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Analytix Reporter

50 Years of TLC-MS

and its complementary use with HPLC demonstrated for honey analysis

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Dear Reader

Chromatography, translated from its Greek roots ***chroma*** and ***graphein***, literally means *writing in colors*, and was first applied in 1903 to separate plant pigments by a column filled with calcium carbonate. Since then, various chromatographic techniques have been developed and routinely used for the analysis of chemical or biological compounds in fields such as pharmaceuticals, environmental monitoring, and food and beverage testing.

As TLC is quick, easy and does not need sophisticated instrumentation, it was a technique that rapidly grew in popularity. Later, with the development of new and powerful instrument platforms, techniques like high performance liquid chromatography (HPLC) and gas chromatography (GC) expanded. With their high resolving capabilities and the degree of automatization possible, the interest in TLC declined for many analysts and TLC enjoys a modest share of applications in analytical labs today. However, its flexibility, high matrix tolerance and the ability to analyze samples in parallel are very strong features of this “old fashioned”, but still effective, efficient and versatile technique.

As a pioneer in TLC, our company introduced the first pre-coated TLC plates at the 1967 Achema tradeshow in Germany, followed by many other product introductions over the years fulfilling the growing analytical needs of our customers.

Advances in mass spectrometry (MS) and ionization technologies over the last few decades have opened up new possibilities for TLC. The hyphenation of TLC with MS was reported for the first time by Prof. R.E. Kaiser in 1969 and has expanded significantly regarding applicability and performance. The introduction of our high purity TLC-MS-grade plate range further fostered the impact of TLC-MS by providing ever greater results.

On the occasion of the 50th anniversary of TLC-MS, our feature article in this issue highlights an application example using honey as matrix to demonstrate how the two liquid chromatography techniques, TLC and HPLC, can complement each other in an analytical workflow to provide convenient, reproducible and accurate results in tricky analyses.

Sincerely yours,

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FOOD & BEVERAGE

50 years of TLC-MS

Thin-Layer Chromatography coupled to Mass Spectrometry and new perspectives by complementary use to HPLC as demonstrated in testing of honey

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Introduction

Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) are known to be convenient, fast and efficient separation techniques enabling analytical methods without the need for complicated sample preparation or high investments. Low cost and short analysis time per sample is given by parallel analysis of many samples on one plate. The high matrix tolerance of TLC offers additional opportunities to existing routine methods, such as cross-checking of HPLC results or complementary method development.

Various different detection approaches such as analyte visualization by application of derivatization reagents or coupling to other methods like UV detection can be used in combination with TLC. In 1969, Prof. R.E. Kaiser has reported the coupling of TLC with mass spectrometry (MS) for the first time.[1] TLC spots were heated and desorbed into a gas stream in front of the inlet of a mass spectrometer. Numerous publications have demonstrated convincing results and contribute strongly to the progress of TLC, today and in the future.[2]

High-performance liquid chromatography (HPLC) is an established analytical technique for quick and highly efficient analyses of a broad range of complex samples. In contrast to TLC, HPLC can suffer from matrix rich samples causing problems such as increased backpressure or column clogging by accumulation of matrix compounds at the column inlet. In addition, the detection of ghost peaks is possible during repeated sample injections under unsuitable gradient conditions.

The joint use of TLC and HPLC is an option to combine the best out of two chromatographic worlds: High matrix tolerance of TLC makes sample preparation facile or even obsolete and HPLC provides excellent peak capacity for the efficient separation of overlapping TLC bands and increases sensitivity, compared to TLC-MS, by band focusing. Combining 2 different phase selectivities can make the TLC-HPLC-MS hyphenation a true 2D-LC method.

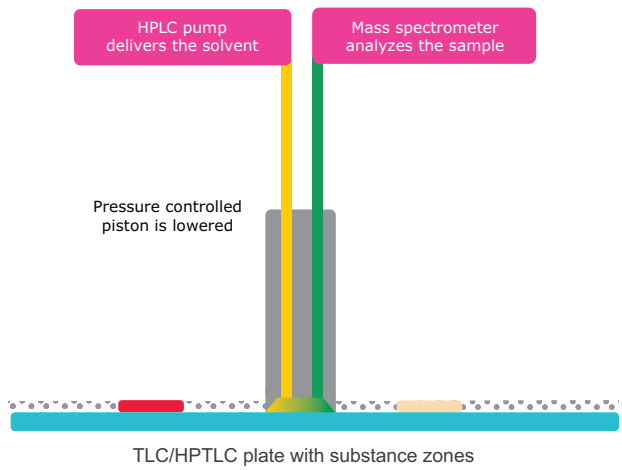
In this article we describe the coupling of thin layer chromatography to mass spectrometry (TLC-MS) and the combination of TLC-MS with high performance liquid chromatography (TLC-HPLC-MS) using as an example the detection of neonicotinoid pesticides in honey.

TLC-MS coupling techniques

The techniques for coupling TLC directly with mass spectrometry can be divided into elution- and desorption-based techniques.[2]

The elution-based approach utilizes a TLC-MS interface that enables the dissolution of the analyte from the silica plate by a solvent and transfer to the mass spectrometer in the liquid phase (see **Figure 1**).

Figure 1. Schematic working principle of elution-based TLC-MS.



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Desorption-based techniques make use of vaporization of the analyte from the TLC surface and transfer to the MS in the gas phase. Vaporization techniques include, gas beam, ion bombardment and MALDI (matrix assisted laser desorption/ionisation) or DART (direct analysis in real time).

Both approaches work offline, and both are performed after a TLC separation is finished and the plate is dried. The sample transfer to the MS is fast and typically takes less than a minute.

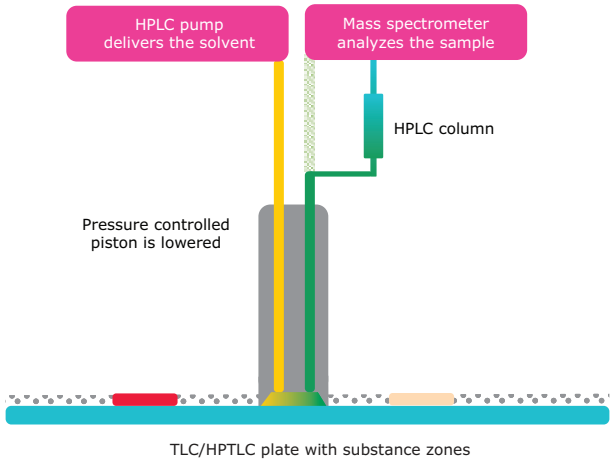
Features and benefits of TLC-MS

- Sample preparation mainly takes place on the TLC plate
- Direct MS analysis of spots or bands of interest – rapid results
- Chromatography is performed separately from MS infusion - high flexibility in choosing mobile phases
- MS-grade plates allow for high resolution separations combined with high sensitivity and reliability in MS detection

Combining TLC-MS and HPLC-MS

A flexible instrument setup allows for direct elution-based TLC-MS and TLC-HPLC-MS measurements (see **Figure 2**). A schematic overview over the entire workflows is displayed in **Figure 3**. A spot can be eluted from the plate and transferred to a HPLC column for detailed analysis. Here the TLC can act either as sample preparation or as the first dimension of 2D-LC.

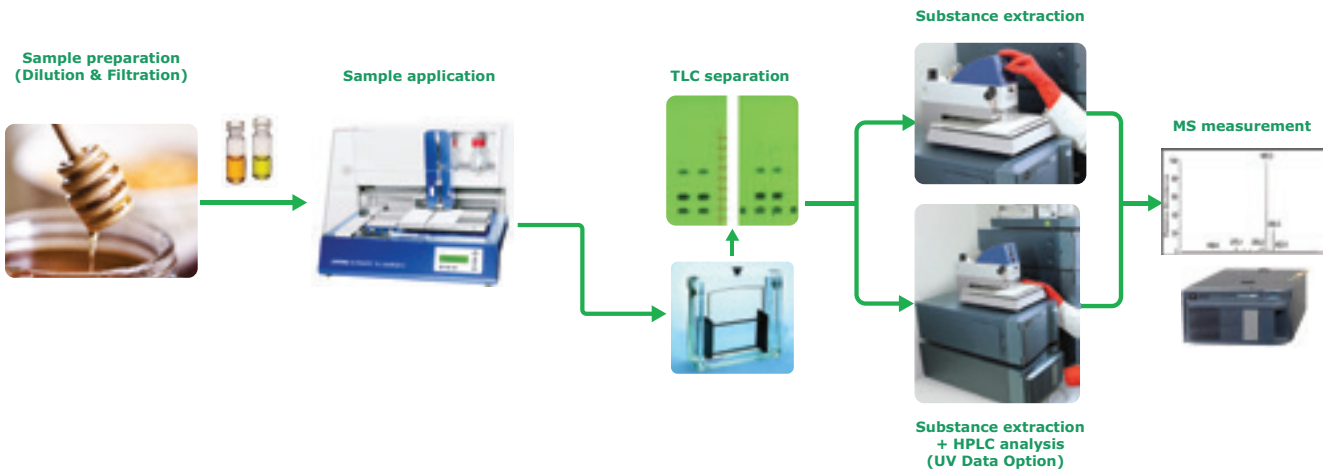
Figure 2. Schematic setup of TLC-HPLC-MS.



Features and benefits of TLC-HPLC-MS

- High matrix tolerance of TLC allows for analyses without complex sample preparation
- Screening and method development capabilities by parallel sample application on one TLC plate and by the option to apply a high variety of staining reagents for visual spot determination during the method development
- Bands overlapping (not resolved compounds) on the TLC plate can be separated by the high separation power of HPLC
- Increased sensitivity by TLC-HPLC-MS compared to TLC-MS

Figure 3. Overview of TLC-MS and TLC-HPLC-MS workflows including instruments and consumables.



Neonicotinoids in Honey

The highly effective group of neonicotinoid pesticides is under discussion regarding negative effects on bee health. (EU) No. 485/2013 prohibits the use and sale of seeds treated with plant protection products containing the neonicotionoids clothianidin, imidacloprid and thiamethoxam. In April 2018 the EU banned these compounds on all outdoor uses (EU) 2018/783-785. European Union maximum residue levels (MRLs) of neonicotionoids authorized in food and feed products are 50 ng/g for acetamiprid, imidacloprid and thiacloprid and 10 ng/g for clothianidin and thiamethoxam.[3]

Honey is a product of natural origin and it is one of the most frequently tested food products. Because of its high viscosity and high sugar content, honey represents a very complex matrix.

Experimental

All TLC analyses were performed utilizing HPTLC Silica gel 60 F_{254} MS-grade plates.

Neonicotinoid standard solutions (NSS) 1 and 2 were prepared by dissolving 0.2 mg/mL and 1 ng/mL, respectively, of each of the seven pesticides nitenpyram, dinotefuran, thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid in acetone.

Sample preparation was done by diluting 1 g honey in 10 mL water/acetone 1/1 (v/v). The samples were applied bandwise (2.5 mm band width) using a CAMAG ATS4.

The thin layer chromatogram development was performed in two steps, using acetonitrile and acetonitrile/methanol 3/1 (v/v) as mobile phases. The development time was 1 and 3 minutes. **Table 2** displays an overview over all applied tracks and obtained hR_f values for the analytes.

Table 1. HPLC conditions

HPLC Column	Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100-2.1 (Cat.No. 1.50648)
Mobile phases	A) Water w/ 0.1 % formic acid
	B) Acetonitrile w/ 0.1% formic acid.
Gradient	100 to 90 % A in 3 min,
	90 to 70 % A in 2 min,
	70 to 60 % A in 7 min,
	60 to 100 % A in 0.4 min,
	100 % A for 3.2 min
Flow Rate	0.25 mL/min
Column Temp	Room temperature
MS mode	ESI (+)
TLC spot elution	100 % water, flow rate 0.25 mL/min

TLC-MS and TLC-HPLC-MS experiments were performed by an elution-based approach, using the CAMAG TLC-MS Interface 2 combined with a Waters Acquity® UPLC H-Class Bio System with an ACQUITY® QDa detector.

Results and Discussion

Analysis of neonicotinoids in honey

In total, 33 tracks of five different samples were applied onto the TLC plate:

a) NSS 1 with a pesticide concentration of 0.2 mg/mL of each neonicotinoid

b) NSS 2 with a pesticide concentration of 1 ng/mL of each neonicotinoid

c) honey sample spiked with 1 mg/g of each neonicotinoid

d) honey sample spiked with 10 ng/g of each neonicotinoid

e) unspiked honey sample

Table 2 displays an overview over all applied tracks and obtained hR_f values for the analytes.

Figure 4A shows the developed TLC plate under irradiation with UV light (254 nm). The neonicotinoids in spiked honey samples are visible at hR_f = 70 (nitenpyram) and hR_f = 93 (dinotefuran, thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid). In order to visualize the high matrix load, the plate was stained with anisaldehyde sulfuric acid reagent (**Figure 4B**). The long, dark smearing zone can be attributed to the high sugar content of the sample. In addition, ninhydrin staining was applied in order to visualize compounds bearing aminofunctions (**Figure 4C**).

After TLC development, elution-based TLC-MS was used to elute the zone at hR_f = 70 and identify it as nitenpyram by subsequent single-quad MS detection. (**Figure 6A**).

The zone at hR_f = 93, resulting from the TLC separation of spiked honey samples and consisting out of six analytes, was eluted from the plate onto the HPLC column. Chromatograms were obtained using UV detection for the two spiked and one unspiked honey sample (**Figure 5** and **Table 3**). In addition, MS detection was utilized to identify the six neonicotinoids (see spectra in **Figure 6 B–G**).

The TLC-HPLC-MS setup was capable of detecting all neonicotinoids in the honey sample spiked at a level of 10 ng/g. As reproducibly (multiple TLC tracks) demonstrated by means of MS, the unspiked honey contained acetamiprid and thiacloprid at levels below the EU limits (MRLs) of 50 ng/g.

For precise quantification by this approach further studies are needed.

(continued on next page)

Table 2. TLC data: Track numbers with applied samples and volumes and obtained hR_f values.

Track	Substance	Application volume [µL]	hR_f
1, 12, 23	Neonicotinoid standard solution 1 – each 0.2 mg/mL	0.5 µL	70: Nitenpyram
3, 14, 25	Neonicotinoid standard solution 2 – each 1 ng/mL	0.8 µL	
4, 15, 26		1.0 µL	
5, 16 ,27		1.2 µL	
2, 13, 24	Honey sample – spiked with 1 mg/g of each neonicotinoid	1.0 µL	93: Dinotefuran, Thiamethoxam, Clothianidin, Imidacloprid, Acetamiprid, Thiacloprid
9, 20, 31	Honey sample – spiked with 10 ng/g of each neonicotinoid	1.0 µL	
10, 21, 32			
11, 22, 33			
6, 17, 28	Honey sample – without spiking	1.0 µL	
7, 18, 29			
8, 19, 30			

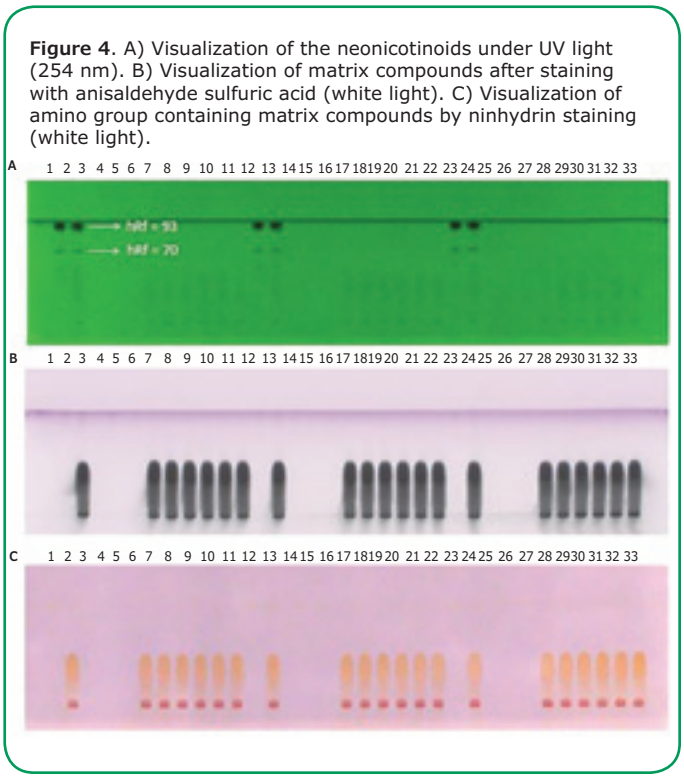


Figure 4. A) Visualization of the neonicotinoids under UV light (254 nm). B) Visualization of matrix compounds after staining with anisaldehyde sulfuric acid (white light). C) Visualization of amino group containing matrix compounds by ninhydrin staining (white light).

Table 3. HPLC retention times of neonicotionoids in spiked honey samples after spot elution and HPLC analysis of the TLC band at hR_f = 93.

Peak	Substance	Retention time [min]
1	Dinotefuran	6.6
2	Thiamethoxam	7.6
3	Clothianidin	8.1
4	Imadacloprid	8.4
5	Acetamiprid	8.8
6	Thiacloprid	10.1

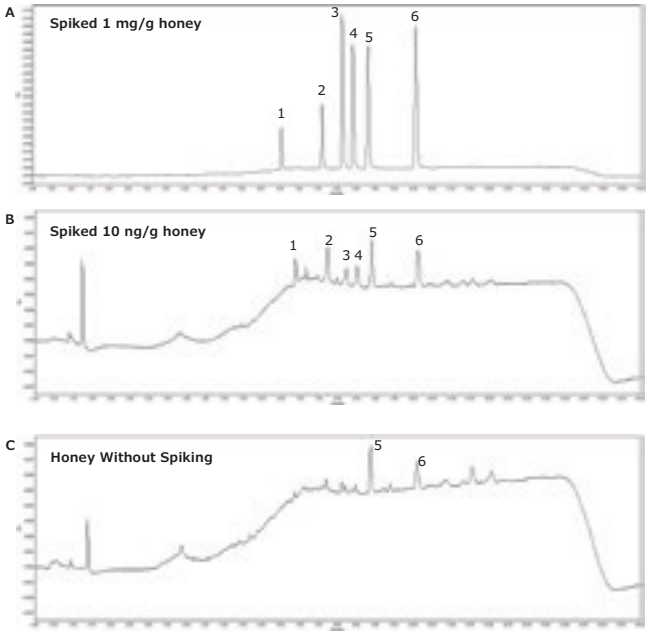
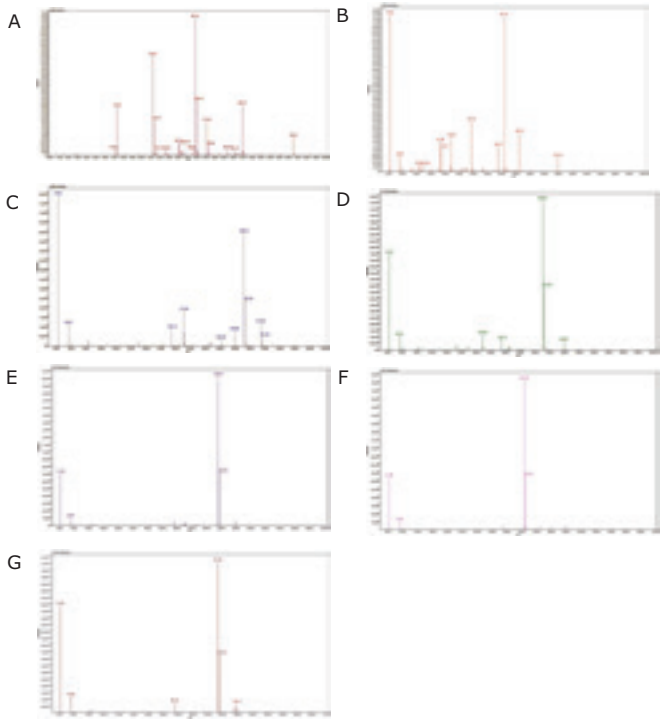


Figure 5. HPLC chromatograms of spiked and unspiked honey samples after spot elution and HPLC analysis of the TLC band at hR_f = 93. A: Honey sample spiked with 1 mg/g. B: Honey sample spiked with 10 ng/g, C: unspiked honey sample. Peak IDs:1: Dinotefuran, 2: thiamethoxam, 3: clothianidin, 4: imidacloprid, 5: acetamiprid, 6: thiacloprid.

Figure 6. Analysis of honey spiked with pesticides. Mass spectra of seven neonicotinoids obtained by analysis of the TLC band at hR_f = 70 using TLC-MS (A: nitenpyram) and by analysis of the TLC band at hR_f = 93 using TLC-HPLC-MS (B: dinotefuran; C: thiamethoxam; D: clothianidin; E: imidacloprid; F: acetamiprid; G: thiacloprid).



Conclusion

An example for the analysis of different analytes in a complex and challenging food matrix was described by using TLC-MS and TLC-HPLC-MS as attractive and flexible methods. Target analytes can easily be separated and detected without time-consuming and labor-intensive sample preparation.

The flexible instrument setup enables the combination of elution-based TLC-MS and TLC-HPLC-MS measurements as complementary chromatographic methods in one setup. The applicability of this combination was demonstrated by means of the analysis of 7 neonicotinoid pesticides. Spiked and unspiked honey samples were analyzed. In the unspiked honey sample acetamiprid and thiacloprid were found at levels below the EU limit (MRLs) of 50 ng/g.

Screening and method development capabilities were shown by the application of 33 tracks (21 honey samples and 11 standard solutions). The high matrix load of the honey samples was visualized by staining with anisaldehyde sulfuric acid and the opportunity to obtain additional selective information was demonstrated by ninhydrin staining for amino group containing compounds.

The versatile TLC-HPLC-MS setup with its TLC strengths of high matrix tolerance, high sample capacity and derivatization flexibility in combination with the high separation power of HPLC enables new approaches especially for the analysis of matrix rich and complex samples.

Featured Products

Description	Cat. No.
HPTLC Silica gel 60 F_{254} MS-grade, 20 x 10 cm	**1.00934**
Purospher® STAR RP-18 encapped (2 µm) Hibar® HR 100-2.1, 100 x 2.1 mm	**1.50648**
Millex® Syringe Filter, Fluoropore™ PTFE, Hydrophobic, Non-sterile, 0.45 µm pore size, 25 mm diameter	**SLFH025**
Solvents & Reagents	
Methanol gradient grade for liquid chromatography LiChrosolv®	**1.06007**
Acetonitril gradient grade for liquid chromatography LiChrosolv®	**1.00030**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Formic acid 98 % - 100 % for LC-MS LiChropur®	**5.33002**
Reference Materials	
Nitenpyram PESTANAL®, 100 mg	**46077**
Dinotefuran PESTANAL®, 100 mg	**32499**
Thiamethoxam PESTANAL®, 100 mg	**37924**
Clothianidin PESTANAL®, 100 mg	**33589**
Imadacloprid PESTANAL®, 100 mg	**37894**
Acetamiprid PESTANAL®, 100 mg	**33674**
Thiacloprid PESTANAL®, 100 mg	**37905**

Related Products

Description	Cat. No.
HPTLC Silica gel 60 F_{254} MS-grade, 20 x 10 cm glass plates	**1.00934**
HPTLC RP-18 F_{254}s MS-grade, 20 x 10 cm glass plates	**1.15161**
HPTLC Silica gel 60 F_{254} MS-grade for MALDI, 5 x 7.5 cm aluminum foils	**1.51160**
TLC Silica gel 60 F_{254} MS-grade, 20 x 10 cm glass plates	**1.00933**
TLC Silica gel 60 F_{254} MS-grade, 5 x 7.5 cm aluminum foils	**1.51022**
TLC RP-18 F_{254}s MS-grade, 5 x 7.5 cm aluminum foils	**1.51015**

To find more information on TLC and HPLC visit
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For a comprehensive overview on our standards visit
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FOOD & BEVERAGE

Approaches to the Analysis of the Oregon List of Pesticides in Cannabis Using QuEChERS Extraction and Cleanup

Edited article, refer to SigmaAldrich.com/Analytix (Issue 5) for full version.

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Introduction

Consumption of cannabis and / or cannabis-based products is currently legal in some form in 33 US states plus the District of Columbia. Testing of the plant materials and products is required by many of these states; however, the specific test methods and target compound lists are not mandated in all cases. In October of 2016, the state of Oregon took a major step forward by requiring that all labs testing cannabis be accredited by the Oregon Environmental Laboratory Accreditation Program (ORLEP) and licensed by the Oregon Liquor Control Commission (OLCC).[1] Consequently, Oregon Administrative Rules (OAR) list specific contaminants to be tested in marijuana samples, along with action levels.[2] The pesticides on this list include carbamate, organophosphorus, macrocylic lactone, neonicotinoid, pyrethroid, and triazole fungicides as well as others. Action levels per OAR vary from 0.2 to 1 µg/g, depending on the specific pesticide. In addition, the state of California, which legalized recreational cannabis in 2016, requires testing for a list of pesticides similar to that on the OAR list, plus 8 additional.[3]

Due to its ease of use and applicability to a wide range of pesticides, the "quick, easy, cheap, effective, rugged & safe" (QuEChERS) approach has been adopted by many testing laboratories for use on cannabis. After extraction, incorporation of a cleanup step is important for removing pigments, as well as other contaminants. QuEChERS cleanup using a mixture of primary secondary amine (PSA), C18 and graphitized carbon black (GCB) is often chosen for this purpose. PSA will remove acidic interferences, C18 hydrophobic interferences and GCB retains some pigments – specifically the green color imparted by chlorophyll. This mixture of sorbents thus retains a wide range of contaminants; however it also has potential to reduce recoveries of target pesticides which are susceptible to hydrophobic retention on C18, or planar enough in structure to be strongly retained by GCB. In previous work done by the author in 2015 with cannabis, an alternative sorbent mix, Supel™ QuE Verde, was evaluated for cleanup in the analysis of various pesticides, and found to offer an advantage with regards to background reduction and recovery.[4] This sorbent mix contains PSA, Z-Sep+, and ENVI-Carb Y. Z-Sep+ is a zirconia coated silica functionalized with C18. The zirconia retains by Lewis acid/base interactions, and has been found to retain certain fatty compounds as well as some pigments. ENVI-Carb Y is a specially manufactured graphitized carbon that is engineered to have weaker retention of small, planar molecules such as certain pesticides. This mixture offers a better balance than traditional PSA/C18/GCB with regards to removal of pigmentation and pesticide recovery. In this application, the pesticide list tested in 2015 has been expanded to include many of those on the OAR list described above. Supel™ QuE Verde was compared directly to PSA/C18/GCB for cleanup and analysis of spiked replicates of cannabis plant material analyzed by LC-MS/MS and GC-MS/MS. Column and mobile phase selection for LC-MS/MS was done based on several factors, which will be described. For the additional pesticides included on the OAR list, Supel™ QuE Verde was found to yield better overall recovery than PSA/C18/GCB.

Experimental

Extraction

Dried cannabis* was pulverized using a IKA T10 Ultra Turrax mixer. 1.9 g was weighed into a 50 mL centrifuge tube and spiked with pesticides at 50 ng/g. After a 10 min equilibration time, the sample was mixed with 10 mL of deionized water and allowed to sit for 30 minutes. 10 mL of acetonitrile was added, and the sample was shaken at 2500 rpm for 30 minutes. The contents of the Supel™ QuE Citrate tube (55227-U) were added, and the sample shaken for 1 minute. The sample was then centrifuged at 5000 rpm for 5 min, and the supernatant removed for cleanup.

**Dried cannabis was supplied courtesy of Dr. Hari H. Singh, Program Director at the Chemistry & Physiological Systems Research Branch of the National Institute on Drug Abuse at the National Institute of Health.*

Cleanup

1 mL of extract was added to a 2 mL tube containing the mixture of cleanup sorbents. Two different sorbent mixtures were used:

1. PSA/C18/GCB/MgSO4 (400 mg/400 mg/ 400 mg/1200 mg)
2. Supel™ QuE Verde (55447-U)

Samples were shaken for 1 minute, centrifuged at 5000 rpm for 3 minutes, and the supernatant removed for analysis.

Analysis

Samples were analyzed by LC-MS/MS and GC-MS/MS using the conditions listed in **Tables 1** and **2**. The same extracts were run on both systems. Pesticides that did not yield response by LC-MS/MS were attempted by GC-MS/MS. Quantitation was done against a 5-point matrix-matched calibration curve prepared in unspiked cannabis extract. Separate curves were prepared for each cleanup. No internal standards were used, thus all recovery values reported are absolute.

Table 1. LC-MS/MS conditions

column:	Ascentis® RP-Amide, 10 cm x 2.1 mm I.D., 3.0 µm (565301-U) with RP-Amide Supelguard cartridge, 2 cm x 2.1 mm I.D., 5 µm (565372-U)
mobile phase:	[A] 5 mM ammonium formate, 0.1% formic acid in 95:5 water:acetonitrile;
	[B] 5 mM ammonium formate, 0.1% formic acid in 5:95 water:acetonitrile
gradient:	10 % B held for 1 min; to 100 % B in 13 min; held at 100 % B for 6 min; to 10 % in 0.5 min; held at 10 % B for 6 min
flow rate:	0.4 mL/min
column temp.:	30 °C
detector:	MRM*
injection:	5 µL
sample:	QuEChERS extract in acetonitrile

Table 2. GC-MS/MS analysis conditions

column:	SLB®-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
oven:	50 °C (2 min), 8 °C/min to 325 °C (10 min)
inj. temp.:	250 °C
carrier gas:	helium, 1.2 mL/min constant flow
detector:	MRM*
MSD interface:	325 °C
injection:	1 µL, pulsed splitless (50 psi until 0.75 min, splitter open at 0.75 min)
liner:	4 mm I.D. FocusLiner with taper

*see online version of article on SigmaAldrich.com/Analytix (Issue 5) for MRM listing

Results and Discussion

HPLC column and mobile phase selection. Typical cannabis samples analyzed by testing labs contain high levels of cannabinoids, often in the range of 20-25% by weight. These compounds will coextract with the pesticides during the QuEChERS process. The acidic forms can be partially retained by some cleanup sorbents (specifically PSA and Z-Sep+), however the neutral forms are not retained well by cleanup sorbents used for pesticide testing. In the case of LC-MS/MS analysis, these co-extracted cannabinoids can build up on the detector, requiring more frequent system maintenance. In this application, column and mobile phase selection were based on conditions that would force elution of the cannabinoids as late as possible in the run, ideally after the pesticides. Under these conditions, the diverter valve on the LC-MS/MS system could be set to flow to waste after elution of the last pesticide. This will then prevent a majority of the cannabinoids from entering the detector.

To facilitate the appropriate HPLC conditions, a screening experiment was designed to study elution of the major cannabinoids compared to the targeted pesticides on several different column chemistries, and using both acetonitrile and methanol based gradients. The columns screened were as follows:

1. Ascentis® Express C18
2. Ascentis® Express RP-Amide
3. Ascentis® Express Phenyl-Hexyl
4. Ascentis® Express Biphenyl
5. Ascentis® Express F5

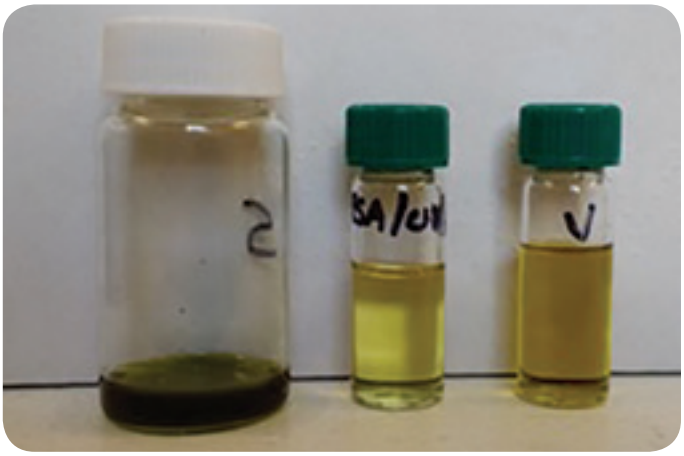
All columns were 10 cm x 2.1 mm I.D., 2.7 µm. The Ascentis® Express Fused-Core version of these chemistries was initially chosen for both efficiency and speed. The HPLC conditions were similar to those listed in **Table 1**, with UV used for detection, and ammonium formate omitted from the mobile phase. Samples were injected in 100% acetonitrile, to emulate samples resulting from the QuEChERS extraction, and as expected, this resulted in poor peak shapes of the earliest eluting pesticides on all five columns. Using an acetonitrile gradient, the Ascentis® Express RP-Amide yielded the least amount of overlap between the pesticide and cannabinoid elution ranges. In addition, comparing acetonitrile to methanol, using the former in the gradient eluted the pesticides faster, resulting in less overlap with the cannabinoids.

To simplify the method as much as possible, the same QuEChERS extract was analyzed by both HPLC and GC. However, as indicated previously in the column screening experiment, injection of 100% acetonitrile into the high aqueous starting conditions of the gradient produced poor peak shapes for the early eluting pesticides. To improve the peak shapes of these compounds, a 3 µm Ascentis® RP-Amide was substituted for the 2.7 µm Ascentis® Express RP-Amide. Installation of a guard column further improved peak shape most likely due to increased retention and improved mixing of the sample with the

mobile phase. (For chromatograms see online version of article on **SigmaAldrich.com/analytix** - Issue 5.) In addition, when working with high background samples, use of a guard column is highly recommended to extend the life of the analytical column.

Background reduction. A comparison of the cannabis extracts before and after cleanup with PSA/C18/GCB and Verde is shown in **Figure 1**. As expected, the co-extracted chlorophyll generated an extract with a deep green color. After cleanup, a majority of the green color was removed, with the extracts appearing yellowish in color. The Verde cleaned extract was slightly darker than the PSA/C18/GCB cleaned extract. Analysis of the extracts by GC/MS in full scan mode is shown in **Figure 2**. The data showed a similar peak pattern between uncleaned and cleaned extracts (both cleanups), but a difference in the amplitude of background peaks (indicated in shaded regions). The predominant peaks eluting in these regions are terpenes (earlier) and cannabinoids (later). Overall reduction in background was compared by summation of total peak area for each chromatogram. Compared to no cleanup, Verde was slightly better than PSA/C18/GCB (35% vs. 31% reduction in background). Specifically in the highlighted regions, Verde showed lower peak amplitudes.

Figure 1. QuEChERS extracts of cannabis before and after cleanup



Elution of cannabinioids. Using the optimized HPLC conditions described previously in the final LC-MS/MS analysis of the cannabis extracts, minimal overlap was observed between two of the major cannabinoids present in the samples and the later eluting pesticides. **Figure 3** shows an EIC of m/z 314.5, taken from a full scan LC/MS analysis of a cannabis extract compared to a TIC of the pesticides of interest in the analysis. The EIC represents the molecular ion of the two major cannabinoids detected in the sample extract; tetrahydrocannabinol and cannabidiol. As indicated, the last pesticide analyzed, pyridaben, eluted just before cannabidiol. The most abundant cannabinoid present, THC, eluted well after. Column flow could be switched to waste after elution of pyridaben, preventing some of the CBD and all of the THC from entering the MS. Other cannabinoids; specifically CBG, CBN, CBDA, CBC, CBGA, and THCAA, are known to elute after CBD on the RP-Amide phase. Thus, if present in the cannabis sample, all of these could also be diverted to waste as well.

Pesticide recoveries. The pesticides included in this study represented a majority of those on the OAR list. Two pesticides from this list, avermectin B1a and naled

Figure 3. EIC, m/z 314.5 from full scan analysis of cannabis extract (green), compared to a TIC of MRM analysis for pesticides (purple).

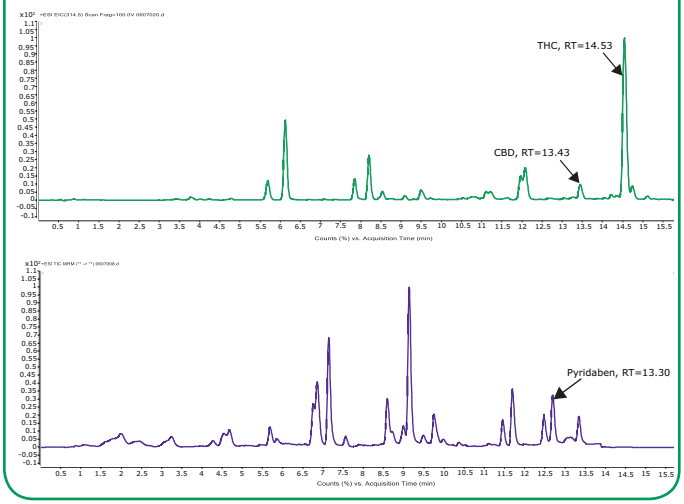
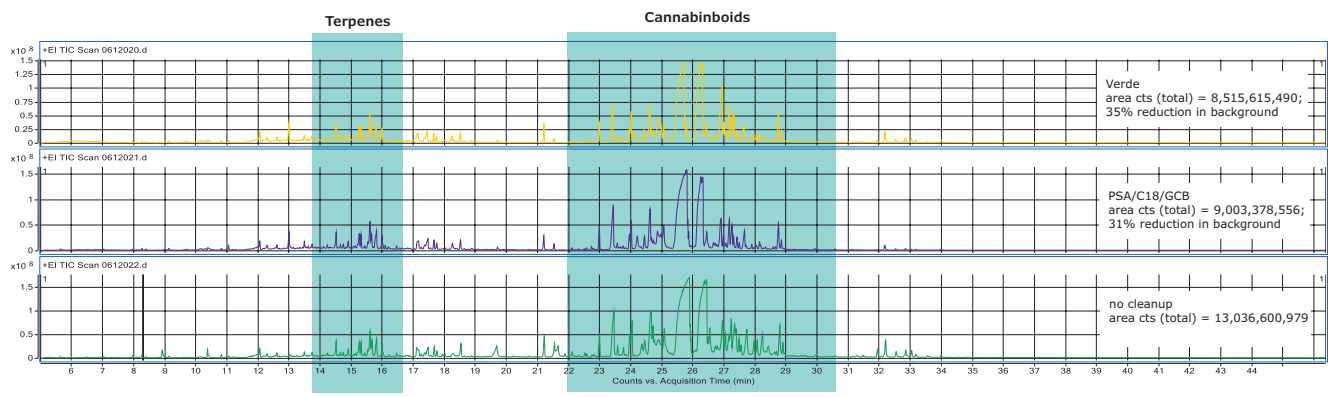


Figure 2. GC/MS scan comparisons of the cannabis extracts



were not analyzed due to lack of response. Avermectin is prone to sodium and potassium adduct formation. The presence of ammonium formate in the mobile phase should reduce this occurrence (as it is monitored as an ammonium adduct). However, even with these measures, others have also reported issues with low level detection of this compound.[4,5] Naled is susceptible to adsorption by PSA, and thus did not make it through the cleanup process with either sorbent mix.

Comparing spike data from the two cleanup methods (**Figure 4**) Supel™ QuE Verde exhibited better overall performance than PSA/C18/GCB. Several pesticides (**Table 3**), specifically bifenthrin, chlorantraniliprole, clofentezin, fenproximate, fludioxinil and hexythiazox showed notably better recoveries using Verde. Although none of these are completely planar in structure, it is possible that recovery was reduced using PSA/C18/GCB

Figure 4. Overall method performance for pesticides spiked at 50 ng/g in cannabis; Supel™ QuE Verde compared to PSA/C18/GCB.

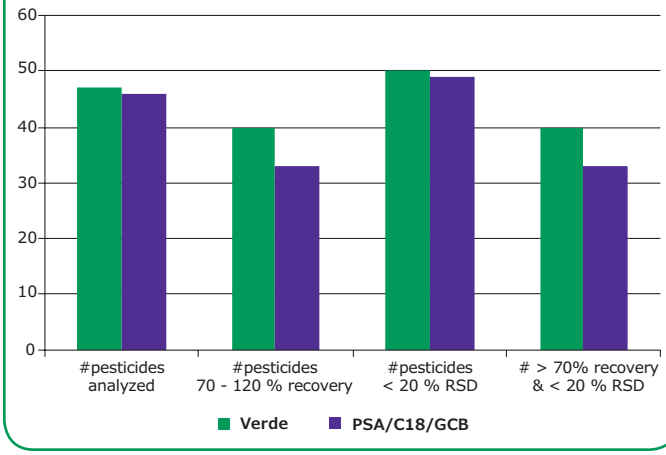


Table 3. Recovery and reproducibility summary; 50 ng/g spiked replicates

Cleanup:	Verde		PSA/C18/GCB		
	% Rec	% RSD	% Rec	% RSD	Analysis
Acephate	72%	12%	82%	6%	LC
Acetamiprid	87%	5%	86%	0.9%	LC
Aldicarb	85%	15%	82%	7%	LC
Azoxystrobin	90%	4%	88%	2%	LC
Bifenazate (D 2341)	50%	6%	50%	2%	LC
Bifenthrin*	74%	4%	57%	9%	GC
Boscalid (Nicobifen)	85%	3%	82%	5%	LC
Carbaryl	87%	7%	87%	2%	LC
Carbofuran	89%	5%	88%	2%	LC
Chlorantraniliprole	87%	6%	72%	1%	LC
Chlorfenapyr	72%	3%	69%	16%	GC
Chlorpyrifos*	87%	5%	71%	5%	GC
Clofentezin	77%	6%	58%	4%	LC
Cyfluthrin*	72%	6%	112%	11%	GC
Cypermethrin*	77%	16%	49%	25%	GC
Daminozide	4%	45%	3%	64%	LC
Diazinon*	92%	3%	88%	10%	GC
Dichlorvos	31%	23%	matrix		LC
Dimethoate	87%	4%	86%	0.4%	LC
Ethoprop (Ethoprophos)	82%	3%	81%	3%	LC
Etofenprox	59%	15	55	8	GC
Etoxazole	76%	2%	69%	2%	LC
Fenoxycarb	85%	6%	84%	2%	LC
Fenpyroximate(E)	74%	3%	59%	2%	LC
Fipronil*	94%	5%	86%	2%	GC
Flonicamid	86%	11%	88%	3%	LC
Fludioxonil	78%	5%	61%	12%	GC
Hexythiazox	72%	3%	63%	4%	LC

Cleanup:	Verde		PSA/C18/GCB		
	% Rec	% RSD	% Rec	% RSD	Analysis
Imazalil (Enilconazole)	49%	5%	66%	2%	LC
Imidacloprid	88%	4%	86%	2%	LC
Kresoxim methyl	84%	13%	79%	7%	LC
Malathion	84%	5%	73%	6%	LC
Metalaxyl	88%	4%	87%	2%	LC
Methiocarb (Mercaptodimethur)	88%	6%	82%	2%	LC
Methomyl	89%	5%	88%	2%	LC
MGK-264	81%	2%	75%	5%	GC
Myclobutanil	87%	3%	88%	2%	LC
Oxamyl	89%	4%	95%	2%	LC
Paclobutrazol	77%	3%	85%	0.5%	LC
Permethrin	54%	2%	58%	6%	GC
Phosmet (Imidan)	90%	5%	79%	7%	LC
Piperonyl butoxide	81%	5%	73%	2%	LC
Prallethrin	72%	10%	67%	7%	LC
Propiconazole	73%	7%	79%	7%	LC
Propoxur	89%	5%	87%	1%	LC
Pyrethrin	71%	6%	67%	29%	LC
Pyridaben	68%	5%	62%	1%	LC
Spinosyn A	42%	10%	42%	2%	LC
Spinosyn D	42%	9%	35%	19%	LC
Spirotetramat	75%	1%	76%	1%	LC
Spiromesifen*	80%	5%	61%	12%	GC
Spiroxamine	26%	1%	27%	3%	LC
Tebuconazole	67%	4%	80%	1%	LC
Thiacloprid	87%	5%	86%	1%	LC
Thiamethoxam	87%	5%	86%	3%	LC
Trifloxystrobin	85%	5%	79%	2%	LC

**See reference 4.*

due to hydrophobic retention on the GCB; which has a higher surface area than the carbon used in the Supel™ QuE Verde mix.

Several pesticides exhibited poor recoveries after both cleanup techniques:

- **bifenazate**: Recovery was around 50% after both cleanups. Bifenazate is susceptible to oxidation to bifenazate-diazine[8], which may have occurred to some degree during the extraction and cleanup process.
- **daminozide:** Very low recovery after both cleanups. This compound is a carboxylic acid, and is thus retained by PSA (present in both cleanups).
- **dichlorvos:** Matrix interference prevented analysis of this pesticide in the PSA/C18/GCB extracts. In the Supel™ QuE Verde cleaned extracts, the peak could be detected, but recoveries were low and variable. The low recovery using Verde is most likely due to retention on the Z-Sep+ portion of the sorbent. This same behavior has been observed in the past with this compound when using zirconia sorbents by both the author and others.[9]
- **etofenprox:** This is a very hydrophobic pesticide (log p= 7.1) and may exhibit poor extraction efficiency and/or retention by the C18 and Z-Sep+ portions of the cleanup sorbents (although less so on the later).
- **imazalil:** This is a relatively polar pesticide, which can be retained by PSA (present in both cleanups). Recovery issues have been observed by others with this compound when using Supel™ QuE Verde for cleanup as well as other zirconia containing sorbent mixtures.[9,10]
- **spinosyn A & D:** Lower recoveries of these large, macrocyclic lactones have been observed when using C18, carbon and zirconia containing sorbents.[11] In the case of zirconia, the use of citrate buffering in the QuEChERS extraction has been observed to increase recovery, possibly by displacement of the analytes from the zirconia.[9]
- **spiroxamine:** Recovery was very low, and about the same level after both cleanups. This could indicate an issue with extraction efficiency.

Conclusions

In the analysis of a majority of the pesticide list required by the state Oregon for cannabis, several recommendations can be made:

- QuEChERS extraction and cleanup can be used; and both LC-MS/MS and GC-MS/MS will be required for analysis.
- Cleanup using Supel™ QuE Verde can be substituted for PSA/C18/GCB. Both cleanups will reduce the green color of the extracts; however Verde was found to produce a slightly lower GC/MS background. Cannabinioids were co-extracted with the pesticides. Significant levels were still present after both cleanups, although slightly less after Verde. Pesticide recovery using Verde was found to be better overall, especially for several pesticides.
- Compared to C18, the Ascentis® RP-Amide column provided less overlap between the elution ranges of the targeted pesticides and the co-extracted cannabinoids. This separation would allow a switch to waste on the LC-MS/MS system after elution of the last pesticide, which would in turn prevent some of the cannabinoids from entering the MS.
- In the LC-MS/MS analysis, a high percentage of aqueous was necessary in the starting mobile phase conditions to increase retention of the more polar pesticides. As a result, injecting extracts in 100% organic resulted in distorted peak shapes for early eluting peaks. Switching from a 2.7 µm Ascentis® Express RP-Amide to a 3 µm Ascentis® RP-Amide (fully porous particle) improved these peak shapes.
- A guard column prior to the Ascentis® RP-Amide will further improve peak shapes when injecting 100% organic, and is recommended to prolong the life of the analytical column.

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11. Han, L.; Sapozhnikova, Y.; Lehotay, S.J., Streamlined sample cleanup using combined dispersive solid-phase extraction and in-vial filtration for analysis of pesticides and environmental pollutants in shrimp. *Anal Chimica Acta* **2014**, *827*, 40-46.

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Ascentis® RP-Amide Supelguard™ guard cartridge 2 cm x 2.1 mm I.D., 5 µm	**565372-U**
Supel™ QuE citrate extraction tube	**55227-U**
Supel™ QuE PSA/C18/ENVI-Carb Tube, 2 mL	**55289-U**
Supel™ QuE Verde, 2 mL	**55447-U**

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FOOD & BEVERAGE

Detect Pyrrolizidine Alkaloid Contamination in Food

New reference materials from Phytolab now available through SigmaAldrich.com

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

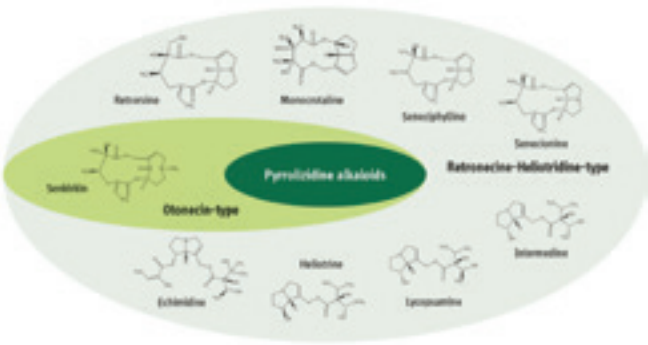
We recently added the portfolio of phytochemical reference materials from PhytoLab to our global product offering. As one of the leading manufacturers internationally, PhytoLab offers over 1,000 extensively documented herbal reference substances of all classes of natural compounds. The portfolio also includes 29 pyrrolizidine alkaloids and N-oxides.

Pyrrolizidine alkaloids are well known contaminants of various foodstuffs such as leaf lettuces, cereals or honey. Just recently pyrrolizidine alkaloids were also found in both herbal teas and common teas. Animal feedstuff can be affected as well. The source of the pyrrolizidine alkaloids are weeds from the families of the Asteraceae (genus Senecio, known as groundsel or ragwort), Boraginaceae and Fabaceae.

Honey can be contaminated with pyrrolizidine alkaloids if plants from these and other families are visited by the honey bees.

From a chemical point of view pyrrolizidine alkaloids are mono- or diesters of 1-hydroxymethyl pyrrolizidine (necine base) and aliphatic mono- or dicarboxylic acids (necic acids). Otonecine-type, platynecine-type, and the diastereomeric retronecine-type and heliotridine-type pyrrolizidine alkaloids are distinguished. A prerequisite for the toxicity is a 1,2-unsaturated necine structure that forms an ester with at least one branched C5-carboxylic acid. Pyrrolizidine alkaloids with these structural characteristics have been found to be genotoxic and carcinogenic in animal experiments. In larger quantities pyrrolizidine alkaloids can cause acute and chronic liver damage.

Structures of typical pyrrolizidine alkaloids from plants



For a reliable analysis of pyrrolizidine alkaloids, well characterized reference substances are essential. Because of great variances in response factors, e.g., in LC-MS analysis, the quantitative analysis of individual pyrrolizidine alkaloids is preferred over a non-selective sum method. Currently we offer 29 different pyrrolizidine alkaloids and their N-oxides, all of them supplied together with a comprehensive certificate of analysis. For a complete and up-to-date product list, please visit our website at **SigmaAldrich.com/phytolab**

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Cat. No.	Description	Package Size
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PHL83433	Erucifoline N-oxide	5 mg
PHL83237	Europine hydrochloride	10 mg
PHL83238	Europine N-oxide	10 mg
PHL80403	Heliotrine	10 mg
PHL83236	Heliotrine N-oxide	10 mg
PHL83234	Indicine hydrochloride	10 mg
PHL83235	Indicine N-oxide	10 mg
PHL82424	Intermedine	10 mg
PHL83446	Intermedine N-oxide	5 mg
PHL83434	Jacobine	5 mg
PHL83435	Jacobine N-oxide	5 mg
PHL80412	Lasiocarpine	10 mg
PHL83220	Lasiocarpine N-oxide	10 mg
PHL89726	Lycopsamine	10 mg
PHL83447	Lycopsamine N-oxide	5 mg
PHL89251	Monocrotaline	20 mg
PHL82629	Monocrotaline N-oxide	10 mg
PHL89775	Retrorsine	10 mg
PHL82630	Retrorsine N-oxide	10 mg
PHL89789	Senecionine	10 mg
PHL82631	Senecionine N-oxide	5 mg
PHL89275	Seneciphylline	10 mg
PHL82632	Seneciphylline N-oxide	5 mg
PHL83436	Senecivernine	5 mg
PHL83437	Senecivernine N-oxide	5 mg
PHL89274	Senkirkin	10 mg
PHL83438	Trichodesmine	10 mg

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FOOD & BEVERAGE

New Food Matrix CRMs from Joint Research Centre (JRC)

Merck continues as authorized distributor of CRMs from JRC

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

The reference material production of the JRC (Joint Research Center), a Directorate-General of the European Commission is situated in Geel, Belgium.

The JRC's mission is to promote a common and reliable European measurement system in support of EU policies. This involves the development and manufacturing of certified reference materials (CRMs, pure compounds and matrix materials) for various applications including environmental analysis, food analysis, clinical chemistry, physical properties or industrial applications.

The collaboration between Sigma-Aldrich in Buchs and the former IRMM (Institute of Reference Materials and Measurements) dates back almost 20 years. With almost two decades of experience in distributing JRC reference materials, we are proud to announce that our status as an authorized distributor of JRC has been renewed through an official candidature for tender in 2018 which was called by the JRC to award distribution contracts for a new contract period. We will hence continue to be a reliable source for these products for our customers.

All the CRMs from JRC are produced according to the specific procedures of the European Commission which are in accordance with ISO 17034 and ISO Guide 35.

Each product is delivered with a certificate containing a certified value (including uncertainty) which is traceable to a SI unit or an internationally accepted reference. The certificate also states the intended use for each CRM. Stability of the CRMs is ensured by storage under controlled conditions and by monitoring programs which have been set up to control CRM stability during their entire shelf life. All the products from JRC can be found listed at SigmaAldrich.com/jrc.

Food Matrix Materials

A considerable portion of the JRC reference materials portfolio consists of Matrix CRMs, products which are of great importance for method development, performance check and the validation of analytical methods by providing real life samples with certified values for specific analytes.

Because the manufacturing of Food Matrix Materials is a very challenging and time consuming process, the number of commercially available products is rather limited and it can be difficult to find the right product for a specific analytical application. To help our customers in finding the right product, we set up a web portal with access to more than 150 Food Matrix Materials from JRC, NIST and BAM, categorized by Matrix Type or Analyte Group (SigmaAldrich.com/foodmatrix).

JRC has recently expanded this product range by two new Food Matrix Materials described below.

Certified Reference Materials for Pesticides in Cucumber ERM-BC403[1]

The number of regulated pesticides is very large. In addition, the variety of different food products that need to be monitored lead to thousands of combinations of matrices and pesticides.

This makes the development and validation of suitable multi-residual analytical methods a big challenge. There are currently not many matrix materials for pesticides in food available on the market to assist with the development of analytical methods. Therefore, the JRC has developed a new matrix material for pesticides in cucumbers.

This new cucumber matrix material ERM-BC403 is certified for the mass fraction of 15 selected pesticides (acetamiprid, azoxystrobin, carbendazim, chlorpyrifos, cypermethrin, diazinon, α-endosulfan, fenitrothion, imazalil, imidacloprid, iprodione, malathion, methomyl, tebuconazole, thiabendazole). Cucumbers from a biodynamic farm were used as base material which was then spiked with the pesticides and then freeze-dried. Prior to analysis, the sponge of dry cucumber must be reconstituted with a specific volume of water. The CRM is provided in sets of two glass vials each containing approximately 3.2 g of dried material.

Certified Reference Materials for Metals in Dark Chocolate ERM-BD512[2]

The tolerable weekly intake (TWI) of Cd has been defined to be 2.5 µg/kg body weight by EFSA[3]. Chocolate and cocoa products are one of the food groups that contribute a major part to dietary Cd exposure. Due to this a new amendment (EC) No. 488/2014 to the Commission regulation (EC) No. 1881/2006 was passed to establish, among others, new maximum levels for Cd in chocolate and cocoa products.

The JRC developed a certified reference material of dark chocolate certified for the mass percentages of cadmium, copper, manganese and nickel. The CRM was produced from commercially available dark chocolate produced in Peru. The chocolate was melted, homogenized and cast into molds to produce pellets of about 0.5 g. Each unit of the CRM contains six vials, each containing one pellet.

References

1. ERM CERTIFICATION REPORT The certification of the mass fraction of pesticides in cucumber: ERM®- BC403
2. ERM CERTIFICATION REPORT The certification of the mass fractions of cadmium, copper, manganese and nickel in dark chocolate: ERM®- BD512
3. J. Alexander, et. al., Cadmium in Food: Scientific Opinion of the Panel on Contaminants in the Food Chain, The EFSA Journal (2009) 980, 1-139.

Featured Products

Description	Cat. No.
Cucumber (pesticides) ERM® certified Reference Material	**ERMBC403**
Dark Chocolate (Cd, Cu, Mn, Ni) ERM® certified Reference Material	**ERMBD512**

For a complete Listing of our food matrix materials visit **SigmaAldrich.com/foodmatrix**

Towards Safer Seafood – New Marine Toxin CRMs

qNMR as an efficient tool to manufacture traceable CRM solutions with limited amount of available material

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

The presence of marine toxins in shell fish and sea food has always been a concern, but has spread in recent years. Increasing global temperatures are suspected to be the reason for an observed expansion of algae species that are commonly the origin of these toxic compounds into areas where they have not been found before. Examples are the emerging of ciguatoxin contaminated fish in the canaries or the increasing occurrence of paralytic shellfish toxins in Northern American lakes[1].

It is therefore very important, that fish and seafood are monitored for contamination by algae toxins. Historically, this has been done by bio-assays such as ELISA or mouse bioassays. However with LC-MS instruments being more sensitive and easier to use, various new methods using LC-MS have been established[2] in recent years. Also, LC-MS is recommended or required as a method by newer regulations such as EC regulation No 15/2011.

Therefore, the access to well characterized reference materials for quantitation of these different toxins has become an increased need in the market. These reference materials should be characterized and prepared according to ISO/IEC 17025 and ISO 17034 to ensure highest precision and accuracy. Unfortunately, isolation, synthesis and purification of those compounds or their stable isotope labeled analogs is very challenging and batch sizes are typically in the range of a few mg.

In order to achieve certification of such small batches according to the above mentioned double accreditation a combined setup of quantitative NMR (qNMR) and Isotope Dilution MS (IDMS) was successfully established.

In a first step, the accurate concentration of a dissolved toxin is determined by ^{1}H-High Performance®-qNMR measurements. Gravimetric dilution and ampule filling delivers the final product with a certified concentration and an associated expanded uncertainty, which can be subsequently applied in an HPLC-IDMS experiment that results in a concentration for the stable isotope labeled analog. Gravimetric IDMS experiments are also carried out to determine the homogeneity and stability contribution to the overall uncertainty.

These concepts were successfully adopted for the certification of multiple toxins despite that they tend to undergo rearrangement or deuterium exchange reactions and often show a low stability at ambient temperature.

Several paralytic shellfish toxins (PST) were developed, for example the well-known Neosaxitoxin or Saxitoxin and their stabile isotope labeled analogs 15N7-Neosaxitoxin and 15N7-Saxitoxin that have been introduced in the previous issue of the Analytix Reporter.

Now we present several additional products to this range such as more paralytic shellfish toxins like GTX-6, lipophilic toxins like Okadaic acid or Pectenotoxin 11 or cyclic imine toxins like Gymnodimine and Pinnatoxins E, F and G.

We have further toxins in our pipeline, please visit our webpage **SigmaAldrich.com/marinetoxins** for an overview on our current offer.

*Trace*CERT® Marine Toxin CRM solutions currently available from Merck (all 0.5 mL)

Analyte	Class	Solution Composition	Cat. No.
Saxitoxin hydrochloride	Paralytic Shellfish Toxin	20 µg/g in HCl	**93665**
$^{15}N_7$-Saxitoxin hydrochloride	Paralytic Shellfish Toxin	10 µg/g in HCl	**30929**
Neosaxitoxin	Paralytic Shellfish Toxin	20 µg/g in HCl	**41619**
$^{15}N_7$-Neosaxitoxin	Paralytic Shellfish Toxin	10 µg/g in HCl	**41206**
N-sulfocarbamoyl neosaxitoxin (GTX6)	Paralytic Shellfish Toxin	20 µg/g in HCl	**07568***
Okadaic acid	Lipophilic Toxin	20 µg/g in methanol	**39302***
Pectenotoxin-11 (PTX-11)	Lipophilic Toxin	20 µg/g in methanol	**80099**
Pinnatoxin E	Cyclic Imines	20µg/g in 0.1% acetic acid in 50% acetonitrile	**80694***
Pinnatoxin F	Cyclic Imines	20µg/g in 0.1% acetic acid in 50% acetonitrile	**40538**
Pinnatoxin G	Cyclic Imines	20µg/g in 0.1% acetic acid in 50% acetonitrile	**40821***
Gymnodimine	Cyclic Imines	20 µg/g in methanol	**80779***

* Coming Soon

For more information and an up-to-date list of marine toxin CRMs please visit **SigmaAldrich.com/marinetoxins**

References:

1. Toxins 2015 Apr; 7(4): 1206–1234.
2. Methods Mol Biol. 2015;1308:277-97

FOOD & BEVERAGE

Is your food healthy?

Measuring Total Sugar (Glucose and Fructose) in Potatoes using Mobile Reflectometry

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

Who doesn't love golden, hot French fries or a steaming baked potato? In fried or baked goods, much of the savory taste and aroma can be attributed to the Maillard reaction. It is what creates the brown compounds that give many cooked foods this flavor. Unfortunately, the reaction between asparagine and reducing sugars (e.g., fructose or glucose) can also produce acrylamide, which is considered toxic and potentially carcinogenic: In the body, acrylamide is converted into glycidamide, which can bind to DNA and cause mutations. High levels of acrylamide can be found in starchy foods, such as potatoes and bread, when cooked at temperatures over 120 °C. This potentially dangerous acrylamide formation can be minimized by ensuring that the levels of reducing sugars are within safe limits, thereby ensuring the quality and nutritional value in processed food.

Precise in-process results—directly on-site

Reflectometry, or remission photometry, is a rapid, sensitive method for quantitating a wide variety of organic and inorganic parameters in water, food, beverages, and environmental samples. Using test strips in combination with a reflectometer, readings can be taken in the laboratory, on the production line, or in the field.

The total free sugar in potatoes can be determined in minutes with the simple Reflectoquant® Total Sugar Test and the RQflex® 20 reflectometer:

Reflectometric determination after enzymatic reaction with glucose-6-phosphate dehydrogenase and diaphorase

Sample preparation

Homogenize the potato in a blender (e.g. Ultraturrax). Weigh 10-20 g of the mashed sample into a 50 mL volumetric flask, and note down the exact sample weight. Add approx. 40 mL distilled water and stir for 10 minutes. Afterwards, make up to the mark with distilled water. Filter through a folded filter.

Analysis

Place 10 mL distilled water, 5 drops of reagent TS-1 and 1 mL filtrated sample in the test vessel and swirl. Press the reflectometer START key and simultaneously dip the test strip into the measurement sample (23 ± 3 °C) for ca. 2 s, ensuring that both reaction zones are immersed. Allow excess liquid to run off via the long edge of the strip on to an absorbent paper towel. Insert the strip immediately into the strip adapter.

After 60 s the strip is measured in the reflectometer. The value [mg/L] will be stored automatically.

The total sugar content can then be calculated as [g/kg] = Measured value [mg/L] x 50 mL / Sample weight [g] x 100

Results and comparison with classic enzymatic method

Sample	Total Sugar [g/kg] Reflectoquant® method	Total Sugar [g/kg] Enzymatic method
1	15.4	17.3
2	5.7	6.4
3	0.4	0.4
4	<0.2	0.1

From Potatoes with 0.2–1.0 g/kg reducing sugar, roasted products of optimum culinary quality can be prepared and, if prepared under conditions minimizing acrylamide formation, acrylamide contents remain below 500 µg/kg.[1]

Featured Products

Description	Cat. No.
Reflectoquant® Total Sugar Test, 50 Tests	**116136**
Reflectometer RQflex® 20	**117246**

Reference:

1. How much reducing sugar may potatoes contain to avoid excessive acrylamide formation during roasting and baking? Biedermann-Brem, S., Noti, A., Grob, K. et al. Eur Food Res Technol (2003) 217: 369. https://doi.org/10.1007/s00217-003-0779-z

RQflex® 20 Reflectometer

Intuitive menu navigation—available in seven languages

Simple and easy to use menu system to access all your methods, results and quality assurance options, along with the ability to tailor your settings to your needs.

Portable Analysis

A light and compact system that acts like a mobile laboratory to perform critical analyses and obtain quantitative results directly on-site. Get results in minutes for easy monitoring of your materials at any stage of your process.

Barcoded test strips with batch specific calibration

For every test condition, each batch of test strips is calibrated and equipped with a barcode for accurate and reproducible results. Obtain consistent results quickly with an average accuracy within ± 10 %.

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For analytical quality assurance (AQA), our comprehensive RQcheck and total system monitoring process ensures accurate and precise measurement results. Calibration is straightforward, so your measurements will always be reliable.

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For maximum reproducibility, a countdown timer with an advanced warning alert before the end of the test strip preparation time can be tailored to fit in with your workflow.

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From ammonium through to nitrate and urea, the Reflectoquant product line offers a comprehensive range of test kits for fast instrumental analysis, e.g., for testing the nitrate content in vegetables or water, determining the freshness of honey, or finding out how much ammonium is in the soil.

For more information and an application overview visit

SigmaAldrich.com/Reflectoquant

SigmaAldrich.com/mobile-lab

Rapid, Flexible and Reliable Analysis for Water, Food and Beverage Industries - Take your Lab to the Sample

COSMETICS & PERSONAL CARE

New Reference Materials for Quality Control of Fragrances and Cosmetics

Neat reference materials for allergenic fragrances listed in the new IFRA method

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

In Analytix Reporter, Issue 3, we presented two certified reference material mixes and a chromatographic application for use with the new IFRA method for the testing of 57 potentially allergenic fragrances.[1] This new method allows the screening of complex fragrance mixtures for the presence of these chemically defined allergens by GC-MS.

We now introduce a number of new individual reference materials as neat compounds for some of the allergenic fragrances contained in the mixes. These reference materials have been certified by quantitative NMR (qNMR) and are traceable to NIST SRMs. The certificate contains information about traceability, homogeneity and stability and the values are given with calculated uncertainties.

Description	Package Size	Cat. No.
Acetyleugenol	100 mg	**36103**
Amyl salicylate	100 mg	**77345**
α-Amylcinnamaldehyde, predominantly trans	100 mg	**78504**
Benzyl cinnamate	100 mg	**78714**
Benzyl salicylate	100 mg	**77069**
D-Camphor	100 mg	**79307**
Cinnamyl alcohol	50 mg	**38382**
(±)-β-Citronellol	50 mg	**76638**
(E)-β-Damascone	10 mg	**79172**
α,α-Dimethylphenethyl acetate	100 mg	**77893**
16-Hexadecanolide	100 mg	**77684**
α-Hexylcinnamaldehyde	100 mg	**78724**
Isoeugenyl acetate	100 mg	**77526**
Linalyl acetate	50 mg	**79308**
DL-Menthol	100 mg	**38278**
4-Methoxybenzyl alcohol	100 mg	**78311**
Methyl 2-octynoate	50 mg	**78700**
(-)-β-Pinene	100 mg	**36327**
Salicylaldehyde	100 mg	**78313**
β,β-3-Trimethylbenzenepropanol	100 mg	**78329**

Find a list of the new reference materials above or online at **SigmaAldrich.com/fragrancestandards**

Reference

1. Analytix Reporter Issue 3 2018.

COSMETICS & PERSONAL CARE

Simplified Determination of Rutin in Anti-Aging Skin Cream Formulations

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Anita Piper, Scientist Instrumental Analytics R&D, anita.piper@merckgroup.com

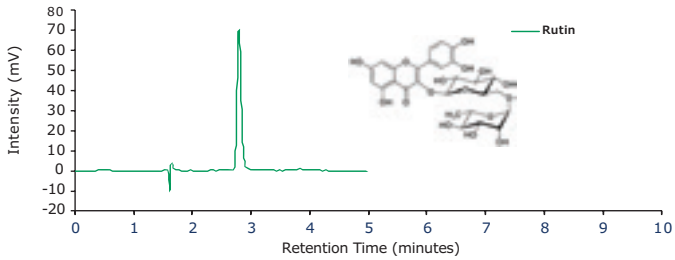
Rutin, also known as rutoside, quercetin-3-O-rutinoside, or sophorin, is a quercetin glycoside found in various plants, including buckwheat and asparagus. The chemical structure contain units of Rutoside and the disaccharide rutinose (α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose).

Rutin is believed to possess antioxidant properties, and/or posses anti aging effects on human dermal fibroblasts and human skin. Like other bioflavonoids, Rutin is investigated for its biological activity and often formulated as skin cream, i.e. semi-solid emulsions of oil and water, products.

HPLC analysis of skin cream formulations can profit from use of monolithic rods over particle based columns, due to higher tolerance against matrix-rich samples and clogging. This application illustrates how Rutin can be determined in skin cream samples using a monolithic Chromolith® HighResolution RP-18 endcapped column with UV detection. Sample preparation of the cream samples would only require ethanol-solubilization and filtration prior to analysis, lowering both the cost and time of analysis.

Experimental Conditions			
Column	Chromolith® HighResolution RP-18e 100x4.6 mm		
Injection volume:	1 µL		
Flow Rate:	1 mL/min		
Temperature:	25 °C		
Pressure Drop:	56 - 85 bar (812-1233 psi)		
Detection	VWD, 5 Hz, Response Time 0.1 s, UV = 220 nm		
Mobile phase A	Phosphate buffer (10 mM, pH 3)		
Mobile Phase B	Methanol		
Gradient	Time (min)	A (%)	B (%)
	0	55	45
	5	55	45
	10	10	90
	10.1	55	45
	15	45	45
Diluent	Ethanol		
Standard solution	Rutin was accurately weighed and diluted to volume with diluent (282 µg/mL).		
Sample preparation	50 mg Day-Cream were weighed into a 25 mL volumetric flask and filled up with ethanol. The cream suspension was placed into an ultra sonic bath for 20 minutes. The sample solution was filtered through a 0.45 µm PTFE filter directly into the HPLC vial prior to analysis and placed in the autosampler.		

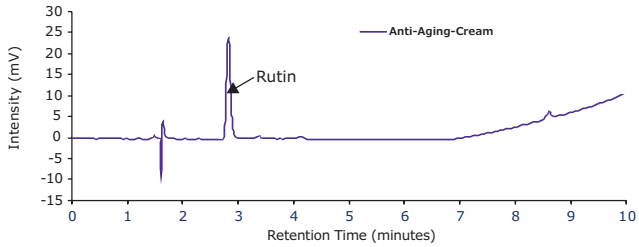
Standard Solution

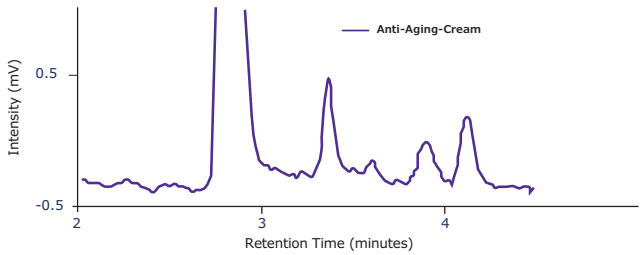


Chromatographic Data [Standard Solution (282 µg/mL)]

No.	Compound	Retention Time (min)	Area (mAU*min)	Tailing Factor
1	t_0 void volume	2.0		
2	Rutin	2.8	2,266	1.2

Cream samples

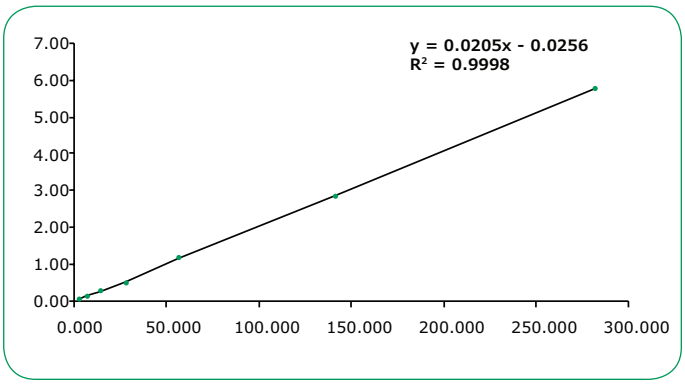




1. Selectivity: Inject standard solution of Rutin and determine the retention time, peak purity, tailing factor and column efficiency				
	Retention Time (min)	Plates (USP)	Tailing Factor	Peak Purity
1 Rutin	2.8	8004	1.2	1.000

2. LOD & LOQ	
Concentration (ppm)	
LOD (ppm)	5.5
LOQ (ppm)	16.7

3. Linearity (area mAU*min)	
Concentration (ppm)	**Mean Area**
2,820	0.06
7,050	0.13
14,100	0.26
28,200	0.50
56,400	1.17
141,000	2.86
282,000	5.77



Featured Products

Description	Cat. No.
Chromatography & Sample Prep	
Chromolith® High Resolution RP-18 endcapped 100-4.6 HPLC column	**1.52022**
Millex® syringe filter units, disposable, Durapore® PVDF, pore size 0.45 µm, non-sterile	**SLHVX13NK**
Reference Materials & Reagents	
Rutin trihydrate analytical standard, neat	**78095**
Rutin hydrate ≥94 % (HPLC), powder	**R5143**
Methanol gradient grade for liquid chromatography LiChrosolv®	**1.06007**
Water for chromatography (LC-MS grade) LiChrosolv® or tap fresh from an appropriate Milli-Q® system	**1.15333**
Ethanol absolute for analysis EMSURE® ACS, ISO, Reag. Ph Eur	**1.00983**
Phosphoric acid ACS reagent, ≥85 wt. % in H_2O	**438081**
Potassium phosphate monobasic (anhydrous, puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., 99.5-100.5 %)	**60220-M**
Sodium phosphate dibasic (puriss. p.a., ACS reagent, anhydrous, ≥99.0 % (T)	**71640-M**

Related Products

Description	Cat. No.
Rutin phyproof® Reference Substance, neat	**PHL89270**
Rutin trihydrate certified reference material, neat	**00300590**
Rutin trihydrate United States Pharmacopeia (USP) Reference Standard	**1606503**

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PHARMA & BIOPHARMA

Threading the Molecular Needle: The Importance of Particle Pore Diameter for Biomacromolecule Separations

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Introduction

Recently, the evolution of middle to large biomacromolecules such as peptides or proteins is apparent in the market of pharmaceutical drugs in addition to traditional active ingredients based on small molecules. The use of peptides for clinical purposes has its origins in the 1920's using insulin and penicillin. In the 1960's, oxytocin and vasopressin were added to the clinician's arsenal. Leuprorelin and octreotide rounded out the first 60 years of peptide-based drugs by being introduced to the clinic in the 1980's.[1]

In the 1980's, the average length of therapeutic peptides was nine amino acids long with molecular weights of less than 5 kDa. But in the 1990's, longer peptides containing 15 – 20 amino acids became common, and, by the turn of the century, proteins with 40 to 50 amino acid residues with molecular weights approaching or exceeding 50 kDa were beginning to emerge en masse from biopharmaceutical companies. Now, in 2019, even larger proteins have taken the market by storm in the form of monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and bispecific antibodies (bsAbs), to name just a few.

A mAb is composed of two light chains (LC) that are tethered to two heavy chains (HC) through disulfide bonds. In addition, since the LC and HC are composed of amino acids with reactive side chains, IgG's can be post-translationally modified through phosphorylation, methylation, oxidation, and nitrosylation, among other modifications. These modifications may change the binding affinity of the mAb so that it binds either the wrong antigen, does not bind any antigen, or associates with the wrong cell surface receptor. In addition, mAbs can also aggregate which can lead to allergic responses in patients. Biopharmaceutical companies need to develop rigorous methods to assess lot-to-lot reproducibility of their candidate biologic drug, and the above-mentioned modifications are known as Critical Quality Attributes (CQAs) that both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) monitor. Due to these stringent requirements from regulatory bodies, much research has been pursued in the past 20 years to develop accurate, robust, and high-throughput methods to assess biopharmaceutical purity and structure.

High performance liquid chromatography (HPLC) has been used extensively in the past several decades to characterize peptides and proteins. Since the advent of ultrahigh performance liquid chromatography-mass spectrometry (UHPLC-MS) in the mid-2000's, there has been much research in both developing new methods to characterize large biomolecules and in developing new column technology to better resolve all the different molecular entities that are present in a heterogeneous mAb therapeutic. Two main types of particle morphology are prevalent in the industry today: fully porous particles (FPPs) and superficially porous particles (SPPs, also called Fused-Core® or core shell particles). To take advantage of the low dispersion of UHPLC instrumentation, columns with sub-2 µm FPPs with pore sizes of 300 Å have been used for the analysis of larger hydrodynamic radii biomacromolecules. These columns have been the industry standard since the mid 2000's. However, these columns suffer limitations when analyzing larger or more complex proteins like mAbs and antibody-drug conjugates (ADCs). The relatively small pore size, in addition to a totally porous architecture, and overall higher surface area, restricts the free diffusion of large molecules through the particle and may cause irreversible adsorption of the protein to the stationary phase. This architecture concomitantly results in an increase in the mass transfer term and longitudinal diffusion term of the Van Deemter equation, leading to peak tailing, loss of resolution, and low recovery.

Since 2017, a new line of 1000 Å columns have been introduced that have been optimized for mAb and ADC characterization. These columns are packed with 2.7 µm SPPs that are composed of a 0.5 µm shell thickness and a 1.7 µm solid silica core. The 1000 Å pore particle permits the analysis of mAbs, ADCs, and other, much larger, biomacromolecules. Advantages over columns packed with FPPs are numerous: the SPP shows a significant advantage in mass transfer, leading to less band spreading; columns packed with SPPs are more uniformly packed than columns composed of FPPs, leading to a lower eddy dispersion (A term in the Van Deemter equation); and larger particle sized SPPs have efficiencies similar to or better than sub-2 µm FPPs, leading to the ability of the analyst to run at higher flow rates with less risk of on-column frictional

heating due to elevated column backpressure. Finally, the B-term (longitudinal diffusion) of the Van Deemter equation is also minimized with SPPs. This is due to the presence of less dead volume in the column. A column packed with FPPs will occupy only 33 % of the column volume whereas a column packed with SPPs will occupy approximately 41 % of the column volume.[2]

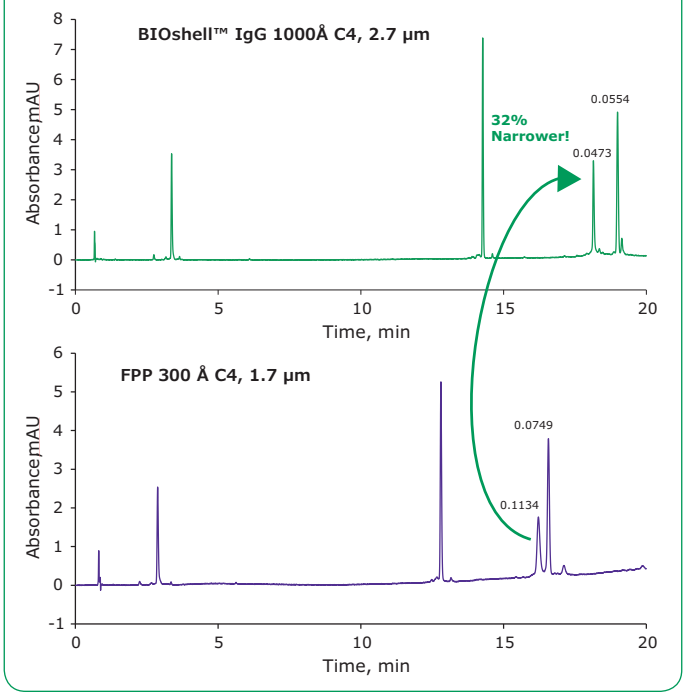
This article will further detail the reasons on why wide-pore diameters are necessary for separating biomacromolecules. Applications involving biomolecules will clearly demonstrate that pore diameters of 450 Å or less can lead to poor results and inadequate resolution of biomolecule variants.

Experimental and Results

All the BIOshell™ IgG 1000 Å SPP columns were obtained from MilliporeSigma*. The experiments were conducted using Shimadzu Nexera X2 UHPLC instruments with PDA detection. Proteins and mAbs were obtained from MilliporeSigma. NISTmAb was purchased from NIST (Gaithersburg, MD). Trifluoroacetic acid was from Pierce Chemicals (Rockford, IL). Acetonitrile was from MilliporeSigma (Gibbstown, NJ). Difluoroacetic acid was purchased from SynQuest Laboratories (Alachua, FL).

Figure 1. Comparison of 1000 Å SPP and 300 Å FPP

Column:	As indicated; 15 cm x 2.1 mm I.D.
Mobile Phase:	[A] Water (0.1 % DFA); [B] Acetonitrile (0.1 % DFA)
Gradient:	23 % B to 50 % B in 24 min
Flow Rate:	0.5 mL/min
Column Temp.:	60 °C
Detector:	UV, 280 nm
Injection:	1.5 µL
Sample:	Protein mixture, varied concentration, water



*The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Larger pores enable improved access to the stationary phase. **Figure 1** shows the comparison of a separation of four proteins using a BIOshell IgG 1000 Å C4 column to a FPP 300 Å C4 column. Increased retention is observed on the BIOshell IgG C4 column indicating better access to the pores/stationary phase. The other advantage of the BIOshell IgG C4 column is that it provides narrower peak widths for all the proteins in the mix. This is especially noticeable for the largest protein in the mix – enolase. The peak width is 32 % narrower on the BIOshell IgG C4 column compared to the FPP 300 Å column.

Another advantage of BIOshell IgG 1000 Å C4 columns is how well they can resolve isoforms of IgG2 mAbs. These mAbs differ in the arrangement of the disulfide bridges that connect the heavy and light chains in the hinge region. In **Figure 2**, six different isoforms of panitumumab (trade name Vectibix) are resolved on the BIOshell IgG 1000 Å C4 column whereas the FPP 300 Å C4 column is only able to resolve approximately two isomers. In addition the back pressure is only 120 bar compared to 205 bar on the FPP 300 Å C4 column. Similar resolution advantages are found when the BIOshell IgG 1000 Å C18 column is compared to a 3 µm FPP 300 Å C18 column as shown in **Figure 3**. In this example, resolution of minor components at the base of the main NIST mAb peak are revealed when the

Figure 2. Improved Resolution of IgG2 Variants

Column:	As indicated; 15 cm x 2.1 mm I.D.
Mobile Phase:	[A] 88:10:2 Water:Acetonitrile:n-Propanol (0.1 % DFA); [B] 10:20:70 Water:Acetonitrile:n-Propanol (0.1 % DFA)
Gradient:	14 % B to 24 % B in 20 min
Flow Rate:	0.2 mL/min
Column Temp.:	80 °C
Detector:	UV, 280 nm
Injection:	2 µL
Sample:	Vectibix, 2 mg/mL, water (0.1 % DFA)

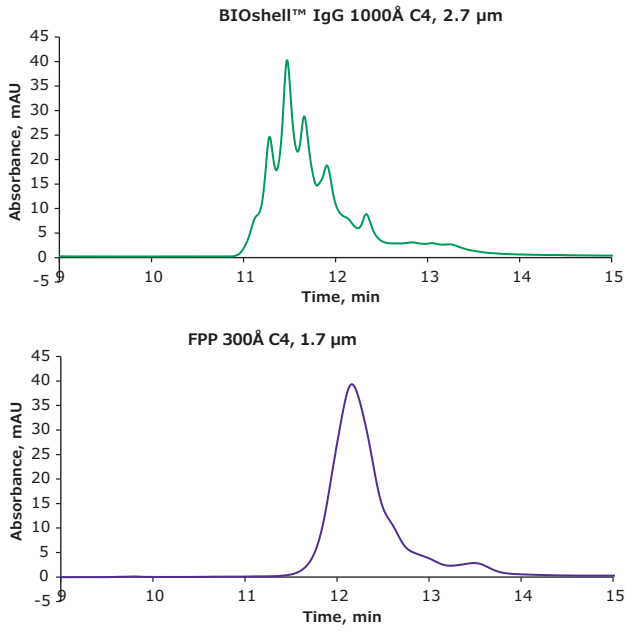
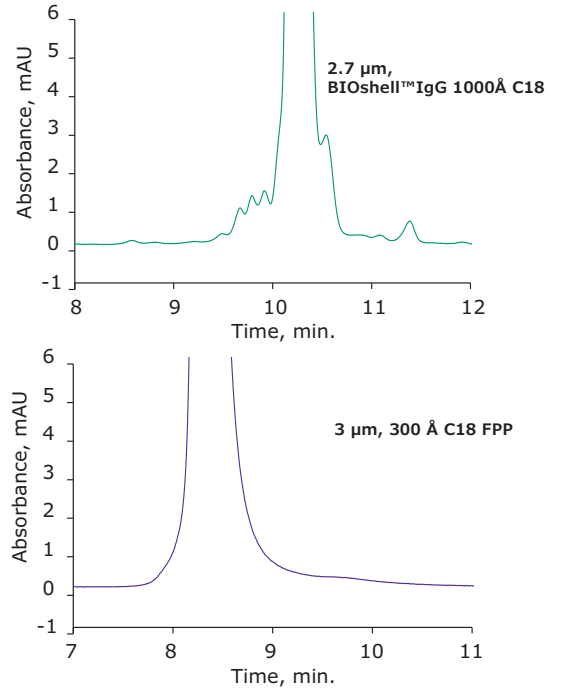


Figure 3. mAb Separation: 1000 Å SPP vs. 300 Å FPP

Column:	As indicated; 15 cm x 2.1 mm I.D.
Mobile Phase:	[A] Water (0.1% TFA); [B] Acetonitrile (0.1 % TFA)
Gradient:	36% B to 44 % B in 16 min
Flow Rate:	0.4 mL/min
Column Temp.:	60 °C
Detector:	UV, 280 nm
Injection:	2 µL
Sample:	NISTmAb, 2 mg/mL, water (0.1 % TFA)



BIOshell IgG 1000 Å C18 column is used. Conversely, none of these minor peaks are visible in the separation when the separation is run on the 3 µm FPP 300 Å column C18 column.

Increased temperature is often used as a means of increasing sample recovery when conducting protein analysis. What has been observed with BIOshell IgG 1000 Å Diphenyl phase is that recovery is equally sufficient at 40 °C as it is at 80 °C. In **Figure 4**, a separation of trastuzumab under 40 °C conditions is compared to a separation on a SPP 450 Å Polyphenyl column. Not only is the peak area greater with the novel BIOshell IgG 1000 Å Diphenyl column, but the resolution and retention are greater, too.

Sample mass on column has always been an experimental variable that chromatographers have had to consider. This variable is further magnified due to the slow diffusion kinetics of large molecules. Column overload can be monitored by observing an increase in peak width as an increasing amount of sample is injected onto the column. **Figure 5** shows the advantages of using a wider pore stationary phase material in terms of band broadening. Much lower peak widths are observed with the BIOshell IgG 1000 Å column than a 300 Å FPP column.

Figure 4. mAb Separation: 1000 Å SPP vs. 450 Å SPP

Column:	As indicated; 15 cm x 2.1 mm I.D., 2.7 µm
Mobile Phase:	[A] Water (0.1% TFA); [B] Acetonitrile (0.1 % TFA)
Gradient:	30 % B to 45 % B in 15 min
Flow Rate:	0.4 mL/min
Column Temp.:	40 °C
Detector:	UV, 280 nm
Injection:	2 µL
Sample:	Trastuzumab, 2 mg/mL, water (0.1 % TFA)

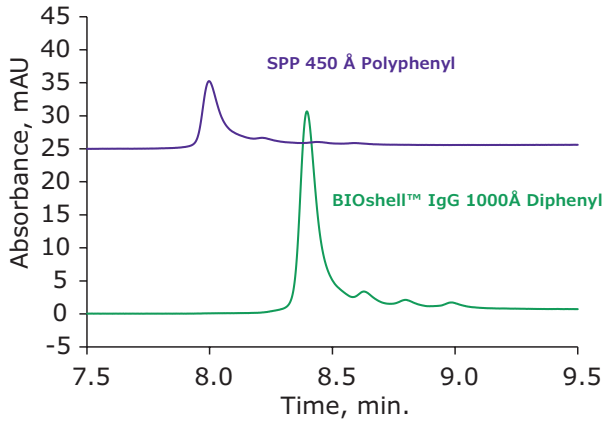
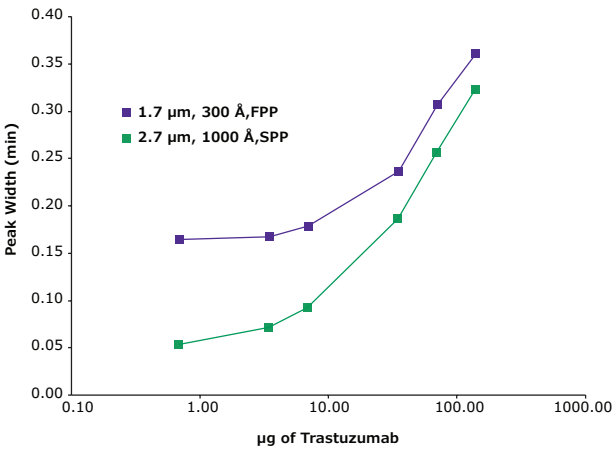


Figure 5. Dependence of Sample Mass on Peak Width

Column:	As indicated; 15 cm x 2.1 mm I.D., C4 Phase
Mobile Phase:	[A] Water (0.1% DFA); [B] Acetonitrile (0.1 % DFA)
Gradient:	27 % B to 37 % B in 10 min
Flow Rate:	0.5 mL/min
Column Temp.:	80 °C
Detector:	UV, 280 nm
Injection:	0.1, 0.5, 1, 5, 10, and 20 µL
Sample:	mAb (trastuzumab), 7 mg/mL, water



Conclusion

Biomacromolecules are complex species requiring cutting-edge chromatographic materials and methods for full characterization. One of the principle parameters of the stationary phase that must be considered when attempting to analyze biomolecules is the pore diameter of the particle. If the pore diameter is too small, steric exclusion will occur resulting in the molecule not being able to access all the available surface area within the pore of the particle. This leads to low chromatographic performance of the method. Combining the high efficiencies garnered from the SPP core-shell architecture with a 1000 Å pore diameter, biomolecules can be completely characterized at a top-down level without fear of steric hindrance or exclusion.

References

1. Ikegami, T.; "Hydrophilic Interaction Chromatography for the Analysis of Biopharmaceutical Drugs and Therapeutic Peptides: A Review Based on the Separation Characteristics of the Hydrophilic Interaction Chromatography Phases." J. Sep. Sci. 2019, 42, 130.
2. Muraco, C. E.; "Improved Biomacromolecule Separations Using Superficially Porous Particles with a 1000 Å Pore Diameter." Chromatography Today, 2018, 4, 27.

Featured Products

Description	Cat. No.
BIOshell™ IgG 1000 Å C4, 15 cm x 2.1 mm I.D., 2.7 µm	**63289-U**
BIOshell™ IgG 1000 Å C18, 15 cm x 2.1 mm I.D., 2.7 µm	**582703-U**
BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 2.1 mm I.D., 2.7 µm	**577421-U**
NISTmAb, Humanized IgG1k Monoclonal Antibody	**NIST8671**

Related Products

Description	Cat.No.
Solvent & Reagents	
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Trifluoroacetic acid for protein sequence analysis	**1.08178**
Guard Columns	
BIOshell™ IgG, C4 2.7 µm, 5 x 2.1 mm Guard Cartridge, pk3	**63291-U**
BIOshell™ IgG, C18 2.7 µm, 5 x 2.1 mm Guard Cartridge, pk3	**581349-U**
BIOshell™ IgG, Diphenyl 2.7 µm, 5 x 2.1 mm Guard Cartridge, pk3	**577431-U**
Ascentis® Express Guard Cartridge Holder, Pk.1	**53500-U**
Acetonitrile with 0.1% (v:v) trifluoroacetic acid for liquid chromatography LiChrosolv®	**4.80448**
Water with 0.1% (v:v) trifluoroacetic acid hypergrade for LC-MS LiChrosolv®	**4.80112**

To read more about our BIOshell line of U/HPLC columns, please visit us at **SigmaAldrich.com/BIOshell**

Certified Reference Materials in Solution for Quantitative NMR (qNMR)

New product line for our qNMR standards range improves user convenience

Alex Rück, Christine Hellriegel, Romana Rigger, Markus Obkircher
Matthias Nold, Product Manager Reference Materials, matthias.nold@merckgroup.com

In recent years, quantitative NMR (qNMR) has become widely accepted as a very efficient and precise method for the quantification of organic compounds and is increasingly used in the pharmaceutical and chemical industry. Its main advantage, when compared to most other analytical techniques, is that the signal integrals are independent of the chemical structure of the compound. This opens the possibility of quantitatively comparing different compounds and thus getting quantitative values that are traceable to internationally recognized primary standards such as those from NIST or NMIJ.

With our new qNMR standard solutions we offer increased convenience to users by providing the qNMR standards already dissolved in a deuterated solvent at a certain concentration. They can be used for internal as well as for external qNMR experiments. In the case of external calibration (e.g., PULCON), no exact weighing will be required.

Our site in Buchs, Switzerland, holds an ISO/IEC 17025 and ISO 17034 (formerly ISO Guide 34) double accreditation as a manufacturer of certified reference materials for quantitative NMR since 2009. This enables us to develop our TraceCERT® product line currently consisting of 300 traceable organic CRMs covering a wide range of analytes such as pesticides, PAHs, phthalates, amino acids, natural products and many more (**SigmaAldrich.com/organiccrm**). The internal standards we use for the certification of these products are also available in our catalog. This range of calibration CRMs for qNMR includes more than 20 traceable CRMs designed for use as internal standards for ^{1}H, ^{31}P and ^{19}F qNMR.[1]

These products have all been provided in neat form to offer maximum flexibility. However, the procedure still requires some critical steps to be performed by the user. The CRM needs to be weighed and dissolved in a deuterated solvent, either directly (for external calibration) or measured together with the analyte (for internal calibration). To avoid this handling step and to add convenience for the end user, we are now launching a series of calibration solutions providing some of the most commonly used calibration standards for ^{1}H quantitative NMR as well as one for ^{31}P quantitative NMR dissolved in a deuterated solvent. The certified value is given as mass fraction (mg/g), allowing a gravimetric sample preparation, which gives the most accurate results. A certified value for the density is also provided, to enable volumetric sampling if desired.

The products are manufactured under ISO/IEC 17025 and ISO 17034 accreditation and are provided in quantities of 1 mL in sealed brown glass ampoules.

New ^{1}H qNMR calibration solutions

Cat. No.	Package Size	Compound	Solvent	Concentration	Chemical Shifts	Nucleus
39387	1 mL	Dimethyl terephthalate solution	DMSO-d_6	5 mg/g	8.1ppm, 3.9ppm	^{1}H
39751	1 mL	Dimethyl terephthalate solution	$CDCl_3$	5 mg/g	8.13ppm, 3.97ppm	^{1}H
76201	1 mL	Fumaric acid solution	D_2O	1 mg/g	6.5ppm	^{1}H
39606	1 mL	Calcium formate solution	D_2O	5 mg/g	8.1ppm	^{1}H
16736	1 mL	Dimethyl sulfone solution	DMSO-d_6	5 mg/g	2.98ppm	^{1}H
39457	1 mL	Benzoic acid solution	DMSO-d_6	5 mg/g	7.5ppm, 7.6ppm, 7.9ppm	^{1}H
79251	1 mL	Phosphonoacetic acid solution	D_2O	5 mg/g	15.7ppm	^{31}P

Find the entire range of qNMR standards at

SigmaAldrich.com/qnmr

References

1. Analytix 2, 2015, page 4 (SigmaAldrich.com/Analytix)

SCIENCE & INNOVATIONS

Titration Goes Digital

Connect your Titrator to SmartChemicals

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

Introduction

Safe and precise titration results will be achieved with a new technology of transferring the data automatically from the reagents and standards to the titrator software.

Copying data from certificates of analysis is slow and creates chances for errors. SmartChemicals eliminate time consuming steps and human errors by transferring all data wireless and instantly to the titrator software. Volumetric Solutions, Karl Fischer Titrants and all standards are embedded with an RFID (Radio Frequency Identification) tag. All relevant data from the Certificate of Analysis are stored on the RFID tag of the SmartChemical.

Just a simple touch transfers all data in seconds

All information needed for method execution, result calculation and documentation are seamlessly transferred as product name, article number, lot/batch no, concentration/assay, shelf life/expiry date. For standards additionally molecular weight, supplier, uncertainty and compliance according to Pharmacopeias and ISO Guide 34.

Previously opened chemicals are also recognized, the initial opening date is stored in the RFID tag and is shown on the touchscreen. Expired chemicals are automatically locked from use.

Benefits using SmartChemicals

- Secure data transfer – ensures complete and correct reagent & standard data in the titration software
- Ease of use – intuitive operation and convenient data transfer in one touch
- Extended quality management –titer determination, shelf life, compliance data, initial opening date
- Improved efficiency – fast data transfer -safes time - no manual writing and no four-eyes principle necessary

Methods using smart chemicals are General Titration and Karl Fischer Titration

Product List

Description	Cat.No.
Titripur® Volumetric Solutions	
Perchloric acid in acetic acid 0.1 mol/L, 1 L	**1090651003**
Potassium hydroxide solution in ethanol 0.5 mol/L, 1 L	**1091141003**
Potassium hydroxide solution in ethanol 0.1 mol/L, 1 L	**1091151003**
Certipur® Volumetric Standards	
Potassium hydrogen phthalate, 80 g	**1024000083**
Benzoic acid, 60 g	**1024010063**
TRIS(hydroxymethyl)aminomethane, 80 g	**1024080083**
Zinc, 100 g	**1024090103**
Aquastar® Karl Fischer Titrants	
CombiTitrant 5, Karl Fischer one component reagents 5 mg H_2O/mL, 1 L	**1880051003** **1024090103**
Aquastar® Water Standards	
Water Standard 1%, 10 x 8 mL ampoule	**1880520013**
Water Standard 1%, 10 x 8 mL ampoule (only North America)	**1880520313**

More products will follow in 2019

SmartChemicals are compatible with Mettler Toledo Excellence Titrator models T5, T7, T9 and Compact Titrator models G10S, G20S, V30S, V20S, V10S

SmartChemicals - the future of titration!

More Information at
SigmaAldrich.com/Titration

Surface Enhanced Raman Spectroscopy (SERS) – A new solution for food quality and safety analysis

Yanqi Qu, Siyue Gao, Lili He, Department of Food Science, University of Massachusetts, Amherst, MA

Winner of the 2018 Merck Lifesciences Award in Food & Beverage Safety 2018 - See full article online at SigmaAldrich.com/Analytix (Issue5)

Surface-enhanced Raman spectroscopy (SERS) is an emerging technology in environmental, agricultural, food, and medical applications.[1–7] In **Figure 1** (a), Raman spectroscopy provides a signature profile of an analyte according to its chemical structure, and with the attachment of nanometallic structures, Raman scattering can be dramatically enhanced. Compared to the standard analytical methods (e.g., HPLC, GC-MS, etc.), SERS showed advantages in simpler sample preparation, faster detection, easier operation, less instrumental complexity, and relative less expensive cost. Additionally, a handled or portable Raman instrument allows SERS to be an on-site solution for the field test.

SERS substrates

Colloidal nanoparticles

Colloidal silver and gold nanoparticles are commercially available and can also be easily fabricated in a lab. Sample preparation can be facilely done by mix a few drops of sample with colloidal nanoparticles and air-dried on solid surfaces for measurement. The biggest concern towards the colloidal nanoparticles is the "coffee ring" that can cause a huge variation.

Solid based substrates

As shown in **Figure 1** (c), nanoparticles can be driven by a "mediating solvent" to self-assembly a monolayer mirror substrate, which features a better signal consistency and an improved quantitative ability. It can be either fabricated with the targets in the solvent or preformed as a dried SERS active platform for sample immersing. In **Figure 1** (b), nanoparticles can also be deposited to targets concentrated on a Nitrocellulose Millipore® membrane. This providing a fast detection of low concentrations of pathogens and contaminants without pre-enrichment.

Application of SERS in food analysis

Pesticides detection

SERS is known for its sensitive and fast detection of many kinds of pesticides in different food or agricultural matricees, with a limit of detection lower than the regulation requirement.[10] In my project, the mirror substrate can detect fonofos pesticide in beverages at a low concentration (i.e. 0.5 ppm) with good recoveries (i.e. 99-106 %), which illustrated the reliability of mirror and SERS in pesticides detection.[8] SERS is also suitable to monitor the distribution and penetration of pesticides on plants (**Figure 1** (d)), also the efficacy of pesticides removal from fruits.[12]

Colorants and adulterants analysis[5]

A SERS database was developed including a wide variety of artificial and natural food coloring agents currently approved or banned in the United States. All colorants showed discriminative SERS signals and can be differentiated or quantified in commercial products.

Wine analysis

By forming the mirror substrate with the red wine extracts in the "mediating solvent", signature peaks in the spectra were successfully matched with condensed tannin, resveratrol, anthocyanins, gallic acid, and catechin, and the unique chemical information creates a specific bar code that could be beneficial for red wine quality assessment and authentication.

Bacteria detection[9,13]

Using the Millipore® filter membrane substrate, we developed a rapid bacteria screening method using SERS to successfully detect Escherichia coli, Salmonella enterica, and Listeria monocytogenes in 80 min in pond water and vegetable rinse water with reliable quantification (i.e. R^2 = 92 %) to a concentration as low as 10 CFU/mL.

References

Please see the online version of the article

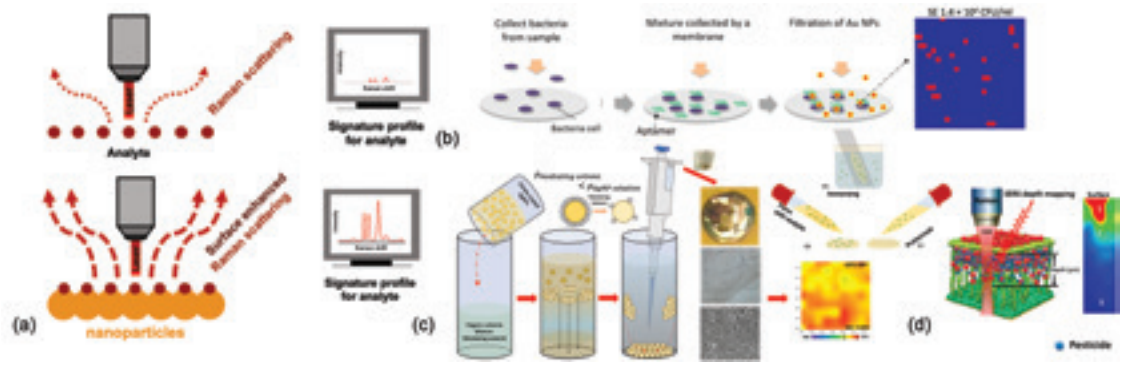
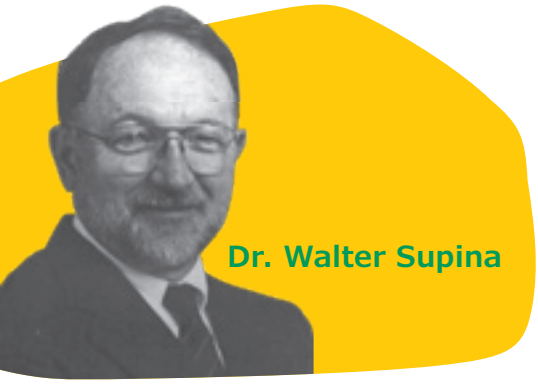


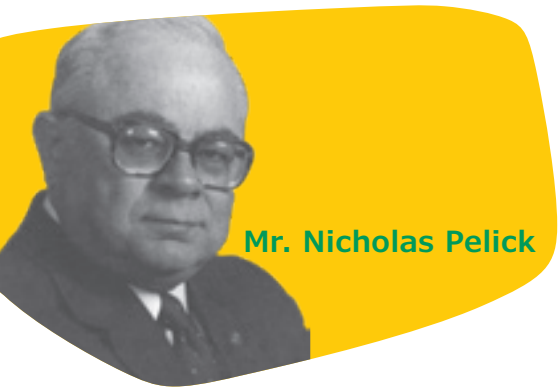
Figure 1. (a) Mechanism of SERS (b) Millipore® membrane substrate for bacterial detection (c) Mirror substrate (d) Monitoring of pesticide penetration using SERS mapping technique.

IN ESSENCE

2019 Pittcon Heritage Award for Supelco® Founders



Dr. Walter Supina



Mr. Nicholas Pelick

Supelco® founders, Dr. Walter Supina and Mr. Nicholas Pelick, received the prestigious 2019 Pittcon Heritage Award for their contributions to the instrumentation and laboratory supplies community; joining such pioneers as Arnold Beckman, James Waters, and Russell and Sigurd Varian, amongst others.

About the Pittcon Heritage Award

The Pittcon Heritage Award is awarded annually by the Chemical Heritage Foundation and the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) to recognize outstanding contributions to the instrumentation and laboratory supplies community.

The long-term objective is to establish the Pittcon Heritage Award as the premiere award in the instrumentation and laboratory supplies community, and to encourage emulation, inspire achievement and promote public understanding of modern sciences, industries and economies.

The award has been presented jointly with Pittcon since 2002 and is given out each year at a special ceremony during Pittcon. The recipient's name and achievements are added to the Pittcon Hall of Fame, which conference attendees can visit at the show each year.

Being a Part of Analytical Chemistry History

In the 1960s, chromatography as a practical analytical tool was in its commercial infancy. As instrument companies came on line to develop and introduce gas chromatographs to new markets, Walter (Walt) and Nicholas (Nick) stepped up to become pioneers at serving customers with the consumables; the columns, standards, reagents, and accessories, to get the most out of those instruments. Recognizing the potential of Gas Chromatography (GC) to provide purified lipid standards for medical research, Walt brought his chemical engineering background and Nick his lipid biochemistry education together to form Supelco® in 1966. Starting with a focused line of packed GC adsorbents and columns and lipid standards, their innovations and growth tracked the emerging chromatography field. Through Supelco Inc., Walt and Nick introduced carbonaceous adsorbents in 1971, capillary GC in 1977, entered HPLC in 1979, air monitoring in 1983, and sample prep in 1985. Supelco Inc., had many "firsts" to the market, with each entry marked with innovations around particles, columns, surface chemistry, and devices that solved particular analytical challenges their customers were facing. While other players have come and gone, the

Supelco® enterprise has remained an entity today, still innovating, and still serving the global analytical community as part of Merck KGaA, Darmstadt, Germany. But through the years and changes, Supelco® never lost sight of the customer. Nick was quoted saying "We made every effort to show the customer that we gave a damn...we would never sell products that were inferior...we never compromised." To drive that home, the Supelco® brand name is now used to represent all analytical (e.g. chromatography, spectroscopy, titration) products in the Merck KGaA portfolio. It is a real testament to the innovation, quality, and customer service culture that Walt and Nick created, and the innovations developed as a result of their passion for the customer and vision of technical leadership are extensive.

Award was accepted by Walt's son, Dick and Nick's daughter, Daria

Important Contributions to Public Understandings

Through teaching…

Few people tried to advance the public understanding of chromatography in its early years more than Walt and Nick did. One example that seems so obvious now, but was quite novel at the time, are the Supelco® chromatography short courses which were developed with their encouragement. The courses were aimed at providing practical instruction to users at all levels. Walt also wrote a book called "The Packed Column in Gas Chromatography". The book was first published in 1974 and was the outgrowth of the column selection short course which Walt began teaching in 1971. The Supelco® team still has that passion for education that we fulfill by conducting workshops, training, webinars and seminars at customer sites, tradeshows, and industry events across the globe. Walt and Nick also taught us about service, and many members of our scientific teams serve on the boards of diverse industry groups and regulatory agencies.

We are honored that the Chemical Heritage Foundation and the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy have awarded Dr. Walter Supina and Mr. Nicholas Pelick with the 2019 Pittcon Heritage Award.

Thank you, Walt and Nick for building a company that stands for quality and innovation—your legacy lives on in the Supelco® analytical product portfolio of Merck.

As we have been celebrating 350 years of Merck, we raise our peaks and say here's to 350 more years of quality analytical products without compromise—made by analytical chemists for analytical chemists.

Because our high standards continue to match yours!

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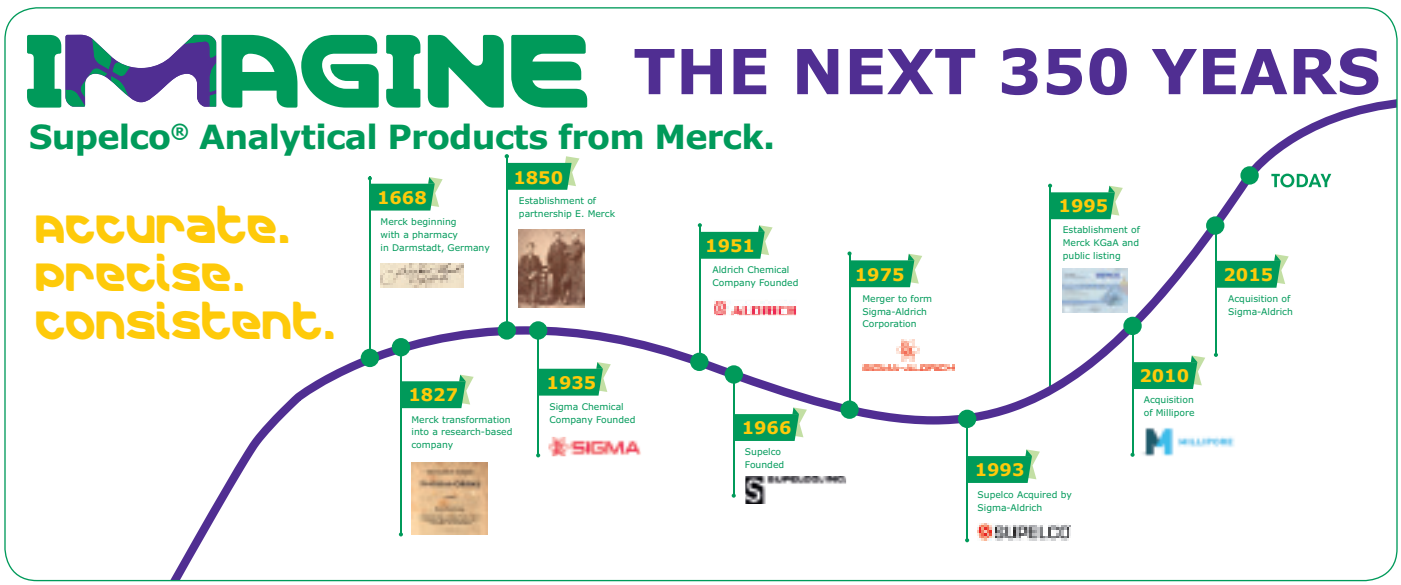

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