U**
Inlet Liner, Split/Splitless Type, Single Taper FocusLiner™ Design (wool packed)	**2879901-U**

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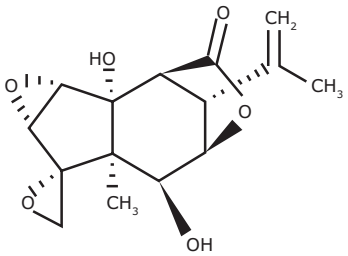
FOOD & BEVERAGE

Sweet Poison

New *Trace*CERT® CRM Solution for Tutin

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



Description	Certified Concentration	Solvent	Package Size	Cat. No.
Tutin *Trace*CERT® CRM	19.98 ug/g	Acetonitrile	0.5 mL	**80718**

Tutin is a toxic oxygenated sesquiterpene occurring in the tutu plant (genus coriaria) that is found in New Zealand. It is responsible for poisoning through contaminated honey, when honeybees collect honeydew produced by sap-sucking vine hopper insects that feed on the tutu plant. Therefore, the Australia New Zealand Food Standards Code has set a maximum level of tutin of 0.7 milligrams per kilogram for honey and honey combs.[1]

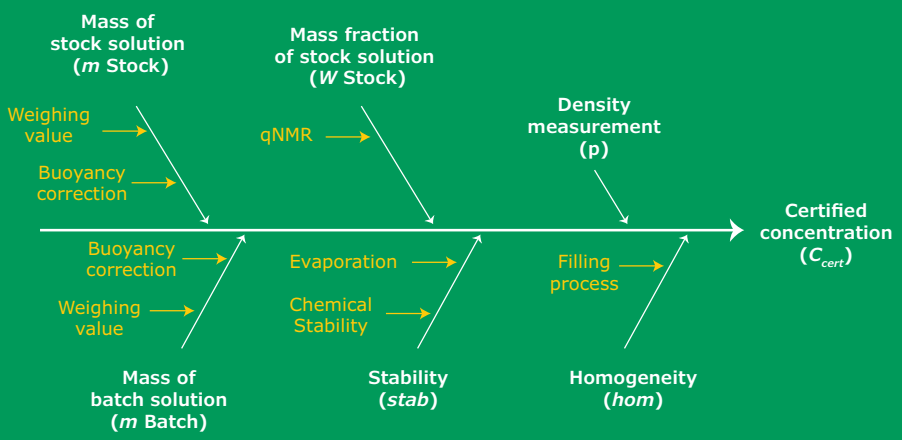
We present a new *Trace*CERT® certified reference material of Tutin as a 20 ppm solution in acetonitrile, suitable as a calibrant for chromatographical methods such as LC/MS. This product is produced in accordance with ISO/IEC 17025 and ISO Guide 34 accreditation and is traceable to NIST SRM through quantitative NMR measurement of a concentrated stock solution in deuterated acetonitrile. The final CRM is then produced by gravimetrical dilution to the final concentration. Accelerated and real time stability tests as well as homogeneity tests have been performed by LC/MS and have been taken into account for the extended uncertainty of the CRM (see **Figure 1**).

For more details and ordering information, please visit **SigmaAldrich.com/tutin**

Figure 1. Cause effect diagram of the main uncertainty contributions

The relative contribution are:

$U(m_{Stock})$	<0.02%
$U(m_{Batch})$	<0.02%
$U(w_{Stock})$	<0.4%
$U(hom)$	<0.8%
$U(Stab)$	<1.8%
$U(p)$	<0.07%
Combined uncertainty	
$U(C_{cert})$	2.0%
Expanded uncertainty:	
$U(C_{cert})$	**4.0%**



References:

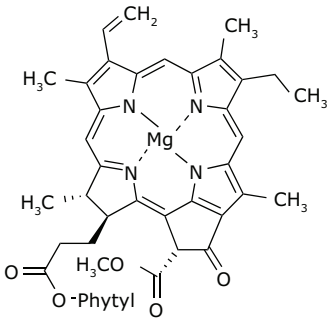
1. http://www.foodsafety.govt.nz/industry/sectors/honey-bee/tutin/

FOOD & BEVERAGE

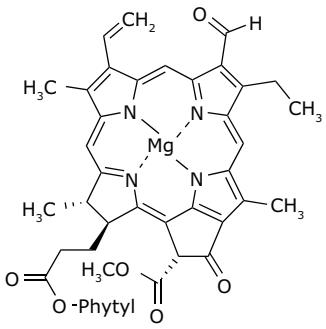
Chlorophyll a and b

New Analytical Standards for Natural Pigments

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



Chlorophyll a 00538



Chlorophyll b 00538

The compounds of the chlorophyll family are natural pigments that are essential for photosynthesis and responsible for the green color of plants and algae. The chlorophylls are all structurally very closely related, containing a porphyrine or chlorin ring with magnesium and, in the case of chlorophyll a and b, a long phytol chain.

Chlorophyll is also applied as a food colorant (E140).

For the complete offering of our analytical standards for carotenoids and other natural pigments, please visit **SigmaAldrich.com/carotenoids**

Description	Package Size	Cat. No.
Chlorophyll a	1 mg	**96145**
Chlorophyll b	1 mg	**00538**

FOOD & BEVERAGE

Water Determination in Instant Coffee

By Karl Fischer Titration

Bettina Straub-Jubb, Global Product Manager Titration, **bettina.straub-jubb@emdmillipore.com**

Foodstuffs include a very diverse group of products. Depending on whether carbohydrate-rich, fatty or protein-rich substances are under investigation, different working techniques are preferable. Complex matrices that dissolve slowly in the Karl Fischer solvent, or instances where the water can only be slowly extracted, necessitate the use of a solubilizer. In addition, titration under heating or the use of a homogenizer to accelerate water release are expedient. Coffee represents such a complex matrix.

Coffee is more than just a drink

In the 17th century, coffee was a luxury food and only affordable for the wealthy. However, since the 19th century it has become a mass produced product and a common daily drink for everyone. Additionally, it is now considered an important trading product and also developing into a life style product. Of primary concern to the consumer is that the coffee tastes good. To ensure this, one quality parameter is the water content. The determination of the water in coffee beans, roasted coffee and instant coffee is of interest as it has an influence on the roasting process of the beans and the quality, taste and shelf life of the coffee powder.

Karl Fischer Titration of Instant Coffee

Instant coffee contains very firmly bonded water. Its extraction by methanol is very slow and sluggish. For volumetric Karl Fischer Titration, the presence of formamide and salicylic acid accelerates the release of water. The salicylic acid has a buffering function to keep the pH in the right range. Additionally, titration under warm conditions, as well as the use of a homogenizer, are favorable. Alternatively, the Karl Fischer oven technique can be used in combination with Coulometry. For the release of water, a temperature of approx. 105 °C is suitable. A direct coulometric titration is not recommended.

Volumetric Karl Fischer Titration procedure

The titration medium is first placed into the cell and titrated dry. As titration medium, 40 mL of Aquastar® CombiMethanol or two component Aquastar® Solvent is filled into the titration cell and 20 mL formamide and 12 g salicylic acid are added. As titrant, Aquastar® CombiTitrant 5 or the two component Aquastar® Titrant 5, if the Aquastar® Solvent is used, can be selected. Then about 0.3 to 0.5 g of the instant coffee sample is added with a weighing boat and the titration is started. The exact sample weight is determined by weighing the boat before and after the sample addition. For a complete dissolution of the sample, a stirring time of three minutes is recommended. To accelerate the water release, the titration medium can be heated up to 50°C, using a double wall titration cell connected to a water bath. If the coffee particles are too large, they may need to be crushed before they are added to the titration cell.

It is recommended to do a regular titer determination (e.g. with Aquastar® Water Standard 1%). It is important that the titer determination is done with the actual titration medium mixture (CombiMethanol or CombiSolvent) containing the formamide and salicylic acid.

Titration instrument parameters:

- Extraction time (stirring time): 180 sec.
- Default titration setting:
  I(pol) = 20 - 50 µA, U(EP) = 100 - 250 mV
  Stop criterion: drift < 20 µL/min

For a reagents list see the Ordering Information.

Karl Fischer Oven Method Procedure combined with Coulometry

A direct coulometric Karl Fischer Titration for instant coffee is not recommended, due to the low water content, the accuracy typically is not sufficient.

With the Karl Fischer Oven technique, the water can be extracted/released from the instant coffee sample and be determined in the coulometric titration cell.
The Karl Fischer reagent Aquastar® CombiCoulomat frit is suitable for both, the cathode and anode compartment of the titration cell with diaphragm, simplifying the handling/method by needing only one reagent. It is recommended to place about 10 mL of the solution into the cathode and 150 mL into the anode cell. Then the coulometer is started and the solvent is titrated dry. After the pre-titration and stabilization of drift, the series of measurements can be started by determining the blank value for the sample vials. Then 0.1 g sample is weighed into a sample vial, which is immediately tightly capped. The vial is either manually or automatically placed into the KF oven and heated to the chosen temperature program. The water thereby released is transferred to the titration cell by means of a gas stream (dry air or nitrogen) and coulometrically analyzed.

An oven standard (e.g. Aquastar® Oven Standard 1%) is recommended to be ran before the first sample determination, and for longer sample series in between, and at the end of your sample determinations to check/verify the performance of the Karl Fischer oven and titration system throughout the measurements.

The Titration Parameters:

Oven settings:

- Temperature: 105 °C Extraction time: 600 sec
- Default coulometer settings for cell with diaphragm:
  End point indication, e.g.:
  I(pol) = 5 - 10 µA, U(EP) = 50 - 100 mV
  Stop criterion: drift < 20 µg/min

Ordering Information

Description	Cat No
Volumetric Titration	
Aquastar® - CombiTitrant 5, one component reagent, 1 mL = approx. 5 mg water	**188005**
Aquastar® – Titrant 5, two component reagents, 1 mL = approx. 5 mg water	**188010**
Aquastar® - CombiMethanol, one component solvent, max. 0.01% water	**188009**
Aquastar® – Solvent, two component solvent	**188015**
Formamide	**109684**
Salicylic acid	**100635**
Aquastar® Water Standards 1% in ampoules	**188052**
Oven Method with Coulometric Titration	
Aquastar® - CombiCoulomat frit, Coulometric Karl Fischer reagent for cells with diaphragm	**109255**
Aquastar® – Oven Standard 1%, solid standard for the Karl Fischer oven method	**188054**

More applications for foodstuffs and beverages can be found at **SigmaAldrich.com/application-note**.

For individual applications and requests, please contact our application lab at **Aquastar@milliporesigma.com**.

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FOOD & BEVERAGE

Our *Trace*CERT® Portfolio Keeps Growing!

New Organic Certified Reference Materials

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

In late 2009, our site in Buchs, Switzerland achieved an ISO 17025 and ISO Guide 34 double accreditation by the Swiss accreditation body SAS, for the manufacturing of organic certified reference materials (CRMs) by quantitative NMR (qNMR). Just recently, our accreditation has been renewed and we are one of the first reference materials suppliers holding the new ISO 17034:2016 which is replacing ISO Guide 34:2009. This method enables us to establish traceability to NIST SRM for basically any organic compound without the need to have a CRM of the same compound available.

Since then, we have continually expanded our portfolio, which is currently comprised of over 250 organic CRMs. These products are marketed under the *Trace*CERT® brand and are suitable as calibrants for all kinds of chromatographic techniques.

Our extraordinary in-house skills in quantitative NMR, which is increasingly used as a quantitative analysis technique in the chemical and pharmaceutical industries, are constantly refined and extended. For our CRMs, we can reach uncertainties as low as 0.5% to 0.1%. Also, qNMR enables us to produce CRM solutions, even when only a very small amount of material is available (see article about the Tutin CRM solution on page 8 of this issue).

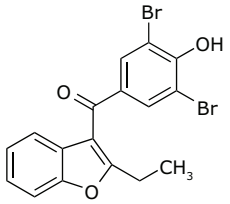
For those interested in more details about this versatile and efficient quantification technique, a large number of publications and technical articles are available.[2] You will also find a detailed technical qNMR brochure available as a pdf download on our website (**SigmaAldrich.com/qnmr**).

We continuously work on the expansion of the organic *Trace*CERT® range. The most recent additions are shown in the table below and include pesticides, vitamins, antibiotics and parabens. Also among the new launches is a CRM for Taurine, for which an interesting analytical application for its detection in shampoo can be found on page 21.

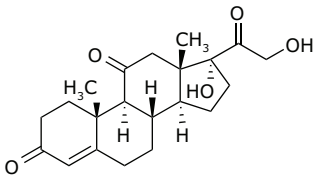
References:

1. Double Accreditation Brings a New Class of CRMs. Analytix, Vol. 2, 2008.
2. Traceable Organic Certified Reference Materials for 13P Quantitative NMR. Analytix, Vol. 2, 2015.

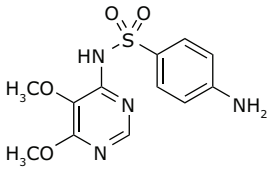
Figure 1. Some chemical Structures of new organic *Trace*CERT® CRMs.



Benzbromarone 09147



Cortisone 39353



Sulfadioxin 74627

Table 1. NEW organic neat CRMs *Trace*CERT® for chromatography

Description	Package Size	Cat. No.
Benzbromarone	50 mg	**09147**
Cortisone	100 mg	**39353**
Denatonium benzoate	100 mg	**93675**
Ethylparaben	50 mg	**79577**
4-Methoxybenzoic acid	100 mg	**93643**
Methylparaben	50 mg	**79721**
2-Methylthiophene	100 mg	**80364**
Pyridoxine hydrochloride	50 mg	**80823**
Sodium benzoate	100 mg	**52451**
Sulfadoxin	100 mg	**74627**
Taurine	100 mg	**93019**
Triazophos	50 mg	**68298**
Trimethoprim	100 mg	**16967**

For more information, please visit
SigmaAldrich.com/organiccrm

NUTRITIONAL SUPPLEMENTS

Chromatographic Testing of Black Pepper

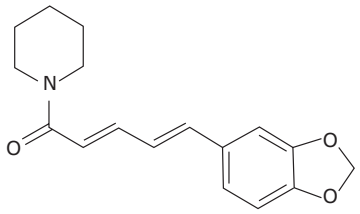
According to the United States Pharmacopeia

Anita Piper, Markus Burholt, Stephan Altmaier, Michael Schulz and Patrik Appelblad, **patrik.appelblad@emdmillipore.com**

Introduction

In truth, what are dietary supplements? They are consumed in large quantities, and are often referred to as vitamins, minerals or even botanicals derived from plants. A dietary supplement is intended to provide nutrients that may otherwise not be consumed in sufficient quantities. Black pepper, a common spice, is also regarded as a dietary supplement and a source of piperine (see Figure 1).

Figure 1. Piperine structure.



Usually when an analytical method is developed, the sample is the focal point of the analysis, driving the selection of the best suited instrumental technique, consumables or accessories needed, and the optimum sample preparation method to analyze the sample and produce reliable results. In other words, decisions are dictated by the sample. In pharmaceutical control, the methodology is regulated by different pharmacopeial bodies, such as the United States, British, European, Chinese and Japanese Pharmacopeias, who establish and update official monograph methods.

With a regulated method, one must follow the monograph instead of just putting the sample at the center of the decision making, so it is purely following the instructions in the monograph. One must know how to apply the dictated product specifications in the methods to get to the point where the value of interest can be reported. There is clearly defined information on what can be changed, the system suitability criteria to meet and how the method should be validated.

In this article, we have chosen powdered black pepper as an example, and have tested it according to the current United States Pharmacopeia (USP) guidelines

Monograph for Black Pepper Extract

The monograph from USP40-NF35 for powdered black pepper extract has a section dedicated to its identification, and another section which outlines its composition analysis. Powdered black pepper is defined as black pepper reduced to powder or very fine powder. It contains not less than (NLT) 2.5% of piperine, calculated on the dried basis. Under the identification test, it refers to a specific method related to its botanical characteristics, which requires high performance thin layer chromatography (HPTLC). The section on compositional analysis refers to high performance liquid chromatography (HPLC).

Identification Using HPTLC

The monograph specifies a chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates), and the mobile phase as a mixture of hexane and ethyl acetate with a volume ratio (v/v) of 5:3. The sample solutions are applied as bands with a bandwidth of 8 mm, using a migration distance of 6 cm. Tracks that were applied to the HPTLC plate are (from left to right):

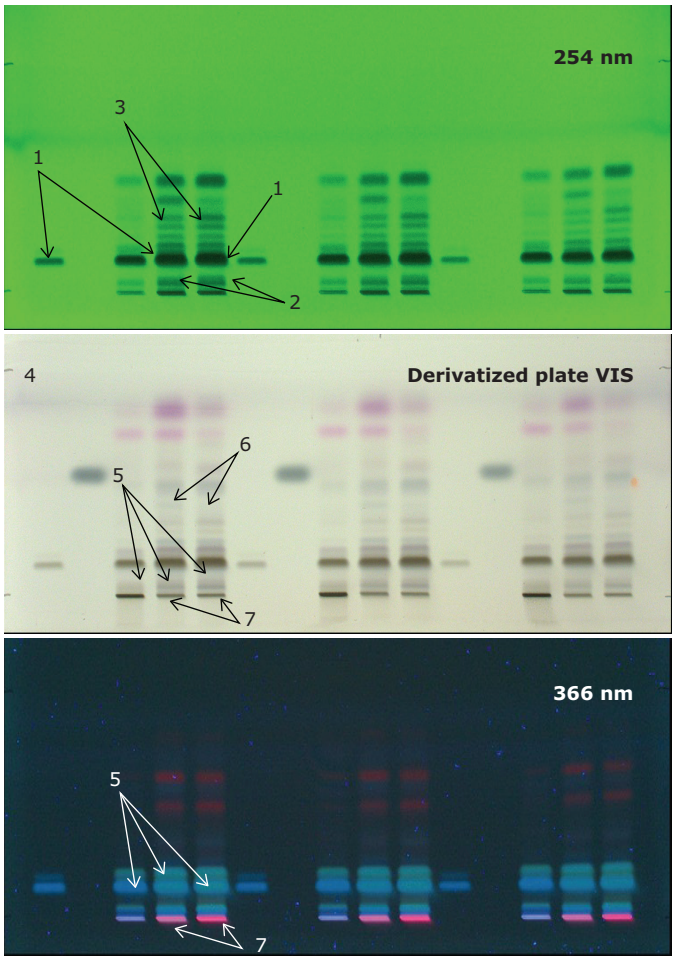
- **Track 1:** 3 µL standard solution A, USP reference standard (RS) of 0.9 mg/mL piperine in methanol.
- **Track 2:** 3 µL standard solution B, a 2 mg/mL borneol standard in methanol.
- **Track 3:** 15 µL standard solution C, USP powdered black pepper extract RS 5 mg/mL in methanol, sonicated, centrifuged, and the supernatant is used.
- **Track 4 & 5:** 7 µL two different commercial pepper samples of the same concentration taken through the same sample preparation steps. (add about 0.5 g of powdered black pepper to 5 mL of methanol, sonicate for 10 minutes, and then use the supernatant).

The resulting HPTLC plate is shown in **Figure 2**.

The conditions & acceptance criteria, i.e., requirements for characterization for black pepper extracts according the HPTLC test in the USP monograph, are identified below:

(continued on next page)

Figure 2. Identification of powdered black pepper by HPTLC as described in USP40-NF35.



UV analysis at 254 nm

Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device. Develop the plate until the solvent front has moved up about 7 cm from the lower edge of the plate. Remove the plate from the chamber, dry, and examine under UV light at 254 nm.

- Under 254 nm, the chromatogram of the sample solution exhibits an intense band at a R_F (retention factor) of about 0.15 corresponding to the piperine band in the chromatogram of standard solution A (Track 1)
- A band at R_F of about 0.02
- Three bands of similar intensity equally spaced located between R_F of about 0.3 and 0.5

Derivatized plate VIS & UV 365 nm

Treat the HPTLC plate with derivatization solution (17 mL of ice-cooled methanol, 2 mL of acetic acid, 1 mL of sulfuric acid, and 0.1 mL of anisaldehyde, mixed in this order), heat for 5 minutes at 100 °C, and examine under white light.

- Under white light, the derivatized chromatogram of the sample solution exhibits main bands similar in position and color to the main bands in the chromatogram of standard solution C.
- These bands include a dark green band of the same color and R_F as the piperine band in standard solution (R_F of about 0.15).
- A weak violet band at R_F of about 0.47 below the position of the band due to borneol in standard solution B.
- A greenish band (in **Figure 2** it is actually pink) in the lower part of the chromatogram at R_F of about 0.07.

It is worth pointing out that other minor bands may be observed in the sample solution and standard solution C chromatograms. No blue bands are detected in the chromatogram of the sample solution at hRF of about 10 and 58 (distinctive from longer pepper). Using the previously mentioned HPTLC plates (glass plate Si 60 F_{254}, 20 x 10 cm, like Cat. No. 1.05642) and following the prescribed recipe in the USP monograph, the acceptance criteria for black pepper under the identification test by HPTLC can be met.

Compositional Analysis by HPLC

The HPLC compositional test described in the monograph, a gradient method, is defining a 4.6 mm x 25 cm, 5 µm, 100 A packing L1 (C18) column. In this study we illustrate that the method system suitability criteria can be met by using a Purospher® STAR, 250 x 4.6 mm, 5 µm, RP-18 endcapped column.

Solution A, or mobile phase A, is a mix of anhydrous potassium dihydrogen phosphate (0.14 g) in 1000 mL of Milli-Q® water incl. 0.5 mL phosphoric acid, while solution B is gradient grade acetonitrile. The gradient profile is shown in **Table 1**. For gradient methods it is important that the column is sufficiently re-equilibrated to obtain good reproducibility. There are several approaches to express the required volume for column re-equilibration. With a flow rate of 1.5 mL/min, an empty tube volume of about 4.2 mL (250 x 4.6 mm column geometry), re-equilibrating is ensured by flushing the column for about 20 minutes or with 30 mL of solvent corresponding to seven complete empty column tube volumes. It can also be expressed in terms of the free/void column volume (depending on packing density and material porosity), which is estimated with 0.1 mL/cm for 4.6 mm ID columns resulting in a „free“ column/solvent volume of 2.5 mL for a 25 cm column. 30 mL solvent thus corresponds to 12 free column volumes. The key is to make sure to flush the column sufficiently to ensure reproducibility.

The sample preparation was done as follows: Black pepper powder (about 2.0 g) was put into a 250 mL flask with a condenser and refluxed with 50 mL HPLC grade methanol for 20 minutes (repeated until last extract was colorless, after which the extracts were combined and concentrated under vacuum). The final

Table 1. Experimental HPLC testing conditions

column:	Purospher® STAR RP-18 endcapped, 5 µm (Cat. No. 1.51456)
mobile phase:	A: anhydrous potassium dihydrogen phosphate (0.14 g) in 900 mL of Milli-Q® water, titrated with 0.5 mL concentrated phosphoric acid. Dilute with water to 1000 mL, mix, filter and degas.
	B: Acetonitrile (gradient grade)
gradient:	95 to 55% A in 8 min, held for 10 min; 55 to 20% A in 7 min; held for 3 min; 20 to 55% A in 7 min; 55 to 95% A in 5 min
flow rate:	1.5 mL/min
pressure:	124-232 bar (1798-3364 psi)
column temp.:	Ambient
detector:	UV 343 & 270 nm
injection:	20 µL
sample:	Black pepper (2 g) extracted with methanol and filtered through a 0.45 µm Millex® PVDF Filter

sample volume was adjusted to 100 mL with methanol, and filtered through a 0.45 µm Millex® PVDF membrane filter, before being introduced into the HPLC system. The system suitability was determined using standard solution A (0.1 mg/mL of USP Piperine RS in methanol) and standard solution B (about 0.5 mg/mL USP powdered black pepper extract RS sonicated, filtered and reconstituted in methanol). Acceptance criteria for the method is defined by the piperine peak tailing factor to be not more than (NMT) 1.5 when analyzing solution A.

Additionally the sample solution chromatogram obtained at 343 nm should exhibit a major peak at the retention time corresponding to piperine (see **Figure 3**). The identification of other piperamide peaks in the sample solution was confirmed by comparing with the standard solution B and the reference chromatogram provided with the lot of USP powdered black pepper extract reference standard. The sample solution chromatogram at 343 nm wavelength shows an additional peak corresponding to piperylene. The chromatogram of the sample solution obtained at 270 nm does not exhibit a peak due to (2E,4E)-N-isobutyldecadienamide at a relative retention time of 1.14 to the piperine peak (distinction from long pepper) (see **Table 2**). Comparing calibration curves for piperine at the two different wavelengths, the 343 nm gives slightly poorer limits of detection (LOD) and quantitation (LOQ) compared to the 270 nm wavelength (data not shown), but both provide an LOD better than 10 ppm.

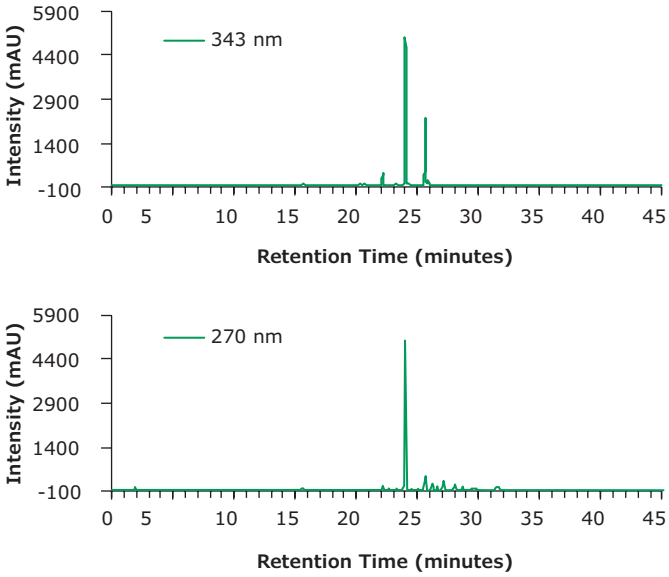
Conclusion

Testing dietary supplement products using regulated chromatographic methods requires an understanding of how different products can be used and operated based on the guidelines of the monograph. In this article we demonstrated that by using suitable HPTLC and HPLC media, appropriate filter, high purity solvents and reagents and following the prescribed USP monograph recipe, you can easily meet the system suitability criteria for black pepper.

Table 2. HPLC performance table

Peak	Compound	RT (min)	Tailing Factor (USP)	RRT
1	t_0 (void volume)	2,5		
2	Piperine	24,0	0,9	1,0
3	(2E,4E)-N-isobutyldecadienamide	25,6	1,0	1,1

Figure 3. Chromatogram



Featured Products

Description	Cat. No.
TLC & HPLC	
HPTLC glass plate Si 60 F_{254}, 20 x 10 cm	**1.05642**
Purospher® STAR RP-18 endcapped 250 x 4.6 mm, 5 µm	**1.51456**
Solvents & Reagents	
Ethyl acetate for liquid chromatography LiChrosolv®	**1.00868**
n-Hexane for liquid chromatography LiChrosolv®	**1.04391**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
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 anhydrous for HPLC LiChropur®	**5.43841**
Ortho-Phosphoric acid 85% for HPLC LiChropur®	**5.43828**
Filtration	
Millex® syringe filter units, disposable, Durapore® PVDF, Pk.1000	**SLHVX13NK**
Standards	
Piperine - United States Pharmacopeia (USP) Reference Standard, 20 mg	**1543200**
Powdered Black Pepper Extract - United States Pharmacopeia (USP) Reference Standard, 1 g	**1509019**

ENVIRONMENTAL

Phosphate in Groundwater and Surface Water

A Rapid and Reliable Determination Method using the Photometric Spectroquant® Test

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Gunter Decker, Senior Global Product Manager, Analytical Point-of-Use Analytics | Photometry, **gunter.decker@emdmillipore.com**

Phosphate in Surface Water

Phosphorus is an essential element for organisms and plants.[1] In natural, uncontaminated waters, it occurs as organically bound phosphate, condensed phosphates or as orthophosphate - often referred to by its chemical formula PO_4-P. The small quantity of phosphorus present in natural waters does not promote the growth of plants. However, a rise in the concentration of phosphorus results in the proliferation of algae, which leads to the eutrophication of the water body.[2, 3]

In the mid-20th century, the anthropogenic contamination of water bodies with phosphate has resulted in widespread eutrophication via fertilizers, wastewater, and washing detergents, among other things. Measures were taken to reduce phosphate concentrations through discontinuation of the use of phosphates in detergents and the precipitation of phosphates in wastewater treatment plants, which brought about a reduction in the phosphate environmental burden of approximately 75%.[2, 4]

A prime example is the case of Lake Constance (Bodensee, Germany). At the end of the 1970s, the concentration of PO_4-P in the lake was 84 µg/L, whereas today this major water body exhibits PO_4-P levels of just 5-6 µg/L.[5, 6]

Analysis of phosphate in surface waters

The analysis of such low concentrations of PO_4-P is challenging.

According to DIN EN ISO 10304-1, the lower working range of the ion chromatography method is 33 µg/L PO_4-P (equivalent to 100 µg/L PO_4), which is considerably higher than the phosphate concentrations usually present in surface waters. Photometry permits significantly more sensitive measurements. According to DIN EN ISO 6878, the lower measurement limit for the photometric determination of phosphorus in water is 5 µg/L PO_4-P, if no laborious extraction procedure is performed.[8]

Spectroquant® Phosphate Test

The Spectroquant® Phosphate Test (Cat. No. 114848) provides an easy-to-use, inexpensive, and sensitive alternative for the reliable quantification of the orthophosphate content in surface waters. The method is analogous to the standard methods for the determination of phosphate, DIN EN ISO 6878, APHA 4500-P E, and EPA 365.2+3.

With the new Prove 600 instrument from the Spectroquant® Prove family, it is now possible to quantify concentrations of PO_4-P at levels as low as 2.5 µg/L by using the 100 mm cuvette, thus allowing the swift and reliable measurement of low PO_4-P concentrations in surface waters.

In addition to the sensitivity of measurement, the Spectroquant® Phosphate Test offers a further advantage. In contrast to the classic photometric method, there is no need for the time-consuming calibration step. All Spectroquant® instruments are pre-programmed with a ten-point calibration curve to enable the exact determination of the phosphate content of the water samples.

The use of various cell sizes (10 mm, 20 mm, 50 mm and 100 mm in the Spectroquant® Prove 600) under application of the Beer-Lambert law, gives an overall measurement range of 0.0025 to 5.00 mg/L PO_4-P, permitting the direct quantification of unknown phosphate concentrations of samples without the need to first establish a calibration curve.

Method comparison of Spectroquant® Phosphate Test

(Cat. No. 114848) and photometry in accordance with DIN EN ISO 6878

Both methods are based on the same principle. Orthophosphate ions react with molybdate and antimony ions in sulfuric acid solution to produce molybdatophosphoric acid. This in turn is reduced with ascorbic acid to form phosphomolybdenum blue (PMB), which is determined photometrically.[9]

In addition to the pre-programmed calibration curve, a further advantage offered by the Spectroquant® test kit, when compared with the standard method, is its ease of use. When performing measurements according to the DIN EN ISO 6878 method, the reagents must be prepared separately. This involves the preparation of at least five separate solutions, not including the standard solutions, which must be prepared for the calibration procedure.[8]

The Spectroquant® test kit already contains the reagents required for the analysis in a ready-to-use form. For the sensitive determination of phosphate in the 100-mm cell, all that is necessary is the addition of two reagents. To a 20 mL sample volume a user needs only to add 20 drops of reagent PO_4-1 and four level spoons of reagent PO_4-2 (using the micro spoon provided). After a reaction time of five minutes, the color intensity of the sample is measured in the photometer, and the concentration of phosphate can be directly read from the photometer display. Detailed instructions are given in the application description, "Sensitive measurement of orthophosphate in groundwater and surface water," which is available online on the product website of the Spectroquant® Phosphate test Cat.No. 1.14848.[10]

As a means to gain expressive statements on the suitability of the Spectroquant® test kit for the determination of the phosphate concentration in surface waters, six samples were investigated for their PO_4-P content using this test kit. For comparison a reference analysis according to the DIN EN ISO 6878 method was also performed. A detection limit of 0.003 mg/L PO_4-P for the DIN method was determined in accordance to DIN 32645.

Table 1. presents the results obtained using the Spectroquant® test kit versus those obtained by the DIN EN ISO 6878 method.

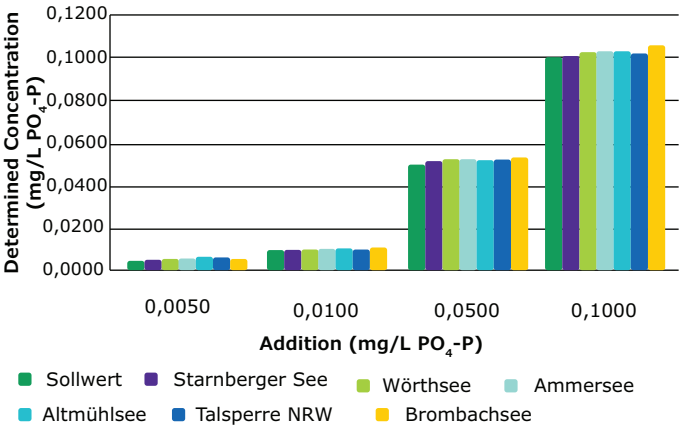
Table 1: Comparison of the measurement results for the Spectroquant® test kit
(Cat. No. 114848) and acc. to DIN EN ISO 6878

Surface water	ß [mg/L PO_4-P]	
	Spectroquant®- Test	DIN EN ISO 6878
Lake Starnberger See	< 0.0025	< 0.003
Lake Wörthsee	< 0.0025	< 0.003
Lake Altmühlsee	0.0126	0.0130
Lake Brombachsee	0.0025	0.0056
Lake Ammersee	< 0.0025	< 0.003
Dam reservoir North Rhine Westfalia	< 0.0025	< 0.003

The two methods show comparable results: in four of the six samples, the PO_4-P concentration was below the measurement range. In only two of the six samples were the measured values within the range. In the sample from the Brombach Lake, the detected concentrations differed by 0.0031 mg/L PO_4-P. In the sample from Altmühl Lake, the deviation between the Spectroquant® result and the DIN result was a mere 0.0004 mg/L PO_4-P. The recovery rate with regard to the DIN EN ISO method was 97%.

In addition to the reference analysis procedure, samples were also measured using the standard addition method. In this investigation, the five lake water samples and one reservoir sample were spiked with four different concentrations of PO_4-P and the recovery in each sample was determined. **Figure 1** shows the results of this investigation.

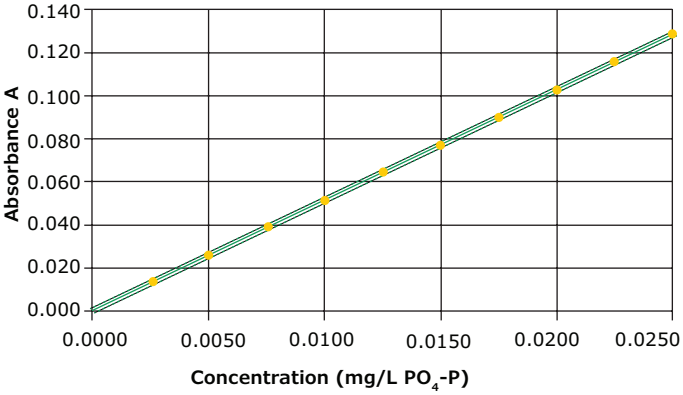
Figure 1: Orthophosphate concentration recovered after standard addition



The added standard concentrations could be recovered in all samples. The average recovery rate was 105% ± 5%.

If it is necessary to enhance the accuracy still further, it is possible to perform a user calibration for the intended measurement range. This is demonstrated by a ten-point calibration curve that was recorded for the measurement range 0.0025 – 0.0250 mg/L PO_4-P. The plotted graph is shown in **Figure 2**.

Figure 2: Calibration curve for the measurement range 0.0025 – 0.0250 mg/L PO_4-P



(continued on next page)

The method shows a good linearity. Compared with the pre-programmed method, the performance characteristics could be considerably improved; achieving a method standard deviation of ±0.0001 mg/L, a method coefficient of variation of ±0.88%, and a confidence interval of ±0.0003 mg/L (see **Table 2**).

Table 2: Comparison of performance characteristics

	Pre-programmed method	User calibration
	0.0025 – 0.5000 mg/L PO_4-P	**0.0025 – 0.0250 mg/L PO_4-P**
Method standard deviation (mg/L)	0.0029	0.0001
Method coefficient of variation (%)	1.2	0.88
Confidence interval (mg/L)	0.006	0.0003

Summary

The Spectroquant® Phosphate Test (**Cat. No. 114848**) is a rapid, inexpensive, and precise alternative to the standard methods for the determination of orthophosphate in groundwater and surface waters. The results are comparable with those obtained using the DIN EN ISO 6878 standard method. By using the standard addition method, it was demonstrated that the test kit is suitable for the quantification of PO_4-P in groundwater and in surface waters.

References

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4. G. Metzner, *Phosphate aus Wasch- und Reinigungsmitteln im kommunalen Abwasser der Bundesrepublik Deutschland*, **2006**.
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6. Bodensee-Wasserversorgung, http://www.zvbwv.de/index.php?id=135 [date of access: 25 August 2016]
7. DIN EN ISO 10304-1:2009-07, Wasserbeschaffenheit – Bestimmung von gelösten Anionen mittels Flüssigkeits-Ionenchromatographie – Teil 1: Bestimmung von Bromid, Chlorid, Fluorid, Nitrat, Nitrit, Phosphat und Sulfat (ISO 10304-1:2007)
8. DIN EN ISO 6878:2004-09, Wasserbeschaffenheit – Bestimmung von Phosphor – Photometrisches Verfahren mittels Ammoniummolybdat (ISO 6878:2004)
9. Package leaflet Spectroquant® Phosphate Test, Cat. No. 114848, January 2016. SigmaAldrich.com/catalog/product/mm/114848 under “Protocols& Articles”

Ordering Information:

Description	Cat. No.
Spectroquant® Phosphate Test Kit	**1.14848**
Spectroquant® Prove 600 UV/VIS Spectrophotometer	**1.73018**
Water for analysis	**1.16754**

SigmaAldrich.com/spectroquant

ENVIRONMENTAL

Achieve Exceptional Resolution of PAHs, Including Several Isomer Sets

Using SLB®-ILPAH Capillary GC Columns

Lisa McCombie, ProductManager GC, **lisa.mccombie@sial.com**;
and Len Sidisky, R&D Manager Gas Chromatography

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment. They belong to a group known as persistent organic pollutants (POPs). Monitoring is important because they are identified as carcinogens. Multiple isomers exist, which are difficult to resolve chromatographically. SLB®-ILPAH is a special purpose column based on an ionic liquid stationary phase. A distinct combination of stationary phase selectivity and efficient column dimensions allow exceptional resolution of PAHs, including several isomer sets. Complete column specifications are listed in **Table 1**.

Table 1. SLB®-ILPAH Column Specifications

Application:	This special purpose and specially tested capillary GC column is designed for the analysis of polycyclic aromatic hydrocarbons (PAHs). It incorporates an ionic liquid stationary phase. Each column is individually tested to ensure resolution of several key sets (phenanthrene / anthracene, benzo[a]anthracene / chrysene / triphenylene, and benzo [b]fluoranthene / benzo[k]fluoranthene / benzo[j]fluoranthene).
USP Code:	None
Phase:	Non-bonded; 1,12-Di(tripropylphosphonium) dodecane bis(trifluoromethanesulfonyl)imide
Temp. Limits:	Subambient to 300 °C (isothermal or programmed)

Resolution Test

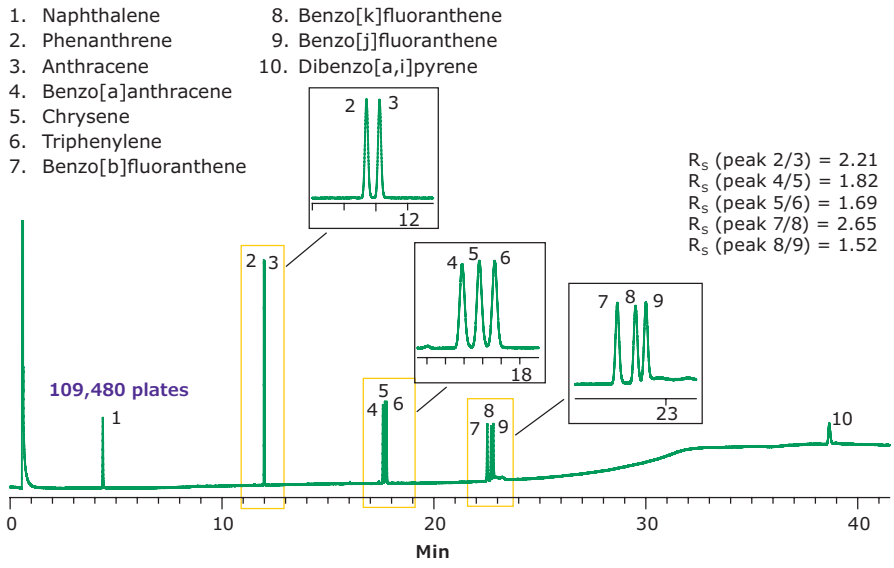
Every SLB®-ILPAH column is specialty tested to ensure it meets stringent resolution requirements for several sets of PAHs. Figure 1 depicts a chromatogram obtained from analysis of the QC test mix. The resolution (RS) results obtained from this chromatogram are:

- 2.21 for phenanthrene/anthracene
- 1.82 for benzo[a]anthracene/chrysene
- 1.69 for chrysene/triphenylene
- 2.65 for benzo[b]fluoranthene/benzo[k]fluoranthene
- 1.52 for benzo[k]fluoranthene/benzo[j]fluoranthene

Column efficiency is also determined by measuring the theoretical plate value of naphthalene. For this chromatogram, it was good, based on the value of 109,480 plates that was obtained.

Figure 1. QC Test Mix

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



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22-Component PAH Mix

Multiple regulatory agencies around the world have promulgated methodologies for the analysis of PAHs. The number of analytes listed in these methods ranges from 16 to 24. To present data more accurately aligned with real-world usage, a 22-component PAH mix which contains the most frequently listed PAHs was analyzed on a SLB®-ILPAH column. The resulting chromatogram is shown in Figure 2. The significant finding is that this column has the necessary selectivity to provide exceptional resolution for several sets of PAHs, such as peaks 5/6, peaks 9/10, and peaks 12/13/14. Also of great interest is that this column can provide baseline separation of dibenz[a,h]anthracene (peak 16) and indeno[1,2,3-cd]perylene (peak 17). This last pair typically co-elutes on other columns, and requires the use of mass spectrometry (MS) for proper identification.

Conclusion

The main strength of ionic liquid GC columns is unique selectivity. This often results in increased resolution compared to columns made with polysiloxane polymer or polyethylene glycol columns. The analysis of PAHs is an example of how an ionic liquid column can achieve a level of separation not possible with other columns. In this case, it is the specially tested SLB®-ILPAH.

Featured Product

Description	Cat. No.
SLB®-ILPAH Capillary GC Column, 20 m × 0.18 mm I.D., 0.05 µm	**29799-U**

Related Product

Description	Cat. No.
SLB®-ILPAH Capillary GC Column, 15 m × 0.10 mm I.D., 0.03 µm	**Inquire**

Multiple applications, product information, real-time availability, and ordering information is available 24 hours a day at
SigmaAldrich.com/il-gc

Did you know?

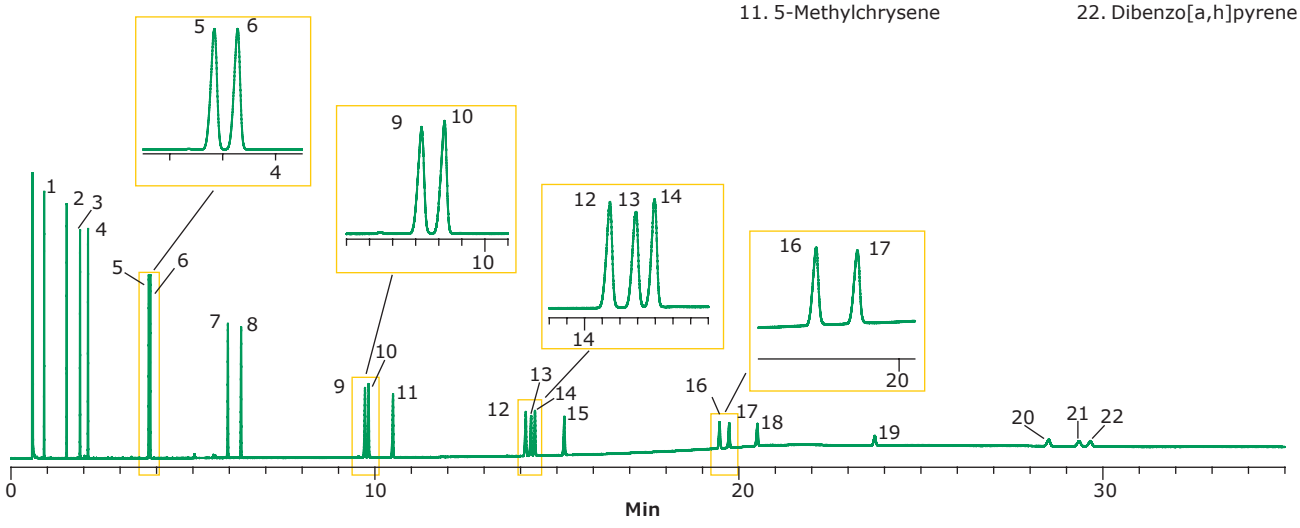
We offer a wide variety of single-component and multi-component PAH mixtures available as certified reference materials (CRMs). Additionally, we have great custom capabilities.

To learn more, visit
SigmaAldrich.com/pahstandards

Figure 2. 22-Component PAH Mix

column:	SLB®-ILPAH, 20 m × 0.18 mm I.D., 0.05 µm (29799-U)
oven:	150 °C, 15 °C/min to 225 °C, 5 °C/min to 300 °C (15 min)
inj. temp.:	300 °C
detector:	FID, 310 °C
carrier gas:	hydrogen, 1.3 mL/min, constant flow
injection:	1 µL, 300:1 split
liner:	2.3 mm I.D., split/splitless type, wool packed straight FocusLiner™ design
sample:	22 analytes, each at 100 µg/mL in methylene chloride

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



COSMETIC & PERSONAL CARE

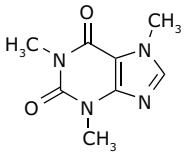
Detection and Determination of Caffeine, Taurine and Arginine in Shampoos by HPTLC

Michaela Oberle, Scientist Instrumental Analytics R&D, **michaela.oberle@emdmillipore.com**
Hans Griesinger, Scientist Instrumental Analytics R&D, **hans.griesinger@emdmillipore.com**

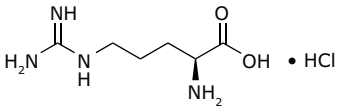
During the last few years a new trend in hair care products (shampoos) has become apparent. The cleaning ability of the product is no longer the primary focus as the cosmetic industry has started to develop products with additional features. Today, anti-dandruff shampoos or shampoos with repair capability are available in every supermarket. With slogans such as “anti-aging for hair”, “youthful hair”, “anti-hair loss system” or “hair energizer”, the cosmetics industry addresses customer needs. One of the reasons for this new focus might be a demographic change in the consumer society. The use of semi-natural additives also seems to be part of the trend. Therefore, there is a need for analytical methods to identify and to quantify these substances.

The application of modern thin-layer chromatography (TLC) offers advantages, especially in the field of cosmetics. Many samples can be analyzed quickly and simultaneously under exactly the same conditions. The ingredients usually can be separated directly from complex matrices and quantified. The separation of the typical cosmetics matrix components such as oils, fats and other components of the formulation is achieved by selecting the appropriate mobile phase, or if applicable, a TLC plate with concentrating zone. Straight forward coupling with other methods such as MS or NMR is also possible. Another advantage is the imaging evaluation, in which the results are presented as a picture and in some cases are self-explanatory.

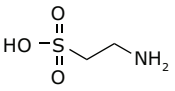
Here the quantification of Caffeine and Taurine in shampoo is described by densitometry. The identity of arginine was detected by HPTLC-MS (data not shown). Since Arginine has not been moved from the starting point, a mobile phase optimization would be needed for precise quantification, which was not performed in this study due to time constraints.



Caffeine



L-Arginine



Taurine

TLC Conditions/Parameters:

Sample and standard solutions

- 1 g of each shampoo sample was stirred with 10 mL of Isopropanol at room temperature and filtered with a 0.45 micron syringe filter.
- Standards: Solutions of Caffeine, Taurine and Arginine 1 mg/mL in Methanol

Layer

- HPTLC silica gel 60 F_{254}, 10 x 10 cm (Cat. No. 1.05628)

Sample application

- Band wise with Automatic TLC Sampler 4, band length 5 mm, distance between bands 10 mm, distance from the lower edge 10 mm, application volume 0.1 – 5 µL

Chromatography

- In a twin through chamber 10 x 10 cm (migration distance 50 mm) with Isopropanol – n-Heptane – Water 7:3:1 (v/v/v) for Caffeine and Isopropanol - Water 8:2 (v/v) for Arginine and Taurine.

Derivatization (only Arginine and Taurine)

- Spraying with Ninhydrin reagent (Cat. No. 1.06705.0100)

(continued on next page)

Documentation

- With DigiStore 2, Caffeine chromatogram (without derivatization) under UV 254 nm; Arginine/Taurine chromatogram (after derivatization) under white light.

Densitometry

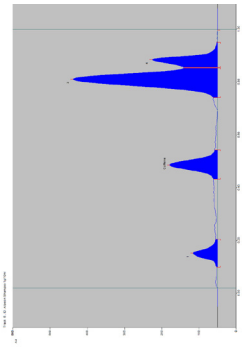
- With TLC Scanner 3, Caffeine chromatogram (without derivatization) with adsorption measurement at UV 254 nm; Arginine/Taurine chromatogram (after derivatization) with adsorption measurement at 600 nm (slit dimension 4 x 0.3 mm, scanning speed 20 mm/s)

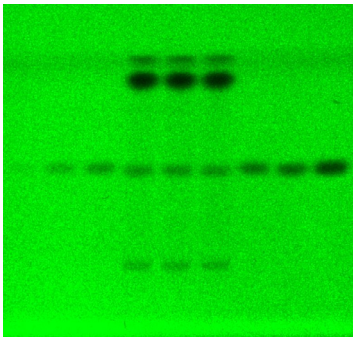
Results and discussion

Caffeine could be identified with a hR_F- value of 54. The quantification using peak areas resulted after triple determination of the shampoo sample 1, and 6 point calibration to an average content of the target substance of 94 mg/g shampoo (track 4: 90 mg, track 5: 91 mg, and track 6: 100 mg), based on the amount in the cosmetic formulation. Quantification was made using the polynomial regression based on the peak area (r = 0.99906, sdv = 3.94%). The amount of caffeine found corresponds to the commonly present amount of 0.1% active ingredient in a formulation.

Figure 1. Scan of track 5 and picture of the whole plate at UV 254 nm

Track	Substance	Application volume µL
1	Caffeine	0.1
2	Caffeine	0.3
3	Caffeine	0,5
4	Shampoo	5
5	Shampoo	5
6	Shampoo	5
7	Caffeine	0.8
8	Caffeine	1
9	Caffeine	2

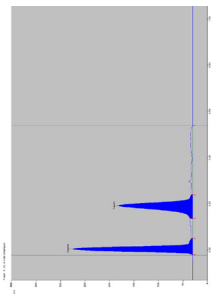


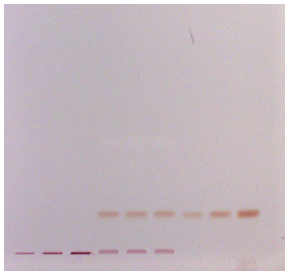


Taurine and Arginine: The plate was derivatized with ninhydrin spray solution and documented under white light. Arginine was not moved under these chromatographic conditions. Taurine could be identified with a hR_F value of 24. The quantification of the peak areas by triple determination of the shampoo sample 2 and a 3 point calibration resulted in an average for the content of taurine of 75 mg/g shampoo (track 4: 72 mg, track 5: 77 mg, track 6: 75 mg, CV = 2.971, n = 3), based on the amount of cosmetic formulation. This corresponds to the usual amount of 0.1% active ingredient in a formulation. Since arginine has not been moved from the starting point, a mobile phase optimization would be needed for precise quantification, which was not performed in this study due to time constraints.

Figure 2. Scan of track 6 at 600 nm and picture of the whole plate after derivatization under white light

Track	Substance	Application volume µL
1	Arginine	0.5
2	Arginine	1
3	Arginine	2
4	Shampoo	5
5	Shampoo	5
6	Shampoo	5
7	Taurine	0.5
8	Taurine	1
9	Taurine	2





Conclusion

The above presented data demonstrates that HPTLC can be used to determine caffeine and taurine in a shampoo matrix. With further method adjustments also arginine potentially can be quantified. In conclusion this study demonstrates that HPTLC is a viable & reliable tool for efficient & economic analysis of active ingredients in cosmetic samples, that typically contain a high matrix load.

Ordering Information

Description	Package Size	Cat. No.
HPTLC silica gel 60 F_{254}	25	**1.05628.0001**
Ninhydrin spray solution	100 mL	**1.06705.0100**
Certified Reference Materials		
L-Arginine monohydrochloride, *Trace*CERT®	100 mg	**90538**
Caffeine, *Trace*CERT®	100 mg	**56396**
Taurine (new), *Trace*CERT®	100 mg	**93019**
Related Products		
2-Propanol gradient grade for liquid chromatography LiChrosolv®	Var.	**1.01040**
Methanol gradient grade for liquid chromatography LiChrosolv®	Var.	**1.06007**
n-Heptane for liquid chromatography LiChrosolv®	Var.	**1.04390**
Millex®-LCR Syringe Filter, 0.45 µm Hydrophilic PTFE Membrane Water from Milli-Q® System	100 & 1000	**SLCR013**

See also page 31

PHARMA & BIOPHARMA

Elemental Impurities

Certified Reference Materials for ICH Q3D, USP<232> & <2232> and Ph.Eur. 5.20

Ingrid Hayenga, Product Manager Reference Materials, **ingrid.hayenga@sial.com**

Metallic contamination in drug products, referred to as elemental impurities, may arise from several sources. They may be added intentionally in synthesis, or may be present as contaminants, (e.g., through interactions with processing equipment or by being present in components of the drug product) and are consequently detectable in the drug product. Since elemental impurities pose a risk to patient health due to toxicological effects, element impurity levels should be controlled within acceptable limits in the drug product.[1]

Evolution of ICH Q3D guidelines

In 2009 the International Conference on Harmonization (ICH) proposed the development of a new harmonized guideline to provide a global policy for limiting metal impurities in drug products and ingredients. This approach should provide clear regulatory guidance on specification limits for elemental impurities worldwide and logically should have an impact on the work of the national regulatory bodies in having transparent and comparable results.

In a step 4 version of its "Guidelines for Element Impurities" document, the ICH categorized the various elemental impurities in four different classifications which were intended to facilitate decisions during the risk assessment process:

Class 1: impurities are significantly toxic to humans and have limited or no use in the manufacture of pharmaceuticals. They can be found as impurities from commonly used materials (e.g., mined excipients). All four elements require evaluation during the risk assessment across all potential sources of elemental impurities and routes of administration.

The class 1 elements are: As, Cd, Hg, Pb.

Class 2: impurities are generally considered route-dependent human toxicants. These impurities are further divided into two sub-classes, 2A and 2B, based on their relative likelihood of occurrence in the drug product.

- Class 2A elements have relatively high probability of occurrence in the drug product and thus require risk assessment across all potential sources of elemental impurities and routes of administration (as indicated). The class 2A elements are: Co, Ni and V.
- Class 2B elements have a reduced probability of occurrence in the drug product related to their low abundance and low potential to be co-isolated with other materials. As a result, they may be excluded from the risk assessment unless they are intentionally added during the manufacture of drug substances, excipients or other components of the drug product.

Class 2B elements are: Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Se and Tl.

Class 3: includes elements which have relatively low toxicity at oral administration but may require a risk assessment if applied via inhalation or parenteral routes.

Class 3 elements are: Ba, Cr, Cu, Li, Mo, Sb and Sn.

Other elements: There are some elemental impurities for which Permitted Daily Exposures (PDEs) have not been established due to their low toxicities and/or differences in regional regulations. If they are present in a drug product, they are addressed by other guidelines and/or regional regulations.

These elements are: Al, B, Ca, Fe, K, Mg, Mn, Na, W and Zn.

Evaluation of USP and EP

Up to 2010, the USP and EP proof of heavy metal contamination in drugs was obtained via a colorimetric analytical method based on the precipitation of a metal sulfide in a sample and comparing it to a lead standard (USP <231> and Ph.Eur. 2.4.8).

Based on the Guideline for Elemental Impurities (Q3D) which was published by the International Conference on Harmonization (ICH) in 2010, the USP proposed three new General Chapters covering impurity limits, analytical procedures in pharmaceutical products and raw materials, and elemental contaminants in dietary supplements.

- Chapter USP <232>, Ph.Eur. 5.20: Elemental Impurities in Pharmaceutical Products - Limits
- Chapter USP <233>: Elemental Impurities in Pharmaceutical Products – Procedures
- Chapter USP<2232>: Elemental Contaminants in Dietary Supplements

(continued on next page)

In January 2015, the USP established January 1, 2018 as the new date of applicability for General Chapters <232>, <233> and <2232>. The implementation should align with limits and timelines set down by other pharmaceutical and medical agencies such as the ICH Q3D Step 4 Guidelines for Elemental Impurities announced on December 16, 2014.

The Pharmacopoeia Europe announced in July 2014 their strategy regarding elemental impurities and the implementation of the ICH Q3D. Nearly one year later, in April 2015, they published their policy on elemental impurities and timelines for revision of general and individual texts. In August of the same year, clarification was given for products outside the scope of ICH Q3D.

The implementation of the guideline compliances should start in June 2016 for products with new marketing authorization, either containing new active substances or already approved substances.

Marketed products, including new mutual recognition applications of already approved substances, should comply with the Guideline from December 2017.

The implementation of the General Test 5.20 and the General Method 2.4.20 replaced the EMA guideline on metal catalysts and metal reagents by the principles of the ICH. The publication was done in the Ph.Eur. Suppl. 9.3 (implementation date January 1, 2018), having no test for elemental impurities in the individual monographs except for substances of natural origin. Given the intrinsic nature of elemental impurities in these substances, they are among the major potential sources of elemental contamination in medicinal products. The Ph.Eur. Commission has also specifically recommended keeping the different tests for elements for which no PDE limits have been established, i.e., those identified as “other elements” in the ICH Q3D guideline in individual monographs.[2]

Analytical methods

Concerning new analytical methods, ICH Q3D does not include any recommendation on instrumental methods but the following analytical procedures are suggested in USP<233> dependent on the expected concentration of the elemental impurity in the product or component:

- Parts-per-million (ppm) concentrations
  ICP-OES or atomic absorption
- Parts-per-billion (ppb) concentrations
  ICP-MS

ICH Q3D limits for elemental impurities

For a total of 24 elements, toxicity limits are specified and defined as maximum PDE levels in mg/day for the four major drug delivery categories. **Table 1** lists the PDE values in µg/day, valid for drug products with an intake of ≤10 g/day.

Table 1. Permitted daily exposure (PDE) for elemental impurities

Element	Class	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalation PDE (µg/day)
As	1	15	15	2
Cd	1	5	2	2
Hg	1	30	3	1
Pb	1	5	5	5
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rb	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	3000	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

Table 2 lists the elements to be considered in the risk assessment.

For the new adapted USP <232> and Ph.Eur.Suppl. 9.3 chapters, we offer three *Trace*CERT® element mixes with element ratio corresponding to the oral concentrations of the ICH Q3D guideline, mix I covers class 1, 2A and some of 2B elements; mix II covers the remaining 2B class elements; mix III covers all class 3 elements.

A second series of three mixes covers the parenteral concentration ratios.

All products with their element respective concentrations (mg/L) are listed in **Table 3**.

Table 4 lists the features of the *Trace*CERT® Certified Reference Material (CRM) solutions.

Table 2. Elements to be considered in the risk assessment

Element	Class	If Intentionally Added (all routes)	If not intentionally added		
			Oral	Parenteral	Inhalation
As	1	Yes	Yes	Yes	Yes
Cd	1	Yes	Yes	Yes	Yes
Hg	1	Yes	Yes	Yes	Yes
Pb	1	Yes	Yes	Yes	Yes
Co	2A	Yes	Yes	Yes	Yes
V	2A	Yes	Yes	Yes	Yes
Ni	2A	Yes	Yes	Yes	Yes
Tl	2B	Yes	No	No	No
Au	2B	Yes	No	No	No
Pd	2B	Yes	No	No	No
Ir	2B	Yes	No	No	No
Os	2B	Yes	No	No	No
Rb	2B	Yes	No	No	No
Ru	2B	Yes	No	No	No
Se	2B	Yes	No	No	No
Ag	2B	Yes	No	No	No
Pt	2B	Yes	No	No	No
Li	3	Yes	No	No	No
Sb	3	Yes	No	No	No
Ba	3	Yes	No	No	No
Mo	3	Yes	No	No	No
Cu	3	Yes	No	No	No
Sn	3	Yes	No	No	No
Cr	3	Yes	No	No	No

Table 4. Features of the TraceCERT® CRMs

*Trace*CERT® Solutions
Unique level of accuracy and lot-specific value
Produced according to ISO Guide 34 and analyzed in our ISO/IEC 17025 accredited lab; traceable to at least two independent references (NIST, BAM or SI unit kg)
Sophisticated packaging and comprehensive documentation including proper uncertainty calculation, expiry date and storage information
Packaged in opaque and gas-tight aluminum foil bags for extended stability. Certificates are included and list up to 70 trace impurities for the *Trace*CERT® products.
250 mL package size*

For more information and to view sample certificates, please visit
SigmaAldrich.com/inorganiccrm

References:

1. ICH Q3D limits from Step 4 version, December 16, 2014 Option 1
2. Thermo Fischer, the Medicine Maker, Edition 4 – August 2016100

Table 3. Suitable Multi-Element CRM Solutions according to ICH Q3D

Element	Class	*Trace*CERT® Elemental Impurities Mix according to ICH Q3D oral			*Trace*CERT® Elemental Impurities Mix according to ICH Q3D parenteral		
		Standard 1	Standard 2	Standard 3	Standard 1	Standard 2	Standard 3
		Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.
		19041	73108	69729	89118	89922	07368
		In 12% HNO_3	In 10% HCl	In 5% HNO_3 & HF<0.5%	In 12% HNO_3	In 10% HCl	In 5% HNO_3 & <0.5% HF
Ag	2B	150 mg/L			10 mg/L		
As	1	15 mg/L			15 mg/L		
Au	2B		100 mg/L			100 mg/L	
Ba	3			140 mg/L			70 mg/L
Cd	1	5 mg/L			2 mg/L		
Co	2A	50 mg/L			5 mg/L		
Cr	3			1100 mg/L			110 mg/L
Cu	3			300 mg/L			30 mg/L
Hg	1	30 mg/L			3 mg/L		
Ir	2B		100 mg/L			10 mg/L	
Li	3			55 mg/L			25 mg/L
Mo	3			300 mg/L			150 mg/L
Ni	2A	200 mg/L			20 mg/L		
Os	2B		100 mg/L			10 mg/L	
Pb	1	5 mg/L			5 mg/L		
Pd	2B		100 mg/L			10 mg/L	
Pt	2B		100 mg/L			10 mg/L	
Rh	2B		100 mg/L			10 mg/L	
Ru	2B		100 mg/L			10 mg/L	

(continued on next page)

The Importance of the Standardization of Volumetric Solutions

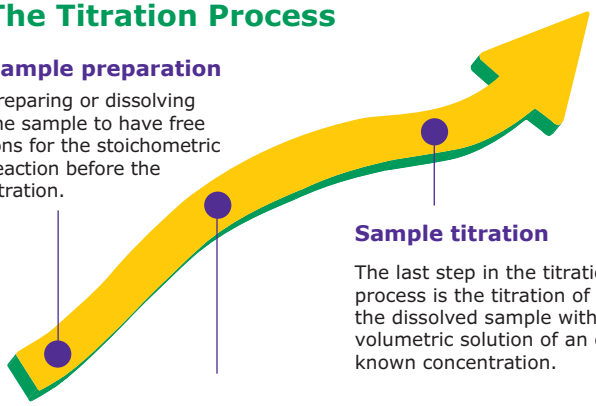
Bettina Straub-Jubb, Global Product Manager Titration, **bettina.straub-jubb@emdmillipore.com**

The so-called titer determination or standardization of a volumetric solution used for titration is one of the most important preconditions for reliable and transparent titration results.

Accurate and reliable titration results are only achievable when we work with the exact concentration of the volumetric solution.

The nominal concentration of a volumetric solution used as a titrant in the titration process is known. The concentration could differ from the real concentration because of a variety of influences. The necessity to determine the real concentration with a titrimetric standard is import in order to obtain correct titration results.

The Titration Process



Sample preparation

Preparing or dissolving the sample to have free ions for the stoichometric reaction before the titration.

Sample titration

The last step in the titration process is the titration of the dissolved sample with a volumetric solution of an exact known concentration.

Titer determination

So-called standardization of the titration system to correct measurement uncertainties through the instrument, the volumetric solution, the handling, the temperature in the lab, and the balance.

Use of a volumetric standard with a known concentration to determine the correct concentration of volumetric solution together with the other uncertainties mentioned above.

The Titer Determination

The titer is defined as the quotient of the nominal concentration of a volumetric solution and the actual concentration. The calculated factor is then used as the correction factor to the titrant. The measured value of the titer is multiplied with the nominal concentration.

$$\text{Titer} = c(x) / c\sim(X)$$

$$c = c\sim(x) \times \text{titer}$$

Titer = factor
c = actual molar concentration of the volumetric solution
c~ = nominal molar concentration of the volumetric solution

The Titration process is influenced by the following factors:

- Measuring method
- Instrument (instrument uncertainty/abrasion of the burette)
- Electrodes (electrode uncertainty/alteration of electrodes)
- Handling
- Balance (weighing error)
- Temperature
- Volumetric solution (change of the concentration, through alteration, carbon dioxide, evaporation of water/solvent)

All the mentioned points could be eliminated with the determination of the titer as a correction factor.

It is important to exactly define both the sample method and the titer method to receive correct titration and titer results. The instrument itself could differ slightly, as well as the burette, which would change the volume, especially with alkaline solutions; therefore, a titer determination must be done with the same instrument used for the sample determination. Depending on usage and treatment of the electrodes, their lifetime and accuracy can vary. Refined distinction in the handling could also influence the results. Another important variation is the volume change of a volumetric solution at different temperatures. The temperature influence for an aqueous solution is 0.02% per degree and 0.1% per degree for organic solutions. Working in an air-conditioned lab is preferred. Also, the accuracy of the balance and the weighing process is important. The weighed portion of a substance must be great enough for a correct titer result. Ideally, if the concentration of the volumetric solution is quite low, one can also prepare a solution of the volumetric standard and weigh in a higher amount of the standard, which reduces the weighing error.

The stability of the titrants themselves are different and depend on the storage conditions. In general, it can be stated that alkaline solutions and Titriplex® solutions are less stable than acid solutions. Alkaline solutions, for example, absorb carbon dioxide from the environment and their concentration can change rapidly. Iodine solutions and potassium permanganate solutions are light sensitive. A di-Ammonium iron(II)-sulfate solution is a quite unstable volumetric solution and needs a more frequent concentration determination. These are only a few examples.

To compensate for all of these influences in your titration process, a regular titer determination is recommended. The frequency is dependent on the kind of solutions and the conditions in the laboratory. Using Titripac® packaging, the solution itself is stable and not influenced by alteration, carbon dioxide, evaporation of water or solvent, or microbes. With Titripac®, the frequency of a titer determination could be reduced. It is recommend to do always a titer determination after opening a new bottle or Titripac®. Depending on the stability of the solutions and the conditions in the lab, the time between further titer determinations can be defined individually by the user. The Certipur® volumetric standards are high purity solid substances determined with exact assays and are directly traceable to NIST (National Institute of Standards and Technology Gaithersburg, USA) volumetric standards. They are recommended for an exact titer determination. It is important to dry them before use.

Conclusion

The base for every accurate and reliable titration method are high quality volumetric solutions and accurate high purity standards for a correct and reliable titer determination.

The Titripur® volumetric solutions portfolio is comprised of high quality solutions for a wide range of applications, and in combination with the high-purity Certipur® volumetric standards and the individual support from our experts, titration processes are made accurate and reliable.

Explore the Titripur® volumetric solutions on our titration page
SigmaAldrich.com/titration

More information on the Certipur® volumetric standards on
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Certipur® volumetric standard

Titripur® volumetric solution

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IN ESSENCE

The 2017 ACS Award in Chromatography Sponsored by MilliporeSigma

The American Chemical Society (ACS) recognized pioneers in the chromatography field by establishing the Award in Chromatography and Electrophoresis in 1959. In 1970, when the award became dedicated solely to chromatography, Supelco, after only three years as a company, began sponsoring this prestigious award. Our uninterrupted sponsorship continues now through MilliporeSigma.[1]

To receive this recognition, the winner must have made an outstanding contribution to the field of chromatography with particular consideration given to developments of new methods. The list of past award recipients is virtually a who's who in chromatography pioneers (**Table 1**). The 2017 winner is Dr. Robert (Bob) Kennedy, the Willard Professor of Chemistry, professor of pharmacology, and Distinguished University Professor at the University of Michigan in Ann Arbor.[2] Dr. Kennedy was cited for the development of innovative techniques in miniaturization of chemical separations and microfluidics for highly sensitive analysis of biological compounds.

Caption: Dr. Kennedy (center) receiving his award from Dr. Paul Ross (right), Director R&D and Technology, Analytical Separations, MilliporeSigma. On the left is ACS President Dr. Allison Campbell.

We posed some questions to Dr. Kennedy and wanted to share his insightful answers with our readers.

MilliporeSigma: There are so many different chemistry disciplines to choose from. What made you gravitate toward analytical chemistry, and separations in particular?

Dr. Kennedy (BK): I started out in organic chemistry as an undergraduate researcher simply because I first learned that undergrads could do research during my organic class. After working on it for a while I found that I was much better at getting the instruments to work than getting the reactions to work. I was always drawn to the more mathematical aspects of chemistry and liked physical/analytical the best. Just something cool about being able to express results in numbers.

MilliporeSigma: What keeps you writing those grants year after year?

BK: I need the money! But seriously, an interesting thing about analytical chemistry is that you are never done. Once you get a good result, there is always more improvement to be had. We have spent years trying to develop "separations-based sensors" to monitor brain chemistry in vivo. We have made great improvements over the state of the art 20 years ago, but still we can't capture many important chemical changes in the brain. We have a long way to go.

MilliporeSigma: High throughput screening has played role in your research, especially as it applies to bioanalysis. Are there any particular bottlenecks in the analytical, and especially LC/MS, workflow that your team is trying to address?

BK: For us it has been about getting the sample into the instrument. There are fast chromatographs and mass spectrometers, but sample injection is slower than the separation. Fixing this is "boring" but necessary.

MilliporeSigma: When you look at global current events, what role do you think we, as chemists, can play toward painting a brighter future for humankind?

BK: I am writing this the day after the "March for Science" which really gets you thinking about exactly this question. It is pretty clear that just about any challenge we face requires some chemistry-based improvements (but also political will and societal drive): feeding the world in a more energy efficient manner, producing clean energy, decreasing the cost of advanced medical diagnostics and medicines, greener synthesis. The other part is constantly educating the public about science and how it works. It is disturbing to see the rise of an "anti-vaccine" movement and forgetting the improvements we have made in air and water quality.

MilliporeSigma: The list of past award recipients is virtually a who's who in chromatography pioneers. Are there any scientists you have followed or benchmarked while developing your own prestigious career?

BK: I really admire all of these people. I would point to Milos Novotny as someone who does something that I try to do. He has several biological problems that he is truly interested in and he invents new analytical techniques to solve those problems; a true "bioanalytical" chemist.

MilliporeSigma: Looking ahead 25 years, what are your predictions on the breakthroughs that the 2042 ACS Award in Chromatography recipient may be cited for? It's interesting to think that person is just starting their career as we write this.

BK: I think about several trends. One is extreme separations; using many dimensions to separate very complex mixtures. I think a future awardee will have a way of performing three or more dimensions of separation in the time we now think of doing a regular chromatogram and resolving 100,000 compounds. A second trend is portable/miniaturized. We are seeing rapid advances and interest in paper microfluidics and other simple devices. Ultimately that will drive a taste for more complex analysis that requires separations in the same environment. Someone will think of a good way to do that. There is also definitely room for improvement in separations of large heterogeneous molecules. Proteoform, molecular machines, and synthetic polymers are examples. Right now we are used to just getting "blobs" but perhaps better resolution is possible. Finally, it is possible that the best advance for separations will come not from "chromatographers" but from mass spectrometry. Improvements in ionization might allow quantitative results with the very high resolution mass spectrometry instruments.

MilliporeSigma: Speaking of the future, do you have any advice for young people considering a career in chemistry?

BK: Do it! It is a great field and there is so much to do. But be open-minded about how your career can go. I've seen people have great success who have started academics and then moved to industry or began in industry and then started their own companies.

References

1. ACS Award in Chromatography: Robert T. Kennedy. Chemical & Engineering News [Online], Vol. 95, Issue 1. http://cen.acs.org/articles/95/i1/ACS-Award-Chromatography-Robert-T.html (accessed April 10, 2017).
2. University of Michigan, Department of Pharmacology. Robert T. Kennedy, Ph.D. Home Page. https://medicine.umich.edu/dept/pharmacology/robert-t-kennedy-phd (accessed April 10, 2017).

Table 1. Recipients of the ACS Award in Chromatography sponsored by MilliporeSigma

Recipient	Year
Robert T. Kennedy	2017
Harold McNair	2016
Milton T. W. Hearn	2015
Susan Olesik	2014
Paul R. Haddad	2013
Wolfgang F. Lindner	2012
Purnendu K. "Sandy" Dasgupta	2011
Udo A.T. Brinkman	2010
Nobuo Tanaka	2009
Frantisek Svec	2008
J. Michael Ramsey	2007
John G. Dorsey	2006
Patrick J.F. Sandra	2005
Shigeru Terabe	2004
William S. Hancock	2003
Edward S. Yeung	2002
Ernest Bayer	2001
Charles W. Gehrke	2000
Daniel W. Armstrong	1999
Georges Guiochon	1998
Peter W. Carr	1997
Stellan Hjerten	1996
Klaus K. Unger	1995
William H. Pirkle	1994
James W. Jorgenson	1993
Josef F. K. Huber	1992
Hamish Small	1991
John H. Knox	1990
Fred E. Regnier	1989
Milton L. Lee	1988
Charles H. Lochmuller	1987
Milos V. Novotny	1986
Leslie S. Ettre	1985
Lloyd R. Snyder	1984
Csaba G. HorvÁth	1983
Barry L. Karger	1982
Marcel J. E. Golay	1981
James E. Lovelock	1980
Evan C. Horning	1979
A. J. P. Martin	1978
Raymond P. W. Scott	1977
James S. Fritz	1976
Egon Stahl	1975
Lockhart B. "Buck" Rogers	1974
Albert Zlatkis	1973
Joseph Jack "J. J." Kirkland	1972*
Julian F. Johnson	1970

*no award was presented in 1971

IN ESSENCE

Analysis of Fipronil and Fipronil Sulfone in Eggs, Chicken Meat and Mayonnaise

Katherine Stenerson, Principal R&D Scientist, **katherine.stenerson@sial.com**

Fipronil is a broad spectrum insecticide that is in the European Union (EU)[1] not permitted to be use with food producing animals. Fipronil sulfone is formed by degradation of fipronil, and is actually more toxic to birds and other organisms than the parent compound.[2] The maximum residue limit designated by the EU for fipronil in eggs 5 ng/g (.005 mg/Kg), reported as a sum of the parent compound and the sulfone degradant.

In this application, QuEChERS extraction and cleanup (see **Figure 1**) followed by GC/MS/MS analysis (conditions listed in **Table 1**) were used for spiked samples that were quantitated against a matrix-matched calibration curve. No internal standard was used, thus recoveries reported are absolute.

Supel™ Que Z-Sep+, used for extract cleanup, was found to significantly reduce levels of co-extracted fatty compounds, including cholesterol. **Figure 2** shows the reduced background after cleanup with Z-Sep+. The fatty acids, eluting in the same retention range as the fipronil and fipronil sulfone, were removed by the Z-Sep+ cleanup, resulting in a clean signal for both compounds at 5 ng/mL in the final extract (**Figure 3**). Recovery and reproducibility of the method was good (**Table 2**). The method was also applied to chicken meat and mayonnaise. The ruggedness test of the GC method was done by repeated injections (>70) of egg sample extracts, resulting in only a small change in signal throughout the run, with a variation of 12%.

Figure 1. Sample preparation procedure, QuEChERS extraction and cleanup with Z-Sep+.

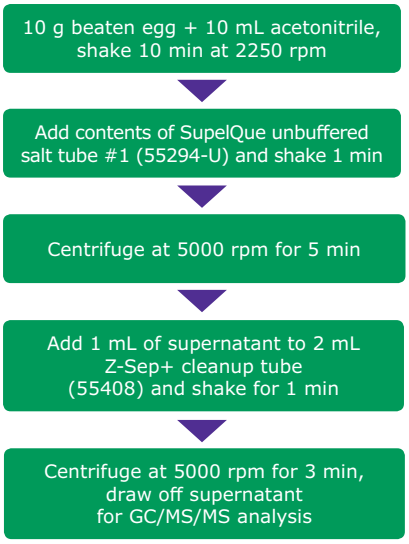

Table 1. GC/MS/MS conditions

column:	SLB®-PAHms, 30 m x 0.25 mm I.D., 0.25 µm (28340-U)
oven:	50 °C (2 min), 15 °C/min to 340 °C (10 min)
inj. temp:	250 °C
carrier gas:	helium, 1.2 mL/min, constant
detector:	MRM, Fipronil: 254.9/228, 350.8/254.8, 366.8/212.8 Fipronil sulfone: 382.8/254.9, 384.8/256.8, 254.9/227.9
injection:	1 µL, pulsed splitless (50 psi until 0.75 min, splitter on at 0.75 min)
liner:	4 mm I.D. FocusLiner™ with taper

Figure 2. GC/MS scan analysis of QuEChERS extract of egg.

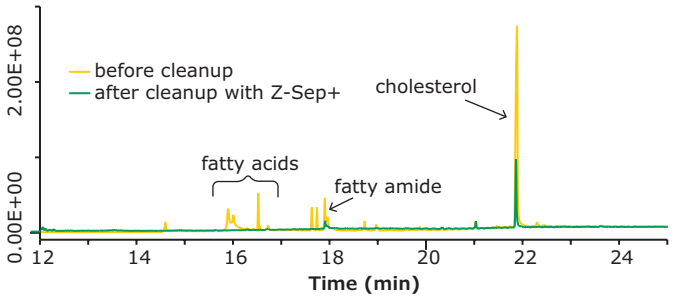

Figure 3. GC/MS/MS analysis of fipronil and fipronil sulfone in eggs at 5 ng/g.

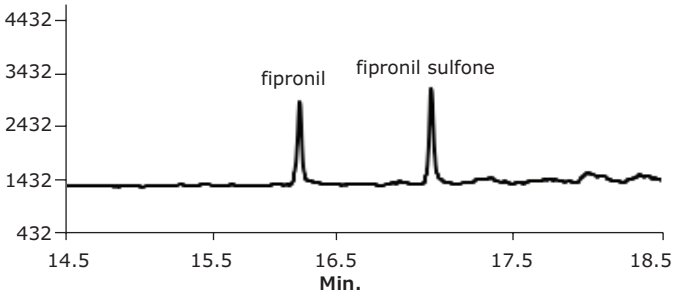

Table 2. Percent recovery and reproducibility (%RSD); spiking level of 5 ng/g.

n=3	Eggs	Chicken Meat	Mayonnaise
Fipronil	91 (0.5)	103 (3)	85 (3)
Fipronil Sulfone	91 (1.8)	116 (2)	87 (4)

For the full application data contact us or visit **SigmaAldrich.com/fipronil**

References

1. Fipronil Egg Scandal: What We Know. bbc.com, 8/11/2017 (accessed 8/15/17).

2 . Madsen, J.E.; Sandstrom, M.W.; Zaugg, Steven D., Methods of Analysis of the U.S. Geological Survey Nation Water Quality Laboratory-A Method Supplement for the Determination of Fipronil and Degradates in Water by Gas Chromatography/Mass Spectrometry; Open-file report 02-462; U.S.G.S.: Denver, CO, 2003.

Featured Products

Description	Cat. No.
SLB®-PAH ms, 30 m x 0.25 mm I.D., 0.25 µm	**28340-U**
Supel™ Que nonbuffered extraction tube #1	**55294-U**
Supel™ QuE Z-Sep + Tube, 2 mL	**55408-U**
Fipronil, Pestanal® analytical standard	**56451-100MG**
Fipronil Sulfone, Pestanal® analytical standard	**32333-50MG**

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