Issue 5 • 2016

Analytix

High Performance Thin Layer Chromatography

HPTLC Fingerprint Applications for *Ginkgo Biloba*

pg. 3

New EC Regulation for Tropane Alkaloids

Testosterone Serum Calibrator Kit

New Vitroids™ Range and Cross Reference Guide

Pesticides as Internal Isotopic-Labeled Standards

Pharmaceutical Reference Materials

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Feature Article

Standards

Mass Spectrometry

Labware

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Every Test Impacts a Life – Results Must Be Accurate!

Sherri Pogue

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Accuracy of analytical results is dependent on many things, but one thing is certain – if the reference materials used are inaccurate, then the results will be inaccurate. Test results have critical implications for the safety and efficacy of pharmaceuticals and dietary supplements. They impact the quality and safety of our food, water, and the air we breathe. Results impact medical diagnoses, occupational and public health, employment, forensic casework, and public policy. Analytical results influence the quality of fuels and industrial materials. In one way or another, these results ultimately impact people – ideally, keeping us safe, free from harm, healthy or on the path toward wellness.

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Kind regards,

Sherri Pogue
Head of Reference Materials
Advanced Analytical Business
Unit Applied Solutions Business
Merck KGaA, Darmstadt, Germany
Life Science

High Performance Thin Layer Chromatography

HPTLC Fingerprint Applications for *Ginkgo Biloba*

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Ginkgo-based products are one of the most commonly used over-the-counter (OTC) herbal preparations for treatment of deficits in memory, concentration and depression from organic brain disease[1]. The two main pharmacologically active groups of compounds present in the Ginkgo leaf are the flavonoids and the terpenoids[2]. Another category of constituents, found in the leaves and fruit pods, is called ginkgolic acid, which is toxic and therefore its presence in medicinal products should be avoided.

In the following, we present HPTLC methods suitable for these three compound groups using CAMAG equipment, Merck KGaA, Darmstadt, Germany TLC plates, analytical standards and Ginkgo extract reference material 05485001. This extract reference is part of a product group of extract reference materials for convenient identification of plant material and quantification of key components manufactured by HWI Analytik and exclusively distributed by Sigma-Aldrich®
(**sigma-aldrich.com/plantextracts**).

Application Note 1: Flavonoids

Numerous flavonol glycosides were identified in *Ginkgo biloba* extracts as derivatives of the aglycones, and together they account for 24% of the Ginkgo compounds. Flavonols (quercetin, kaempferol and isorhamnetin) are usually found only in small amounts in the leaves[3]. Moreover, the flavonoid content in the leaf is known to vary between seasons, with greater amounts found in the fall than in the spring[4].

The quality of *Ginkgo biloba* extracts is generally evaluated by determination of a minimum content of the terpene lactones and total flavonoids, expressed as the three flavonols (Q/K/I), after hydrolysis of the various flavonol glycosides with acid and heat[5]. Simplification of quality control methods might lead to economically driven adulteration with inexpensive synthetic compounds such as rutin, which, after hydrolysis, is converted into quercetin, or quercetin itself.

Scope:
Identification of flavonoids in the HPTLC fingerprint of *Ginkgo biloba* extracts and leaf obtained with the HPTLC method of the USP monograph on Ginkgo leaf[6] by comparison of the R_F values of the reference substances and the matching zones in the reference extract.

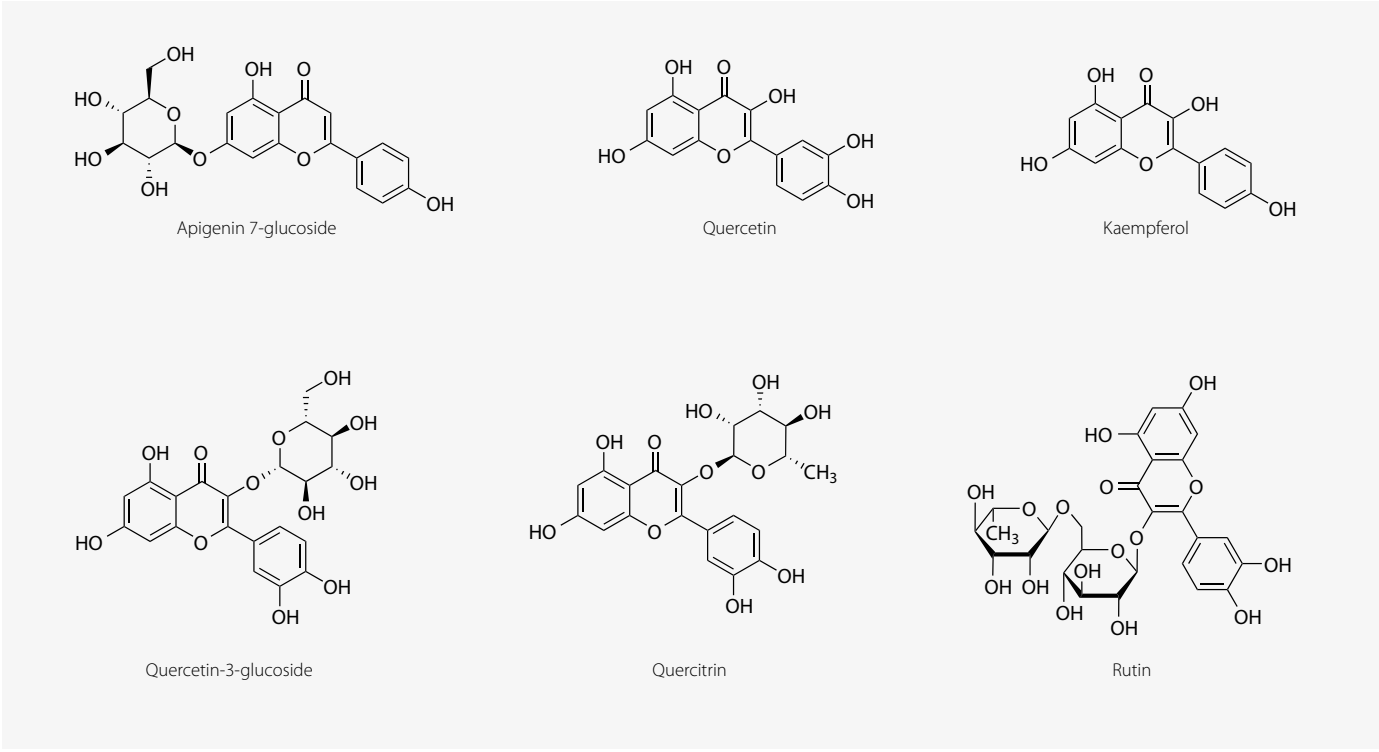


Figure 1. Flavonoids Present in *Ginkgo Biloba*

Recommended CAMAG Devices:
Automatic TLC Sampler 4 (ATS 4) or Linomat 5, Automatic Developing Chamber (ADC 2), TLC Visualizer, Chromatogram Immersion Device, TLC Plate Heater and visionCATS.

Sample:
Drug: In a 25 mL flask, 1 g powdered raw material is refluxed with 10 mL methanol for 10 min. After cooling, the mixture is centrifuged.
Extract: 0.1 g of dry extract is sonicated with 10 mL methanol for 10 min and filtered. The supernatant is used as test solution.

Standards:
Standard solutions were prepared in a concentration of 0.25 mg/mL in methanol.

Derivatization Reagent:
Natural Products reagent (NP reagent): 1 g diphenylborinic acid aminoethylester is dissolved in 200 mL ethyl acetate.

Polyethylene glycol (PEG reagent): 10 g polyethylene glycol 400 (macrogol) are dissolved in 200 mL dichloromethane.

Chromatography Following USP <203>[7]:

Stationary phase:	HPTLC Si 60 F_{254} 20×10 cm
Sample application:	3 µL each of test solution and 2 µL of standards are applied as 8 mm bands, 8 mm from lower edge of plate and 20 mm from the left edge, using the ATS 4.
Developing solvent:	Ethyl acetate, glacial acetic acid, formic acid and water (100:11:11:26 v/v/v/v)
Development:	Development was performed with ADC 2, saturated for 20 min. with the mobile phase (filter paper). Prior to the development, the plate was exposed to a relative humidity of 33% (with a saturated solution of $MgCl_2$).
Developing distance:	70 mm from lower edge of the plate
Plate drying:	5 min in a stream of cold air
Detection:	The plate is heated at 100 °C for 3 min, then dipped (speed: 3, time: 0) while still hot in NP reagent, dried in a stream of cold air, then dipped (speed: 3, time: 0) in PEG reagent
Evaluation:	Documentation under UV 366 nm after derivatization

Results:

Under UV 366 nm after derivatization (**Figure 2**), the zones corresponding in color and position to the standards rutin, quercetin-3-glucoside, apigenin-7-O-glucoside and quercitrin are seen in all samples in different intensities. The standards quercetin and kaempferol are seen in all three extracts (track 7, our reference extract, track 8 and track 9); however, in different intensities. The extract on track 9 represents a characteristic fingerprint of a sample adulterated with quercetin, kaempferol and isorhamnetin due to the presence of an intense yellow/green zone at the position of quercetin and kaempferol (just below the solvent front). Ginkgo leaf (track 10) shows a fingerprint similar to the reference extract on track 7; however, due to the extraction processes and chlorophylls, the red zone at the solvent front is not seen in the reference extract. The fingerprint on track 11 is characteristic of golden fall ginkgo.

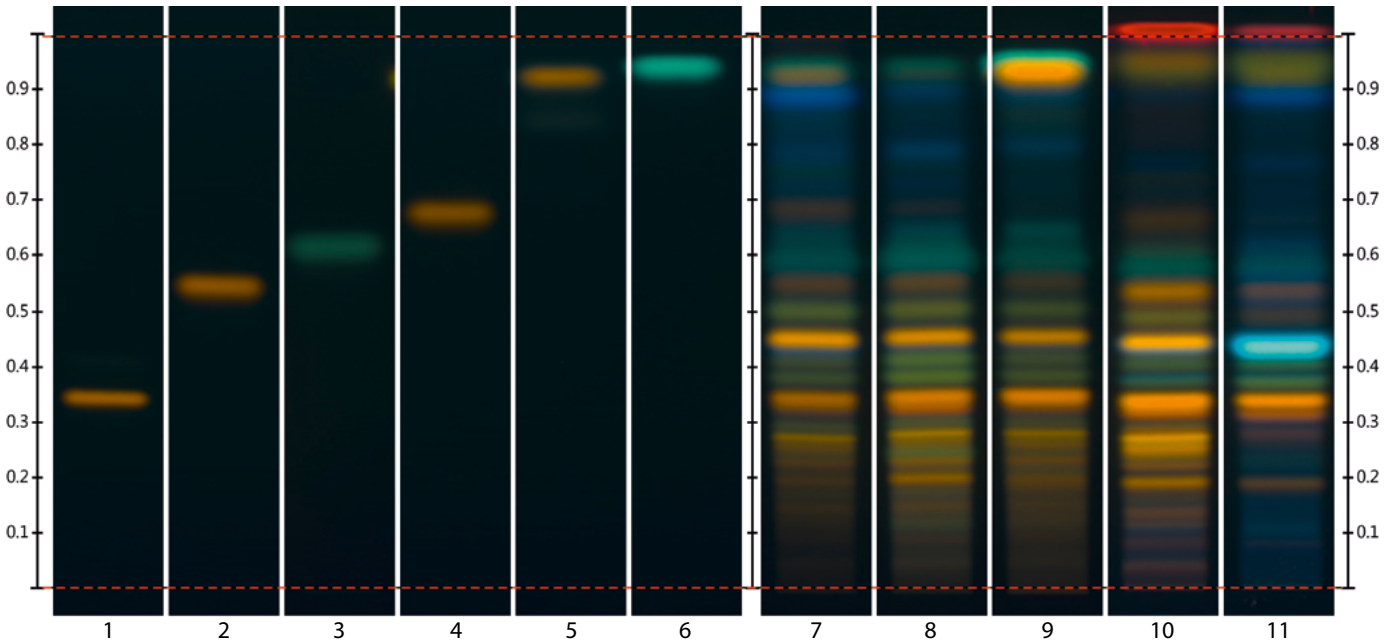
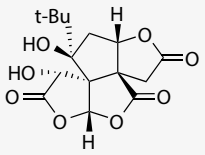
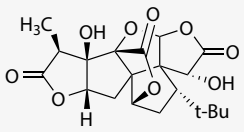


Figure 2. Chromatogram under UV 366 nm after Derivatization. Track 1: Rutin; Track 2: Quercetin-3-glucoside; Track 3: Apigenine-7-O-glucoside; Track 4: Quercitrin; Track 5: Quercetin; Track 6: Kaempferol; Track 7: *G. Biloba* Leaf Powdered Extract 05485001; Track 8: *G. Biloba* Leaf Dry Extract; Track 9: *G. Biloba* Leaf Dry Extract Adulterated with Quercetin; Track 10: *G. Biloba* Powdered Leaf; Track 11: *G. Biloba* Powdered Leaf (Golden Ginkgo)

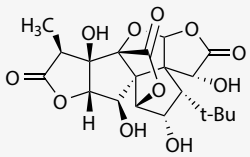
Application Note 2: Ginkgo Terpene Lactones



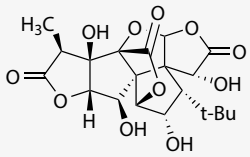
Bilobalide



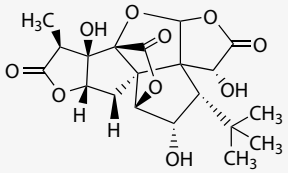
Ginkgolide A



Ginkgolide B



Ginkgolide C



Ginkgolide J

Figure 3. Ginkgolide and Bilobalide Lactones Present in *Ginkgo Biloba*

Two types of terpenoids are present in ginkgo as lactones: ginkgolides and bilobalides (**see Figure 3**). Together they account for 6% of the ginkgo compounds and are present only in this species[2, 8]. Due to the low UV absorption of the terpene lactones, HPLC analyses require special detectors[9]. For detection in HPTLC, only a simple derivatization step is required.

Scope:
Identify the presence of ginkgolides (A, B, C and J) and bilobalide in the fingerprints of *Ginkgo biloba* extracts and leaf obtained with the HPTLC method of the USP monograph for ginkgo leaf by comparison of the R_F values of the reference substances and the matching zones in the reference extract.

Required or Recommended CAMAG Devices:
Automatic TLC Sampler 4 (ATS 4) or Linomat 5, Automatic Developing Chamber (ADC 2), TLC Visualizer, TLC/HPTLC Sprayer or CAMAG Derivatizer, TLC Plate Heater and visionCATS.

Sample:
Drug: In a 25 mL flask, 1 g powdered raw material is refluxed with 10 mL methanol for 10 min. After cooling, the mixture is centrifuged.
Extract: 0.1 g of dry extract is sonicated with 10 mL methanol for 10 min and filtered. The supernatant is used as test solution.

Standards:
Standard solutions were prepared in a concentration of 1.0 mg/mL in methanol.

Plate Impregnation with Sodium Acetate Solution:
8 g of sodium acetate are dissolved in 200 mL of ethanol, water (3:2 v/v). HPTLC plates are immersed into the solution for 2 seconds and allowed to dry at room temperature in the hood for 5 min. The plates are then heated at 90 °C for 30 min.

Derivatization Reagent:
Acetic anhydride is directly used for spraying.

Chromatography Following USP <203>[6]:

Stationary phase:	HPTLC Si 60 F_{254} 20×10 cm.
Sample application:	5 µL each of test solution and 3 µL of standards are applied as 8 mm bands, 8 mm from lower edge of plate and 20 mm from the left edge using the ATS 4.
Developing solvent:	Toluene, ethyl acetate, acetone, methanol (20:10:10:1.2 v/v/v/v).
Development:	Development is performed with ADC 2, saturated for 20 minutes with the mobile phase (filter paper). Prior to the development, the plate is exposed to a relative humidity of 33% (with a saturated solution of $MgCl_2$).
Developing distance:	70 mm from lower edge of the plate.
Plate drying:	5 min in a stream of cold air.
Detection:	The plate is sprayed evenly with acetic anhydride and heated at 180 °C for 10 min.
Evaluation:	Documentation under UV 366 nm after derivatization.

Results:

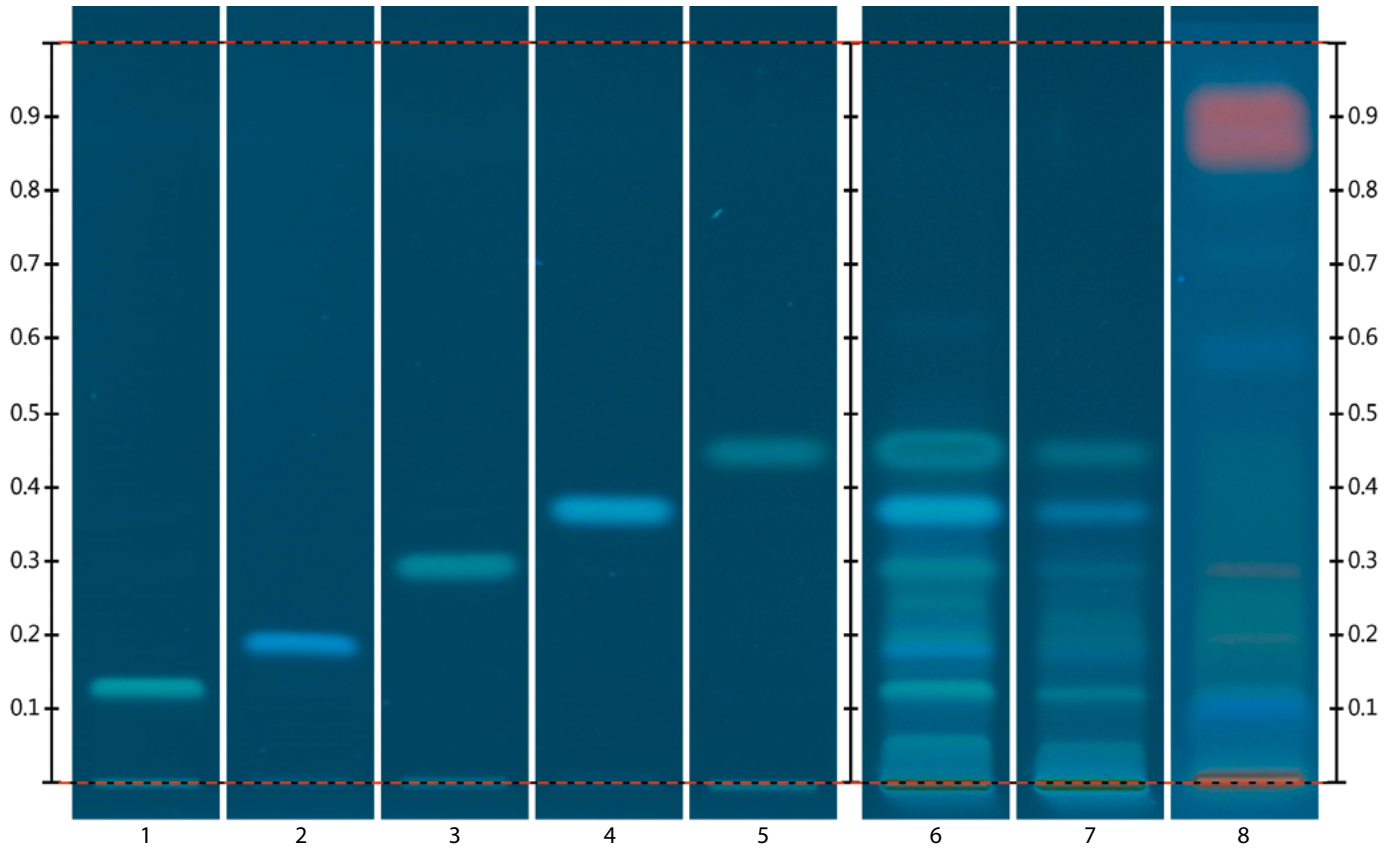
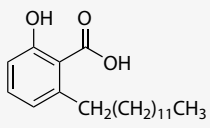


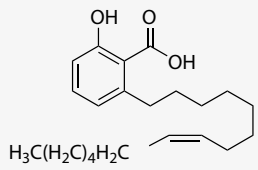
Figure 4. Chromatogram under UV 366 nm after Derivatization. Track 1: Ginkgolide C; Track 2: Ginkgolide J; Track 3: Ginkgolide B; Track 4: Ginkgolide A; Track 5: Bilobalide; Track 6: *G. Biloba* Leaf Powdered Extract 05485001; Track 7: *G. Biloba* Leaf Dry Extract; Track 8: *G. Biloba* Powdered Leaf

In **Figure 4**, the zones, due to the standards Ginkgolides C, J, B, A and Bilobalide, are seen in both extracts (track 6, our reference extract from HWI Analytik, and track 7). These zones are barely seen in leaf (track 8) due to the presence of a matrix that disturbs the chromatogram.

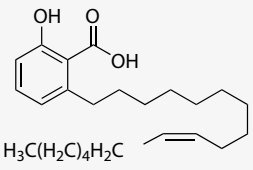
Application Note 3: Ginkgolic Acids



Ginkgolic acid C13:0



Ginkgolic acid C15:1



Ginkgolic acid C17:1

Figure 5. Ginkgolic Acids Present in *Ginkgo Biloba*

The compound class of ginkgolic acids can have toxic effects such as allergies[10-11]; therefore, during the preparation of ginkgo-based products, except for the crude drug products, the undesired ginkgolic acids are usually eliminated[12].

Scope:
Identification of ginkgolic acids (C15:1, C13:0 and C17:1) in the HPTLC fingerprint of *Ginkgo biloba* extracts and leaf obtained with the HPTLC method of the HPTLC Association[13] on ginkgo leaf by comparison of the R_F values of the reference substances and the matching zones in the reference extract.

Required or Recommended CAMAG Devices:
Automatic TLC Sampler 4 (ATS 4) or Linomat 5, Automatic Developing Chamber (ADC 2), TLC Visualizer and visionCATS.

Sample:
Drug: In a 25 mL flask, 1 g powdered raw material is refluxed with 10 mL methanol for 10 min. After cooling, the mixture is centrifuged. Extract: 0.1 g of dry extract is sonicated with 10 mL methanol for 10 min and filtered. The supernatant is used as test solution.

Standards:
Standard solutions were prepared in a concentration of 0.4 mg/mL in methanol.

Chromatography Following USP <203>[7]:

Stationary phase:	HPTLC Si 60 F_{254} 20×10 cm.
Sample application:	5 µL each of test solution and standards are applied as 8 mm bands, 8 mm from lower edge of plate and 20 mm from the left edge using the ATS 4.
Developing solvent:	Toluene, ethyl acetate, gracial acetic acid (40:10:1 v/v/v).
Development:	Development is performed with ADC 2, saturated for 20 min with the mobile phase (filter paper). Prior to the development, the plate was exposed to a relative humidity of 33% (with a saturated solution of $MgCl_2$).
Developing distance:	70 mm from lower edge of the plate.
Plate drying:	5 min in a stream of cold air.
Evaluation:	Documentation under UV 366 nm.

Results:

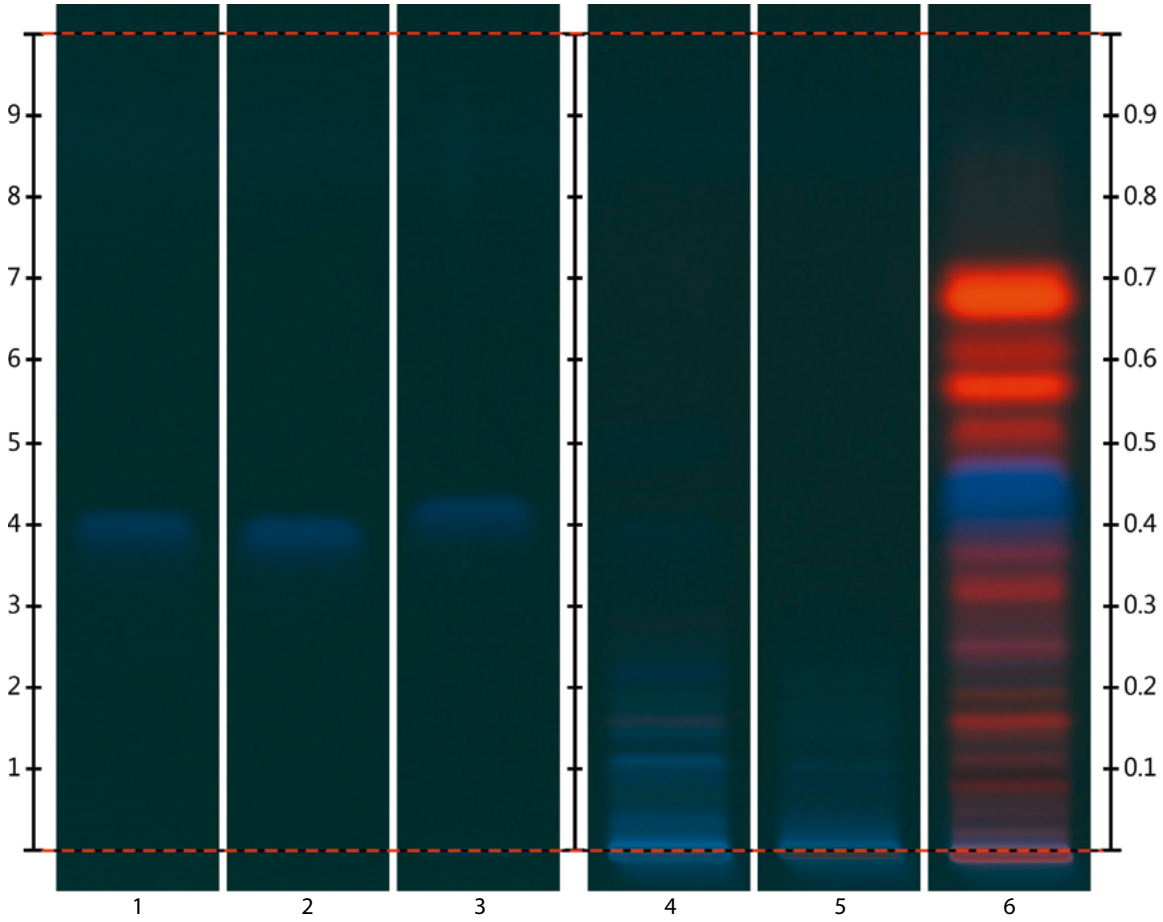


Figure 6. Chromatogram under UV 366 nm. Track 1: Ginkgolic Acid C15:1; Track 2: Ginkgolic Acid C13:0; Track 3: Ginkgolic Acid C15:1; Track 4: *G. Biloba* Leaf Powdered Extract 05485001; Track 5: *G. Biloba* Leaf Dry Extract; Track 6: *G. Biloba* Powdered Leaf

As observed in **Figure 6**, only the *G. biloba* powdered leaf sample (track 6) shows a wide blue fluorescent zone due to unresolved ginkgolic acids between R_Fs 0.38 and 0.49.

Conclusions:

The examples shown above demonstrate that HPTLC is a very powerful and efficient tool for fast analysis of complex compound mixtures such as plant materials. Besides consuming little time and low quantities of solvents, the method allows for simultaneous analysis of multiple samples and in addition does not require a time-consuming sample preparation procedure, thus making HPTLC a valuable alternative to other chromatographical methods. Further information on instrumentation for HPTLC can be found at:
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We are very proud to offer all consumables needed for the described applications including reference materials and TLC plates ready from stock. Find all used products listed below.

Cat. No.	Product Group	Description	Package Size
00720585	Flavonoids	Apigenine-7-O-glucoside	10 mg
00550580		Kaempferol	10 mg
00200595		Quercetin	50 mg
16654		Quercetin 3-glucoside	10 mg
00740580		Quercitrin	25 mg
78095		Rutin	25 mg
49962	Ginkgolic Acids	Ginkgolic acid C13:0	10 mg
02580585		Ginkgolic acid C15:1	10 mg
01390590		Ginkgolic acid C17:1	10 mg
00760595	Terpene Lactones	(-)-Bilobalide	10 mg
00770590		Ginkgolide A	25 mg
94970		Ginkgolide B	10 mg
01490590		Ginkgolide C	25 mg
89556		Ginkgolide J	5 mg

Table 1. Analytical Standards Used for Applications

Cat. No.	Description	Quantitative Markers	Qualitative Markers	Package Size
05485001	*Ginkgo biloba* extract	Bilobalide, Ginkgolide A	Bilobalide, Ginkgolide A, Ginkgolide B, Ginkgolide C	150 mg

Table 2. Ginkgo Plant Extract Reference Material Used for Applications

Cat. No.	Description	Dimensions	Package Size
1.05642.0001	HPTLC glass plate Silica gel 60 F_{254}	20×10 cm	50 Plates

Table 3. TLC Plates Used for Applications

A list of our complete offering of phytochemical standards can be found at **sigma-aldrich.com/medicinalplants** and all our extract reference materials including an example certificate can be viewed here: **sigma-aldrich.com/plantextracts**. All plant extract reference materials are delivered with a certificate giving the exact mass fractions for the quantitative markers. Additional qualitative markers are confirmed. A chromatographical method is also provided, including a chromatogram with peak assignation.

Our HPTLC plates enable significant faster results at high precision in outstanding quality.

Learn more about the features of High Performance Thin Layer Chromatography plates at
www.merckmillipore.com/hptlc

References:

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New EC Regulation for Tropane Alkaloids

Overview of Standards and CRMs for Atropine and Scopolamine

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In February 2016, the European Commission issued the new Regulation 2016/239 as an amendment to Regulation (EC) No. 1881/2006 to define maximum levels of tropane alkaloids in cereal-based foodstuffs for infants and young children[1].

Tropane alkaloids have a bicyclic structure and naturally occur in plants of various families such as Brassicaceae, Solanaceae and Erythroxylaceae. The best-known representatives of this compound class are (-)-hyoscyamine and (-)-scopolamine. These two alkaloids are known to have anticholineric activity. The racemic mixture of (-)-hyoscyamine and its inactive enantiomer (+)-hyoscyamine is called atropine.

Tropane alkaloids are known to be present in the genus Datura, a plant which is widely distributed in temperate and tropical regions. The seeds of Datura have been found as contaminants in lots of linseed, soybeans, sorghum, millet and buckwheat, and therefore the European Commission has defined maximum levels for these compounds in cereal-based foods. Both atropine and scopolamine are not allowed to be present at levels higher than 1.0 µg/kg.

We offer both atropine and scopolamine as analytical standards or CRM solutions as well as CRM solutions of the deuterated forms to be used as internal standards in stable isotope dilution analysis LC-MS experiments.

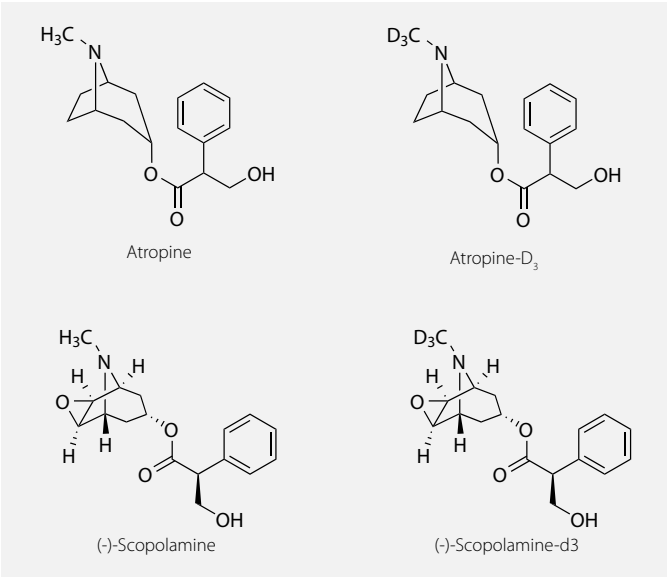

Figure 1. Chemical Structures of Atropine and (-)-Scopolamine and Structures of the Corresponding Deuterated Standards

Cat. No.	Description	Concentration	Quality Grade	Package Size
A-046	Atropine	Solution, 1.0 mg/mL in acetonitrile	Certified reference material	1 mL
37019	Atropine	Dried down solution, 100 µg/mL after reconstitution with 1 mL of water	Analytical standard	0.1 mg
A-077	Atropine-D_3	Solution, 1.0 mg/mL in acetonitrile	Certified reference material	1 mL
37022	(–)-Scopolamine hydrochloride	Dried down solution, 100 µg/mL (free base) after reconstitution with 1 mL of water	Analytical standard	0.1 mg
S-098	(–)-Scopolamine hydrochloride	Solution, 1.0 mg/mL in acetonitrile	Certified reference material	1 mL
S-099	(–)-Scopolamine-D_3 hydrochloride	Solution, 1.0 mg/mL in acetonitrile	Certified reference material	1 mL

Table 1. Tropane Alkaloid Reference Materials

Reference:

[1] Commission Regulation (EU) 2016/239 of 19 February **2016**.

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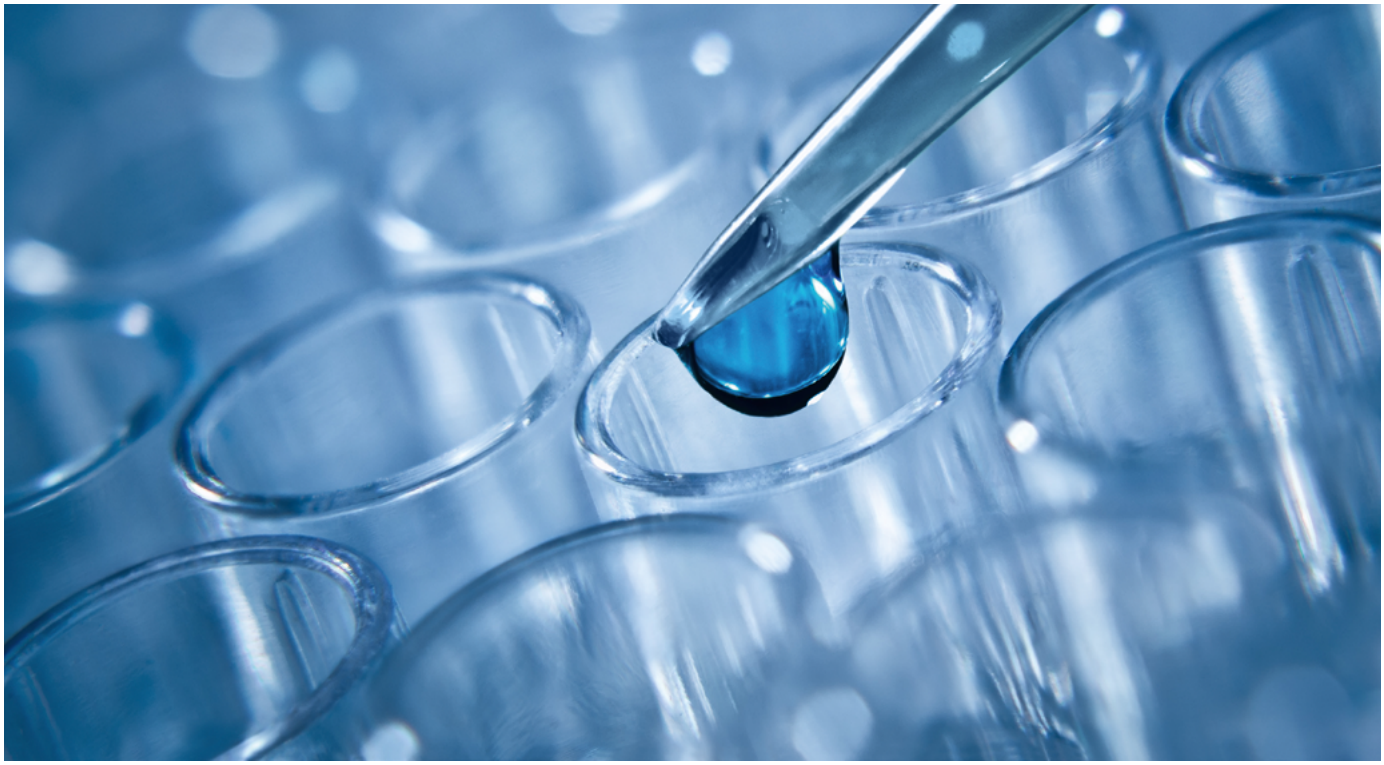
Overview of Analytical Standards and Certified Reference Materials of Substances of Very High Concern (SVHC)



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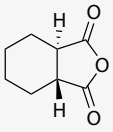
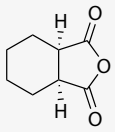
Substances of very high concern (SVHC) are hazardous chemicals regulated by REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), Article 57. It can be proposed that their use be subject to authorization under the REACH Regulation within the European Union. The decision to add a substance to the SVHC candidate list is made by national REACH Competent Authorities, or by the European Chemicals Agency (ECHA) at the request of the European Commission (EC). All suppliers of industrial goods must inform the ECHA if an article contains more than 0.1% (w/w) of SVHCs.

We offer a comprehensive portfolio of analytical standards and certified reference materials for SVHC or SVHC candidates for quality control and analytical testing. The products marked with an asterisk (*) are available in CRM grade (*Trace***CERT®**) certified by quantitative NMR (qNMR) under ISO Guide 34 and ISO 17025 (see also article on page 17).

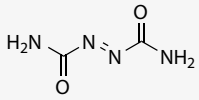
You can also find the products listed on our webpage at **sigma-aldrich.com/svhc**



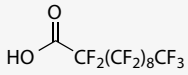
trans-1,2-Cyclohexanedicarboxylic anhydride



cis-1,2-Cyclohexanecarboxylic anhydride



Azodicarboxamide



Perfluoroundecanoic acid

Figure 1. Some Recent Product Additions of SVHC Reference Materials

Cat. No.	Description	CAS No.	Package Size
08267*	Acrylamide	79-06-1	100 mg
46130	4-Aminoazobenzene	60-09-3	250 mg
31629	*o*-Aminoazotoluene	97-56-3	250 mg
31598	4-Aminobiphenyl	92-67-1	250 mg
31597	*o*-Anisidine (2-Methoxyaniline)	90-04-0	250 mg
07671*	Anthracene	120-12-7	100 mg
79997	Azodicarboxamide	123-77-3	50 mg
46364	C.I. Basic Violet 3	548-62-9	250 mg
42438*	Benzyl butyl phthalate (BBP)	85-68-7	50 mg
31598	Biphenyl-4-ylamine	92-67-1	250 mg
67261*	Bis(2-ethylhexyl)phthalate (DEHP)	117-81-7	100 mg
36934	Bis(methylglycol) phthalate	117-82-8	250 mg
34120	Bis(pentabromophenyl) ether (BDE No 209) solution 50 µg/mL in isooctane:toluene (9:1)	1163-19-5	1 mL
07369	*trans*-1,2-Cyclohexanecarboxylic anhydride	14166-21-3	50 mg
18139*	4,4'- Diaminodiphenylmethane (MDA)	101-77-9	50 mg
18281*	Dibutyl phthalate (DBP)	84-74-2	50 mg
02562	1,2-dichloroethane	107-06-2	1 mL, 5 mL
04143	Diethylene glycol dimethyl ether	111-96-6	1 mL, 5 mL
49617	Dihexyl phthalate	84-75-3	250 mg
43540*	Diisobutyl phthalate	84-69-5	100 mg
72405	1,2-dimethoxyethane,ethylene glycol dimethyl ether (EGDME)	110-71-4	1 mL, 5 mL
72336	*N,N*-dimethylacetamide	127-19-5	1 mL, 5 mL
72438	*N,N*-dimethylformamide	68-12-2	5 mL
18191*	2,4-dinitrotoluene	121-14-2	100 mg
45453	Dinoseb (6-*sec*-butyl-2,4-dinitrophenol)	88-85-7	100 mg
59832*	Dipentyl phthalate (DPP)	131-18-0	50 mg

Cat. No.	Description	CAS No.	Package Size
75768	C.I. Direct Red 28	573-58-0	25 mg
79109	2-ethoxyethanol	110-80-5	1 mL, 5 mL
01913*	2-ethoxyethyl acetate	111-15-9	100 mg
43861	Furan	110-00-9	1 mL
08483	Hexahydro-4-methylphthalic anhydride	19438-60-9	100 mg
45531	Imidazolidine-2-thione	96-45-7	250 mg
08485	Methoxyacetic acid	625-45-6	100 mg
88907	2-methoxyethanol	109-86-4	1 mL, 5 mL
46111	6-methoxy-*m*-toluidine (*p*-cresidine)	120-71-8	250 mg
78769	1-Methyl-2-pyrrolidone (NMP)	872-50-4	5 mg, 10 mg
66681	4,4'-Methylene-bis(2-chloroaniline)	101-14-4	250 mg, 1 g
46106	4,4'-methylenedi-o-toluidine	838-88-0	100 mg
45922	4-methyl-m-phenylenediamine	95-80-7	250 mg
56671	Methyloxirane (Propylene oxide)	75-56-9	1 mL, 5 mL
07559*	Michler's base (*N,N,N',N'*-tetramethyl-4,4'-methylenedianiline)	101-61-1	50 mg
56614*	Michler's ketone (4,4'-bis(dimethylamino) benzophenone)	90-94-8	100 mg
46383	Musk xylene (5-*tert*-butyl-2,4,6-trinitro-*m*-xylene) (solution, 100 µg/mL in acetonitrile)	81-15-2	2 mL
06084	Nitrobenzene	98-95-3	1 mL, 5 mL
46117	4,4'-oxydianiline	101-80-4	250 mg
33824	Pentadecafluorooctanoic acid (PFOA)	335-67-1	100 mg
07435	C.I. Solvent Blue 4	6786-83-0	50 mg
442858	4-(1,1,3,3-tetramethylbutyl)phenol	140-66-9	500 mg
45979	*o*-toluidine	95-53-4	250 mg
46267	Trichloroethylene	79-01-6	5 mL
47794	1,2,3-trichloropropane	96-18-4	1 g
96382*	Tris(2-chloroethyl) phosphate	115-96-8	100 mg

Table 1. Analytical Standards and Certified Reference Materials (Marked with *) of Substances of Very High Concern (SVHC)

Testosterone Serum Calibrator Kit

New CE-Marked Matrix Calibrator Kit for IVD Use Now Available in Europe

Norman Hardt, Product Manager Reference Materials
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Development of accuracy-based calibrators in biological matrices for clinical diagnostic applications requires reference measurement calibrators and materials with high accuracy and sensitivity. Testosterone presents a unique challenge with the wide range of endogenous levels across female, male and age-based patient populations. The critical importance of testosterone in physiological development and function underscores its significant role in endocrine disorders and diseases. Clinical assessment of testosterone levels in males and females is performed for a variety of diagnostic applications, from low testosterone levels in men to suspected polycystic ovary syndrome (PCOS) and its impact on fertility and pregnancy in women. Testosterone measurements also assist in the diagnosis of androgen-secreting tumors in women as well as monitoring of drug treatment responses in men with prostate cancer[1].

Recent literature reports that reference ranges for testosterone assays, as well as the methodologies used to develop them, can vary significantly among laboratories. According to these studies, variation in testosterone results may be due to underutilization among laboratories of testosterone reference ranges specific to clinically relevant populations and the potential limited use of serum-based specimen calibration programs[2].

Our Cerilliant® brand offers a certified reference material (CRM) grade testosterone calibrator kit in stripped serum, designed to bracket male and female testosterone clinical reference ranges, including ten levels from 2–2,000 ng/dL along with a blank and ^{13}C-labeled internal standard for *in vitro* diagnostic (IVD) use in LC-MS/MS-based laboratory developed tests (LDTs). All kit components can be ordered separately as needed.

Our Testosterone Serum Calibrator Kit is manufactured and tested to the highest industry standards. This kit is affixed with a CE mark, demonstrating its conformance to the *in vitro* diagnostic (IVD) Medical Device Directive (98/79/EC) for availability in Europe. FDA 510(k) clearance of the CRM grade Testosterone Serum Calibrator Kit, for availability in the US, is expected by the end of 2016. Cerilliant is accredited to ISO Guide 34 and ISO/IEC 17025, certified to ISO 13485 and ISO 9001, and compliant with ISO 17511 and ISO 15194.

References:

[1] a) Wolf, O.T. and Kirschbaum, C. Hormones and Behavior, **2002**, 41, 259–266. b) Mitchell Harman, S.; Jeffrey Metter, E.; Tobin, J.D.; Pearson, J. and Blackman, M.R. *The Journal of Clinical Endocrinology and Metabolism*, **2000**, 86, 724–731. c) Schatzl, G.; Madersbacher, S.; Thurridl, T.; Waldmüller, J.; Kramer, G.; Haitel, A. and Marberger, M. Prostate, **2001**, 47, 52–58.

[2] a) Le, M.; Flores, D.; May, D.; Gourley, E. and Nangia, A.K. *The Journal of Urology*, **2016**, 5, 1556–1561. b) Köhler, T.S. *The Journal of Urology*, **2016**, 5, 1561.

Cat. No.	Description	Package Size
T-107	10 levels, Blank & ^{13}C-labeled internal standard	One of each of the individual standards
T-096	2 ng/dL–20 pg/mL – 0.07 nM in stripped serum	1 mL
T-097	4 ng/dL–40 pg/mL – 0.14 nM in stripped serum	1 mL
T-098	9 ng/dL–90 pg/mL – 0.31 nM in stripped serum	1 mL
T-099	17.5 ng/dL–175 pg/mL – 0.61 nM in stripped serum	1 mL
T-100	35 ng/dL–350 pg/mL – 1.21 nM in stripped serum	1 mL
T-101	52.5 ng/dL–525 pg/mL – 1.82 nM in stripped serum	1 mL
T-102	150 ng/dL–1.5 ng/mL – 5.20 nM in stripped serum	1 mL
T-103	500 ng/dL–5.0 ng/mL – 17.34 nM in stripped serum	1 mL
T-104	750 ng/dL–7.5 ng/mL – 26.00 nM in stripped serum	1 mL
T-105	2,000 ng/dL–20 ng/mL – 69.30 nM in stripped serum	1 mL
T-106	0 ng/dL–0 ng/mL – 0 nM in stripped serum	1 mL
T-095	Testosterone-2,3,4-$^{13}C_3$ – 10 µg/mL in acetonitrile	1 mL

Table 1. Testosterone Serum Calibrator Kit and Single Components

JRC – IRMM Becomes JRC Directorate F: Health, Consumers and Reference Materials

For RM-related Activities

Coralie Leonard, Product Manager Reference Materials
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Since July 1, 2016, JRC activities related to chemical, physical and life sciences (e.g., chemicals, food safety and authenticity, nanomaterials, consumer products, nutrition, public health issues, and reference materials) will be performed in the new JRC Directorate F: Health, Consumers and Reference Materials.

So far, these activities have been carried out by two JRC Institutes, namely the Institute for Health and Consumer Protection (IHCP) based at the JRC-Ispra site and the Institute for Reference Materials and Measurements (IRMM) based at the JRC-Geel site.

The Directorate F: Health, Consumers and Reference Materials is one Directorate of the European Commission's (EC) Joint Research Centre (JRC). One of its objectives is the support of EU policies with scientific advice concerning measurements and standards through development of reference methods or certified reference materials. The reference materials of the European Commission's Joint Research Centre cover several areas including clinical chemistry, the environment, genetically modified organisms (GMOs), industrial raw materials, occupational hygiene, and physical properties as well as food and agriculture.

New Reference Materials from the European Commission's Joint Research Centre

Sigma-Aldrich® is proud to be an authorized distributor of reference materials from the European Commission's Joint Research Centre. Please find below two of the newest product additions: IRMM-359 and ERM-CE100.

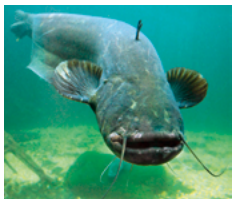
IRMM-359: Certified Reference Material for Staphylococcal Enterotoxins Detection in Cheese

Staphylococcal enterotoxins (SEs) are released into foods by microorganisms such as *Staphylococcus aureus*, causing foodborne illnesses. In 2011, 345 foodborne outbreaks were caused by staphylococcal enterotoxins, thus representing 6% of all foodborne outbreaks reported.

Cheese is one of the foods associated with staphylococcal food poisoning outbreaks, particularly cheeses fabricated from raw (unpasteurized) milk. Therefore, a raw cow-milk cheese, variety Tomme de Savoie, is the base of this CRM. The final product, IRMM-359, consists of a spiked and lyophilized cheese powder.

ERM-CE100: First Biota CRM Ever Available for Hexachlorobutadiene (HCBD) Testing

In support of the Directive 2013/39/EU, ERM-CE100 was developed as a fresh-like biota matrix CRM. The objective of the European Commission's Joint Research Centre has been to develop a naturally contaminated, fresh-like biota matrix material, rather than an artificially contaminated matrix (spiked).



The catfish (*Silurus glanis*) was selected for the production of ERM-CE100. This fish can reach large sizes and is a predator positioned high in the trophic chain, which potentially leads to bioaccumulation and biomagnification of organic pollutants, in particular hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD).
Hexachlorobenzene and hexachlorobutadiene are two substances which are considered global environmental pollutants and are listed as Persistent Organic Pollutants (POP) by the Stockholm Convention. HCB and HCBD are among the Priority Substances that Member States are expected to assess, monitor and control in EU water resources. ERM-CE100 is the first biota Certified Reference Material available for such testing.

In the table below, please find the complete list of recent additions to this product range.

Cat. No.	Description	Analyte/Format
ERMAD454K	Alanine transaminase	Lyophilized enzyme, freeze dried
IRMM359	Cheese powder	Microbiological properties and pathogens (SEA)
ERMAD455K	Creatine kinase MM isoenzyme	Lyophilized enzyme, freeze dried
ERMCE100	Fish tissue	Organic pollutants
ERMAE671	202Hg labeled methylmercury in 2% ethanol	Certified for isotope abundance ratio
ERMAD453K	Lactate dehydrogenase isoenzyme 1	Lyophilized enzyme, freeze dried
ERMBF439A	Maize GMO Standard DP-øø4114-3	Blank
ERMBF439B	Maize GMO Standard DP-øø4114-4	100% DP-004114-3
ERMBF439C	Maize GMO Standard DP-øø4114-5	0.1% DP-004114-3
ERMBF439D	Maize GMO Standard DP-øø4114-6	1% DP-004114-3
ERMBF439E	Maize GMO Standard DP-øø4114-7	10% DP-004114-3
ERMAD483	pDNA calibrant	Porcine
ERMAD482	pDNA calibrant	Ruminant
ERMEC681M	Polyethylene	High-level element content
ERMEC680M	Polyethylene	Low-level element content
ERMCC144	Sewage sludge	Trace elements
ERMCA100	Water	PAHs

Table 1. Latest Reference Materials from the JRC Available

New Vitroids™ Range and Cross Reference Guide

What are Vitroids and LENTICULE® Discs?

Coralie Leonard, Product Manager Reference Materials
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Vitroids and LENTICULE discs contain viable microorganisms with a certified colony forming units (CFU) count. They are reference materials (RMs) and Certified Reference Materials (CRMs) compliant with ISO Guide 34:2009 and tested in an ISO 17025 accredited laboratory. They are traceable to an authenticated reference strain from either NCTC®, NCPF® or CECT®.

Derived from CECT® strains

These RMs and CRMs consist of pure cultures of bacteria or fungi in a solid water soluble matrix; they are stable from one to three years in a viable state. The intra-batch variation is low (down to 4% standard deviation). Each product is provided with a downloadable comprehensive certificate of analysis that contains the mean number of CFU with an expanded uncertainty and details about the method used.

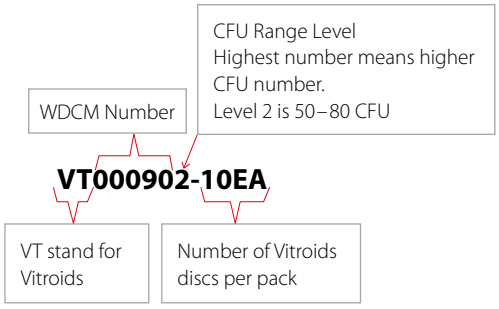
New Vitroids Range Based on WDCM Numbers

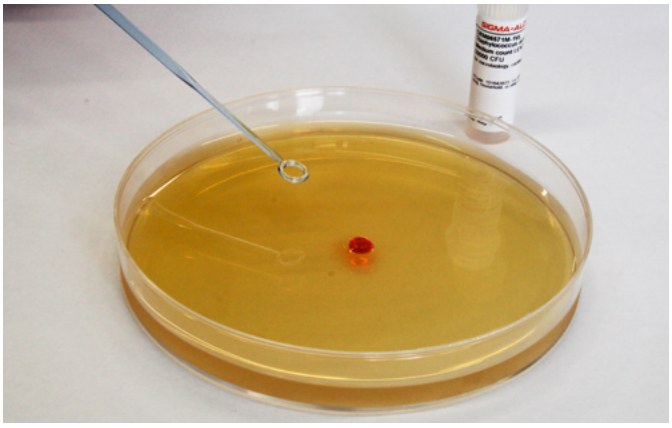
Important changes to the Vitroids range of Microorganism Certified Reference Materials have been made.

A new range of Vitroids has been launched. These new products are based on the same technology and production process. Therefore they are designed to be used following the same methods and protocols as for the previous range. They are also Certified Reference Materials according to ISO Guide 34 and retained the same excellent standard deviation.

However, this new range utilize just one passage for each product. They also are conveniently matched to WDCM numbers and have cfu ranges that more closely align with ISO 11133. To achieve this, we have chosen to derive the new products from CECT® Spanish Type Culture Collection strains instead of ATCC strains.

Example of Our New Part Number





When available, the WDCM numbers have been used to match our old part number to the new strain used for our new Vitroid range. Where there is no WDCM number available for a specified strain, then the closest related product has been used using the CECT strain description directly on the CECT website.

We have also expanded the range with the new strain below:

Strain Name	WDCM	New Part No.	CECT®	CFU Range
Bacillus cereus	00001	VT000013	193	80–120 CFU
Enterobacter aerogenes	00083	VT000834	194	130–270 CFU
Escherichia coli	00090	VT000906	515	3,000–7,000 CFU
Fluoribacer bozemanae	-	VT072767	7276	50,000–150,000 CFU
Pseudomonas aeruginosa	00026	VT000267	111	50,000–150,000 CFU
Salmonella enterica subsp. Enterica serovar Enteritidis	-	VT000303	4300	80–120 CFU

A cross-reference table with the previous range is available online at **sigma-aldrich.com/europe/new-vitroids-cross-reference.html**

Pesticides

Newest Additions for the Isotope-Labeled and the Pesticide Metabolite Portfolios

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Isotope-labeled pesticides comprise a large number of substances that belong to many completely different chemical groups with different structures and, consequently, numerous differences between their modes of action, uptake, biotransformation, and elimination. They are widely used to combat diseases and pests, but may also adversely affect the production of vegetable and animal foodstuffs. Residues of these compounds can sometimes find their way to human consumers or to environmental compartments. Statutory maximum residue levels for pesticides in food and water have been defined in most countries to guarantee consumer safety and to regulate the presence of pesticides in the environment. The determination of pesticide residues is a requirement to support the enforcement of legislation, ensure trading compliance, conduct residue monitoring programs in dietary components and in environmental samples, and to study their mode of action and movement within the environment[1].

Cat. No.	Description	Package Size
89928	Azinphos-methyl-(dimethyl-d_6)	5 mg
89936	Carbofuran-3-hydroxy-(2,2-dimethyl-d_6)	5 mg
04311	Maleic hydrazide- $^{13}C_4$	10 mg
05690	Morpholine-2,2,3,5,5,6,6,-d_8 hydrochloride	5 mg
05567	*N*-acetyl-d_3-glufonisate	5 mg
05357	Nicosulfuron-(*N*,*N*-dimethyl-d6)	5 mg
93163	Oxyfluorfen-(ethoxy-d_5)	5 mg
19847	Paclobutrazol-(phenyl-d_4)	5 mg
93103	Paraoxon-ethyl-d_{10} (diethyl-d_{10})	5 mg
05374	Phosmet-(dimethyl-d_6)	5 mg
05351	Propoxur-(isopropoxy-d_7)	5 mg
05585	Sulcotrione-d_4 (benzoyl-3-d, mesyl-d_3)	5 mg
78827	TDCPP-d_{15}	5 mg
80814	Tembotrione-(methyl-d_3)	5 mg

Table 1. Newest Isotope-labeled Pesticides

Solid environmental and food samples are often very complex matrices, and the number of compounds that co-elute with the analytes generates a very important problem in those analyses – the matrix interferences.

As a result of the matrix effects, the response of an analyte in a pure solvent standard can differ significantly from that in a matrix sample. Therefore, for accurate results, the matrix effect must be either eliminated or compensated.

One method of compensation is the use of an appropriate calibration technique. Calibration with an isotope-labeled internal standard is well-suited for this purpose.

We are continually expanding our portfolio of isotope-labeled standards, with the most recent additions listed in **Table 1**.

Pesticide Metabolites

Besides the isotope-labeled group of pesticide standards, we also continually expand our portfolio of pesticides and pesticide metabolites (**Table 2**).

Sulfonylurea, for example, is one of the most important groups of herbicides being used worldwide for control of broadleaf weeds in crops and vegetables.

Nicosulfuron, a sulfonylurea herbicide, has been available for commercial use since the 1990s and is widely used. However, with its widespread application, residue has been reported in soil and surface waters. This group of herbicides is mainly degraded or transformed by microorganisms or chemical hydrolysis in water and soil. *N*,*N*-Dimethyl-2-sulfamoylnicotinamide is one of the main metabolites of nicosulfuron.

You can find a complete listing of our products at
sigma-aldrich.com/pesticides

Cat. No.	Description	Package Size
92397	2,3-Dichlorophenoxyacetic acid	50 mg
80827	*N*,*N*-Dimethyl-2-sulfamoylnicotinamide	10 mg
92529	2-(Octylsulfanyl)ethan-1-ol	25 mg

Table 2. Newest Pesticide Metabolites

Reference:

[1] Pico, Y. et al. *Mass Spectrometry Reviews*, 23, **2003**, 45–85.

Organic *Trace***CERT®** CRMs

The Newest Product Additions to Our Organic Neat Certified Reference Material Portfolio

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For accredited testing labs, the availability of reliable and traceable Certified Reference Materials (CRMs) is crucial since the use of CRMs for calibration is demanded by ISO/IEC 17025. For the certification of our organic *Trace***CERT** products, high-performance quantitative NMR (HP-qNMR) is applied as a relative primary method to achieve traceability to NIST SRM. If you would like to learn more about qNMR and our in-house capabilities in this field, please refer to the references cited below.

The organic *Trace***CERT** reference materials are characterized by:

- Certified content by quantitative NMR (qNMR)
- Manufactured under ISO/IEC 17025/ISO Guide 34 double accreditation
- Superior level of accuracy, calculated uncertainties, and lot-specific values
- Traceability to NIST SRM
- Comprehensive documentation delivered with the product (certification according to ISO Guide 31)

The portfolio of organic *Trace***CERT** products comprises over 250 items. Our newest additions to the rapidly growing organic *Trace***CERT** product portfolio are included in **Table 1**.

A complete product listing can be found at
sigma-aldrich.com/organiccrm

Cat. No.	Description	Package Size
02827	1,3-Dinitrobenzene	100 mg
75918	2,6-Di-*tert*-butyl-4-methylphenol	50 mg
43658	Dopamine hydrochloride	50 mg
79838	Nicotinic acid	50 mg
42224	4-Nitrophenol	50 mg
07754	Octanoic acid	50 mg
94551	4-*tert* Octylphenol	100 mg

Table 1. New Product Additions to the *Trace***CERT** Product Range

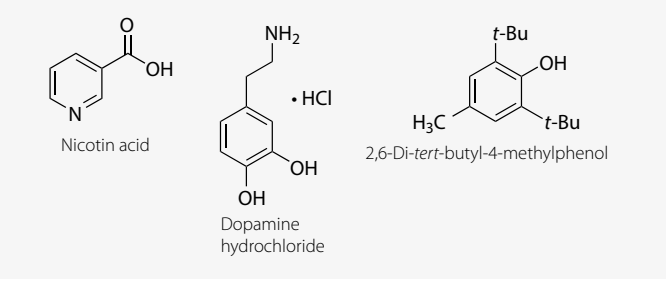

Figure 1. Selected Chemical Structure of New *Trace***CERT** CRMs

References:

[1] Weber, M.; Hellriegel, C.; Rück, A.; Wüthrich, J.; Jenks, P. Using high performance 1H-NMR (HP-qNMR®) for the certification of organic reference materials under accreditation guidelines – Describing the overall process with focus on homogeneity and stability assessment, *JPBA 93*, **2014**, 102–110.

[2] Weber, M.; Hellriegel, C.; Rück, A.; Sauermoser, R.; Wüthrich, J. Using high performance quantitative NMR (HP-qNMR®) for certifying traceable and highly accurate purity values of organic reference materials with uncertainties < 0.1%, *Accred. Qual., Assur. 18*, **2013**, 91–98.

[3] Weber, M., Hellriegel, C.; Rück, A.; Wüthrich, J.; Jenks, P.; Obkircher, M. Method development in quantitative NMR towards metrologically traceable organic certified reference materials used as 31P qNMR standards, *Anal. Bioanal. Chem.*, **2015**, 407, 3115–3123.

Pharmaceutical Reference Materials

Primary and Secondary Standards

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The quality and accuracy of reference materials is essential to the manufacture of quality medicines and foods. We offer an expanded portfolio of pharmaceutical reference standards for both Primary Pharmacopeial Standards and Secondary Standards.

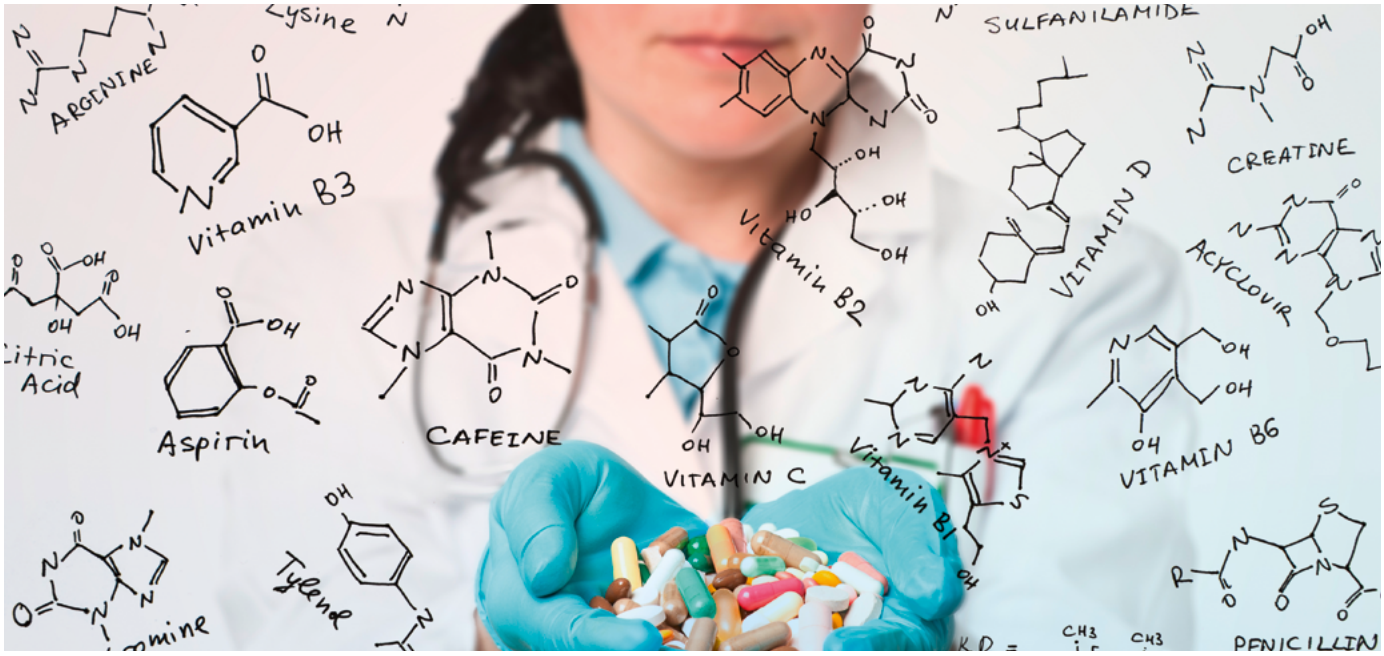
Primary Standards

Pharmacopeial Reference Standards (also known as Primary Standards) are highly characterized physical specimens used in testing by pharmaceutical and related industries to help ensure the identity, strength, quality and purity of medicines (drugs, biologics and excipients), dietary supplements and food ingredients. Pharmacopeial Reference Standards are closely tied with the documentary standards, or monographs, published by the pharmacopeia. Each standard has a specific designated use which is to be implemented in accordance with the official methods prescribed by the corresponding pharmacopeia. The USP catalog of Reference Standards now consists of more than 3,600 items ranging from drug substances, related impurities, residual solvents, biologics, excipients, botanicals, polymers, near-IR and dissolution calibrators, photomicrographs and melting point standards. The European Pharmacopoeia Reference Standards catalog includes chemical, herbal and biological reference standards and reference spectra, currently numbering over 2,700. We offer both the USP and EP Pharmacopeial Reference Standards. These items can be found at **sigma-aldrich.com/pharmaceuticalstandards**

Secondary Standards

Although not a Primary or Pharmacopeial Standard, a Secondary Standard is a reference material that is traceable to and qualified against a Primary Standard, usually obtained from a national or international metrology institute or recognized national authority such as the U.S. Pharmacopeial Convention or the European Pharmacopoeia. Secondary Standards may be used as reference standards in routine analysis, including the analysis and qualification of drug substances, dosage forms, excipients and impurities by compendial methods, as well as R&D, method development, and process and equipment validation studies. Our line of Secondary Standards is produced under clean room conditions using appropriate cGMP procedures, including batch record documentation, calibration, line clearance, label control, etc. Secondary Standards are fully characterized ISO Guide 34 Certified Reference Materials that offer complete analytical certification and direct traceability to Primary Standards (where available) by both comparative assay (HPLC, GC, UV, etc.) and identity (FTIR, HPLC, etc.) analytical procedures. Where Primary Standards are available from the different authorities, traceability of Secondary Standards is maintained to these Primary Standards (typically the USP, EP and BP), which allows for a single harmonized reference standard that can be used to meet the requirements of the multiple compendia.

To see our full library of Pharmaceutical Secondary Standards, visit **sigma-aldrich.com/pharmastandards**



Metformin – New Impurity Standards Available for the Commonly Used Diabetes Medicine

Standards for Use in Pharmaceutical Quality Control

Nicolas J. Hauser, Product Manager Reference Materials
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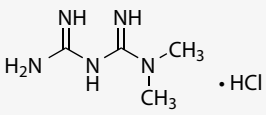
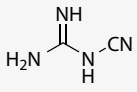
Metformin is a widely used medication prescribed for the treatment of type 2 diabetes. Both the USP (US pharmacopeia) and the EP (European pharmacopoeia) provide monographs for this drug. We provide the full range of Metformin impurities (or "related compounds") defined in the monographs as listed in **Table 1**. This includes the pharmacopeia compendial standards (if available), or our in-house-produced pharmaceutical Secondary Standards. The line of Secondary Standards is comprised of fully characterized ISO Guide 34 Certified Reference Materials that offer complete analytical certification and direct traceability to Primary Standards (where available) by both comparative assay (HPLC, GC, UV, etc.) and identity (FTIR, HPLC, etc.) analytical procedures. For the Metformin itself, the compendial standards from USP and EP, as well as a Secondary Standard traceable to both the USP and the EP reference standard, are available (**Table 2**).

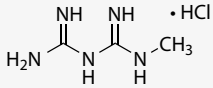
A complete, up-to-date list of pharmaceutical impurity standards can be found at
sigma-aldrich.com/pharmaimpurities



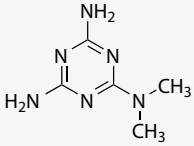
Metformin hydrochloride



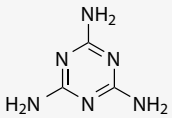
Related Compound A



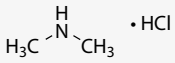
Related Compound B



Related Compound C



Related Compound D



Related Compound F

Cat. No.	Description	Chemical Name	Product Quality	Package Size
1396310	Metformin Related Compound A	Cyanoguanidine	USP Reference Standard	30 mg
Y0001590	Metformin Impurity A	Cyanoguanidine	EP Reference Standard	25 mg
PHR1331	Metformin Related Compound A	Cyanoguanidine	Secondary Pharmaceutical Standard	500 mg
1396331	Metformin Related Compound B	1-Methylbiguanide hydrochloride	USP Reference Standard	25 mg
PHR1505	Metformin Related Compound B (EP Impurity E)	1-Methylbiguanide hydrochloride	Secondary Pharmaceutical Standard	50 mg
PHR1969	Metformin Impurity B	(4,6-diamino-1,3,5-triazin-2-yl)guanidine Nitrate	Secondary Pharmaceutical Standard	50 mg
1396342	Metformin Related Compound C	*N,N*-Dimethyl-1,3,5-triazine-2,4,6-triamine	USP Reference Standard	25 mg
PHR1506	Metformin Related Compound C (EP Impurity C)	*N,N*-Dimethyl-1,3,5-triazine-2,4,6-triamine	Secondary Pharmaceutical Standard	50 mg
PHR1274	Metformin Impurity D	Melamine	Secondary Pharmaceutical Standard	1 g
Y0001600	Metformin Impurity F	Dimethylamine hydrochloride	EP Reference Standard	2 mL
PHR1532	Metformin Impurity F	Dimethylamine hydrochloride	Secondary Pharmaceutical Standard	200 mg

Table 1. Metformin Impurities

Cat. No.	Description	Product Quality	Package Size
1396309	Metformin Hydrochloride	USP Reference Standard	200 mg
M0605000	Metformin Hydrochloride	EP Reference Standard	50 mg
PHR1084	Metformin Hydrochloride	Secondary Pharmaceutical Standard	500 mg

Table 2. Primary and Secondary Standards Available from Sigma-Aldrich®

Halogenated High Performance MALDI-MS Matrices

Rationally Designed Matrix Derivatives Allow for Increased Sensitivities

Dr. Thorsten W. Jaskolla, Head of Quality Control, Dr. Franz Köhler Chemie GmbH
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Importance of the Matrix for MALDI-MS Analysis

The matrix has to enable codesorption and ionization of the matrix and analyte molecules. These processes require energy which is applied as laser radiation and necessitate a sufficient absorption of the matrix at the irradiation wavelength.

One major drawback is that only a fraction of the analyte is ionized and, therefore, detectable. Typically used matrices such as α-cyano-4-hydroxycinnamic acid (CHCA) can have severe limitations in challenging cases with low analyte amounts, peptides with scarce post-translational modifications, weakly basic or small peptides or other hard-to-protonate analyte classes.

Optimization of Matrix Structures

It has been demonstrated that proton transfer reactions from protonated matrices to counteranions of positively precharged analytes as well as to neutral analytes are the dominant reactions leading to analyte protonation[1]. To increase the efficiency of these processes, the strength with which the positively charged proton is bound to the matrix must be lowered. This can be achieved by insertion of electron-withdrawing halogens into the matrix structure, which decrease the electron density, see **Figure 1**[2]. As a result, the proton affinity as a measure of the bond strength between proton and matrix decreases from 866 kJ/mol for the standard matrix CHCA to 842 kJ/mol for 4-chloro-α-cyanocinnamic acid (ClCCA) and to 837 kJ/mol for α-cyano-2,4-difluorocinnamic acid (DiFCCA)[3]. This reduction is accompanied by increasing reactivities, especially at irradiation wavelengths of 337 nm. Simultaneously, the absorption profiles of the matrices are shifted to shorter wavelengths, which limit the number of possible matrix halogens in cases where standard laser systems with fixed wavelengths are used. Consequently, higher halogenated matrix derivatives require the use of matrix mixtures including an absorber.

Typical Applications

The higher reactivities of the halogenated MALDI matrices open up a multitude of possible applications, such as more sensitive protein identification using peptide mass fingerprinting. Regardless of a protein's nature, the use of halogenated matrices allows for detection of a higher number of and more intense peptides, resulting in substantially higher sequence coverages. This advantage is independent of the cut-specificity of the chosen protease or the digested protein amount[4], see **Figure 2**. A comparison between standard CHCA and the halogenated ClCCA matrix for the example of 50 fmol of a tryptic α-/β-casein digest is given in **Figure 3**. The higher sensitivity of ClCCA results in detection of more peptides, an increase of the highest absolute analyte intensity from 160 to about 4,400 counts, and in a higher number of phosphopeptides with a tenfold increase in signal-to-noise ratio, on average[2]. In addition, halogenated matrices with higher sensitivities enable lower amounts of "one hit wonders" of analytes which are at the border of detection using conventional matrices[4]. Lowering the detection limit allows for analysis of otherwise undetectable acidic and low-abundance peptides with rare post-translational modifications or peptides that are generated by uncommon protease cut-specificities[5].

MALDI analyses in the negative-ion mode are usually less common due to typically lower sensitivities. Depending on the nature of the analyte, halogenated matrices also allow for more sensitive analyte anion detection, see **Figure 4**. In addition to ClCCA:DiFCCA mixtures, 4-bromo-α-cyanocinnamic acid (BrCCA) and its mixtures with ClCCA or DiFCCA are also well-suited for negative-ion mode analysis.

The higher reactivities of halogenated matrix derivatives also enable sensitive detection of other substance classes such as phospholipids, e.g., sphingomyelins or phosphatidylcholines[7]. Halogenated phospholipids as products of inflammatory processes and intermediates of lipid peroxidation are of low basicity and exclusively detectable by halogenated matrices such as ClCCA in MALDI-MS, see **Figure 5**.

The lower internal analyte energy using ClCCA and other halogenated derivatives better preserves fragile sidechain modifications in the MS mode than "hotter" matrices such as CHCA[8]. Nevertheless, resulting from the higher analyte ion intensities,

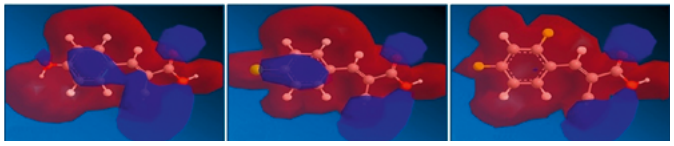


Figure 1. Molecular Electrostatic Potential of Neutral CHCA, ClCCA, and DiFCCA Matrices. Areas with Low Electron Densities Are Illustrated in Red, Regions with High Density in Blue. The Matrix Structures Were Energetically Optimized by Density Dunctional Theory, B3LYP/6-311++G(3df,3pd)

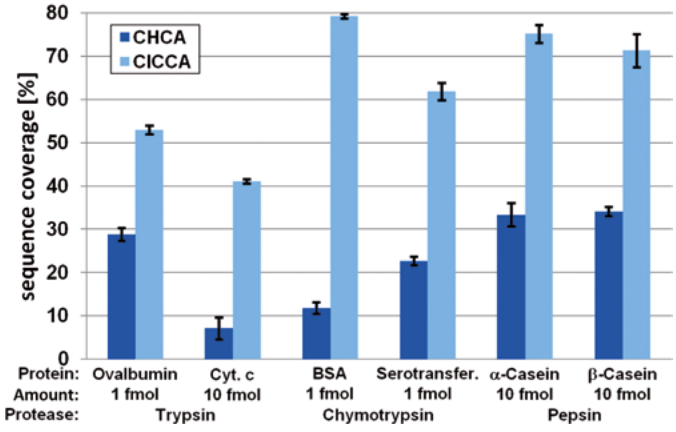


Figure 2. Achievable Sequence Coverages of Proteins Digested by Several Proteases in Different Amounts. CHCA and ClCCA Were Used as Matrices. All Values Refer to the Average of Three Independent Digestions. For More Information, See Reference 4

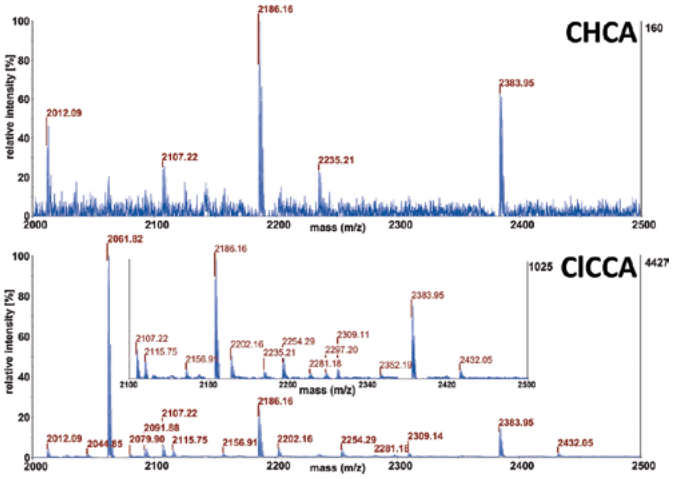


Figure 3. MALDI Mass Spectra of a Tryptic In-solution Digest of β-casein (Containing 10% α-casein) Using CHCA or ClCCA as Matrix. m/z Section 2,000–2,500 Da; Shots per Spectrum, 500; Polarity, Positive; Total Sample Load, 50 fmol. For More Information, See Reference 2

fragmentation can yield a wealth of information under CID-MS/MS conditions using these derivatives, which is especially helpful for *de novo* approaches or automatic database analyses of fragment ion spectra, see **Figure 6**.

Sigma-Aldrich® is proud to be the only authorized distributor of halogenated MALDI matrix materials.

Find our complete product offering for MALDI-MS products at
sigma-aldrich.com/maldi

References:

[1] Jaskolla, T.W. Karas, M.J. *Am. Soc. Mass Spectrom*, **2011**, *22*, 976–988.
[2] Jaskolla et al. *Proc. Natl. Acad. Sci. U.S.A.*, **2008**, *105*, 12200–12205.
[3] Soltwisch et al. *Anal. Chem.* **2012**, *84*, 6567–6576.
[4] Jaskolla et al. *J. Proteome Res.*, **2009**, *8*, 3588–3597.
[5] Papasotiriou et al. *J. Proteome Res.*, **2010**, *9*, 2619–2629.
[6] Teuber et al. *Chem. Phys. Lipids*, **2010**, *163*, 552–560.
[7] Jaskolla et al. *J. Am. Soc. Mass Spectrom.*, **2009**, *20*, 867–874.
[8] Leszyk, J. D. *J. Biomol. Tech.*, **2010**, *21*, 81–91.

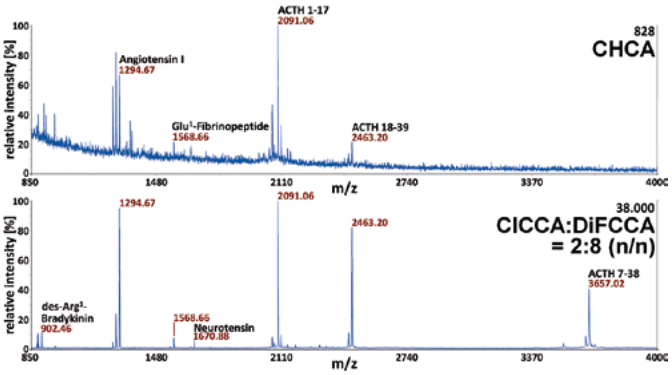


Figure 4. MALDI Mass Spectra of AB Sciex Peptide Mass Standard Calibration Mixtures I and II (0.5 µl, Prepared as Given in the AB Sciex Protocol and Further Diluted by a Factor of 8) Using CHCA or ClCCA:DiFCCA = 2:8 (n/n) as Matrix. Shots per Spectrum, 500; Matrix, 10 nmol Each; Mass Spectrometer, Voyager-DE STR; λ = 337 nm; Polarity, Negative

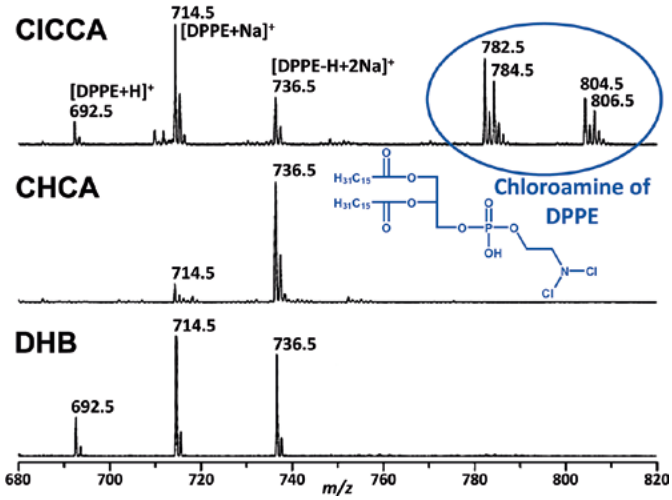


Figure 5. Positive-ion Mode MALDI-TOF Mass Spectra of Dipalmitoylphosphatidylethanolamine (DPPE) and Its Dichlorinated Counterpart Recorded with ClCCA, CHCA, and 2,5-dihydroxybenzoic Acid (DHB). For Details, See Reference 6

Cat. No.	Description	Synonym	Typical Application	Package Size
89063	4-Bromo-α-cyanocinnamic acid	BrCCA	Peptides, Phosphopeptides, Phospholipids, Chlorinated Lipids, Drugs, Fragile Analytes, Ionic Liquids (Quantification), ME-SIMS, CID-MS/MS	100 mg
55841	4-Bromo-α-cyanocinnamic acid - α-Cyano-2,4-difluorocinnamic acid mixture	BrCCA:DiFCCA		100 mg
68914	4-Bromo-α-cyanocinnamic acid - 4-Chloro-α-cyanocinnamic acid mixture	BrCCA:ClCCA		100 mg
94141	4-Chloro-α-cyanocinnamic acid	ClCCA		100 mg
39379	4-Chloro-α-cyanocinnamic acid - α-Cyano-2,4-difluorocinnamic acid mixture	ClCCA:DiFCCA		100 mg
77646	α-Cyano-2,4-difluorocinnamic acid	DiFCCA		100 mg
77081	α-Cyano-4-fluorocinnamic acid	FCCA		100 mg
03841	α-Cyano-4-hydroxycinnamic acid - α-Cyano-2,4-difluorocinnamic acid - α-Cyano-2,3,4,5,6-pentafluorocinnamic acid mixture	CHCA:DiFCCA:PentaFCCA		100 mg
38419	α-Cyano-2,3,4,5,6-pentafluorocinnamic acid	PentaFCCA		100 mg

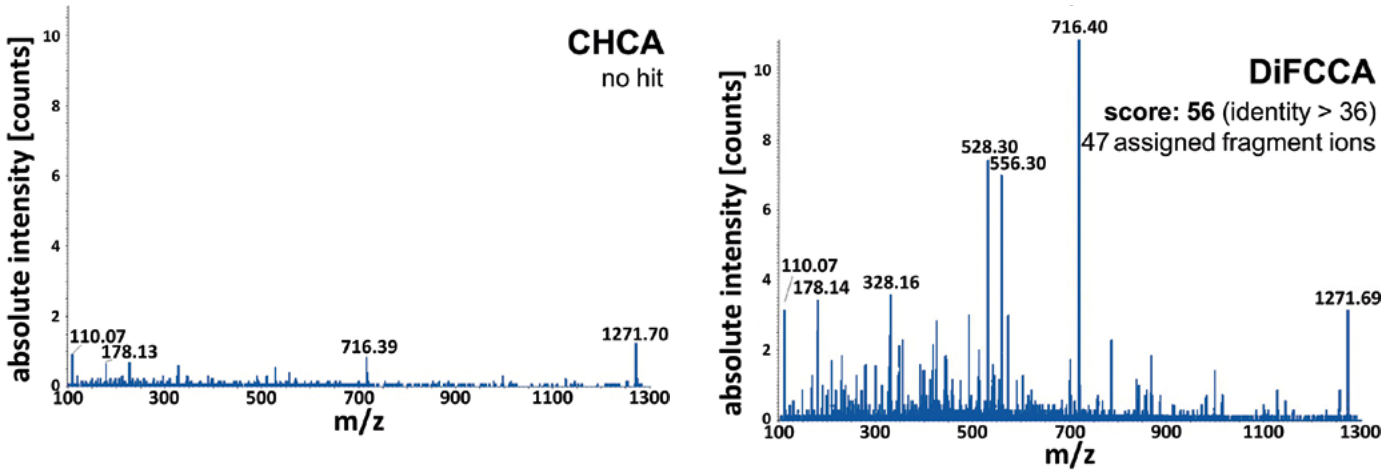


Figure 6. CID-MS/MS Spectra of Protonated Peptide LFTGHPETLEK (m/z 1271.66) Resulting from a Myoglobin Digest (50 fmol). Mass Spectrometer, QStar Pulsar I (MALDI-QqQ-OTOF); Collision Gas, Argon; Collision Energy, 90 eV; Data base, NCBInr; Organism, Other Mammals; Enzyme, Trypsine; Max. No. of Missed Cleavages, 1; Variable Mod., CAM-C and Met.-Ox.; Precursor Tolerance, 25 ppm; MS/MS Tolerance, ±0.1 Da

Diatoxanthin and Diadinoxanthin

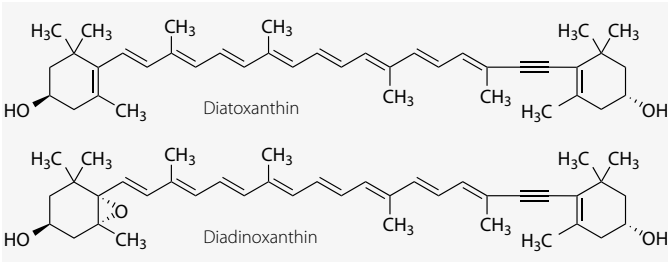
New Carotenoid Analytical Standards

Matthias Nold, Product Manager Analytical Standards
matthias.nold@sial.com

Diatoxanthin and Diadinoxanthin are two xanthophylls present in diatoms, a group of marine algae and major component of phytoplankton. These carotenoids are involved in the xanthophyll cycle, which plays a key role in stimulating energy dissipation within light-harvesting antenna proteins in order to reduce the amount of energy that reaches the photosynthetic reaction centers.

These two products have been added to our expanding range of analytical standards for carotenoids.

For the complete offering of carotenoid standards, please visit **sigma-aldrich.com/carotenoids**



Cat. No.	Description	Package Size
79449	Diatoxanthin	1 mg
08379	Diadinoxanthin	1 mg

Table 1. New Carotenoid Standards

NEW Hellma® Bestcellers

Quality Exceeding Expectations

Daniel Weibel, Product Manager Trace Organic Analysis & Sensorics
daniel.weibel@sial.com

Introduction

Cells from Hellma® Analytics are available in different materials ranging from inexpensive optical glass to high-performance Suprasil® 300 quartz. The cell selected for a specific application should exhibit high transmission in the spectral range of interest to facilitate the highest level of sensitivity in the measurement.

We offer a comprehensive range of high-quality absorption and fluorescence cells from Hellma® Analytics. Learn more at **sigma-aldrich.com/hellma**

Cat. No.	Size	Hellma Type	Material	Spectral Range Limit (nm)	Optical Path Length (mm)	Chamber Volume (µL)
Ultra Micro, Micro and Semi Micro						
Z802921	Ultra Micro	105.210-QS	Suprasil® quartz	200–2500	5	2.5
Z803030	Ultra Micro	105.210-QS	Suprasil® quartz	200–2500	5	2.5
Z802719	Ultra Micro	105.020-QS	Suprasil® quartz	200–2500	10	120
Z802824	Ultra Micro	105.025-QS	Suprasil® quartz	200–2500	10	120
Z802603	Micro	115B-QS	Suprasil® quartz	200–2500	10	400
Z802387	Micro	108.002-QS	Suprasil® quartz	200–2500	10	500
Z802492	Micro	108.002B-QS	Suprasil® quartz	200–2500	10	500
Z802263	Micro	104.002B-QS	Suprasil® quartz	200–2500	10	700
Z802166	Micro	104.002B-OS	Optical® glass	320–2500	10	700
Z801062	Semi Micro	104-QS	Suprasil® quartz	200–2500	5	700
Z801496	Semi Micro	104B-QS	Suprasil® quartz	200–2500	10	1400
Z801178	Semi Micro	104-QS	Suprasil® quartz	200–2500	50	7000
Z801275	Semi Micro	104-QX	Suprasil® 300 quartz	200–3500	10	1400
Z801607	Semi Micro	108-QS	Suprasil® quartz	200–2500	10	1000
Z801712	Semi Micro	108B-QS	Suprasil® quartz	200–2500	10	1000
Z801836	Semi Micro	114-OS	Optical glass	320–2500	10	1400
Z801380	Semi Micro	104B-OS	Optical glass	320–2500	10	1400
Macro						
Z800074	Macro	110-QS	Suprasil® quartz	200–2500	5	1750
Z800171	Macro	110-QX	Suprasil® 300 quartz	200–3500	1	350
Z800295	Macro	110-QX	Suprasil® 300 quartz	200–3500	2	700
Z800406	Macro	110-QX	Suprasil® 300 quartz	200–3500	5	1750
Z800511	Macro	110-QX	Suprasil® 300 quartz	200–3500	20	7000
Z805653	Macro	110-OS	Optical glass	320–2500	1	350
Z805777	Macro	110-OS	Optical glass	320–2500	2	700
Z805882	Macro	110-OS	Optical glass	320–2500	5	1750
Z805998	Macro	110-OS	Optical glass	320–2500	50	17500
Z800848	Macro	404.000-QX	Suprasil® 300 quartz	200–3500	10	7000
Z800627	Macro	402.000-OG	Optical glass	360–2500	10	6000
Z800732	Macro	402.000-OG	Optical glass	360–2500	20	12000
Z805009	Macro	100-QX	Suprasil® 300 quartz	200–3500	1	350
Z805114	Macro	100-QX	Suprasil® 300 quartz	200–3500	2	700
Z805238	Macro	100-QX	Suprasil® 300 quartz	200–3500	5	1750
Z805335	Macro	100-QX	Suprasil® 300 quartz	200–3500	20	7000
Z800945	Macro	100-QX	Suprasil® 300 quartz	200–3500	40	14000
Z805440	Macro	100-QX	Suprasil® 300 quartz	200–3500	50	17500
Z805556	Macro	100-QX	Suprasil® 300 quartz	200–3500	100	35000

Table 1. Latest Additions to the Hellma Absorption Cuvette Product Line

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Fax: (+972) 8 948 4200

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Fax: (+81) 3 5796 7315

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Fax: (+52) 722 276 1601

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Free Fax: 0800 937 777
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Fax: (+61) 2 9841 0500

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Fax: (+47) 23 17 60 10

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Fax: (+46) 8 742 4243

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