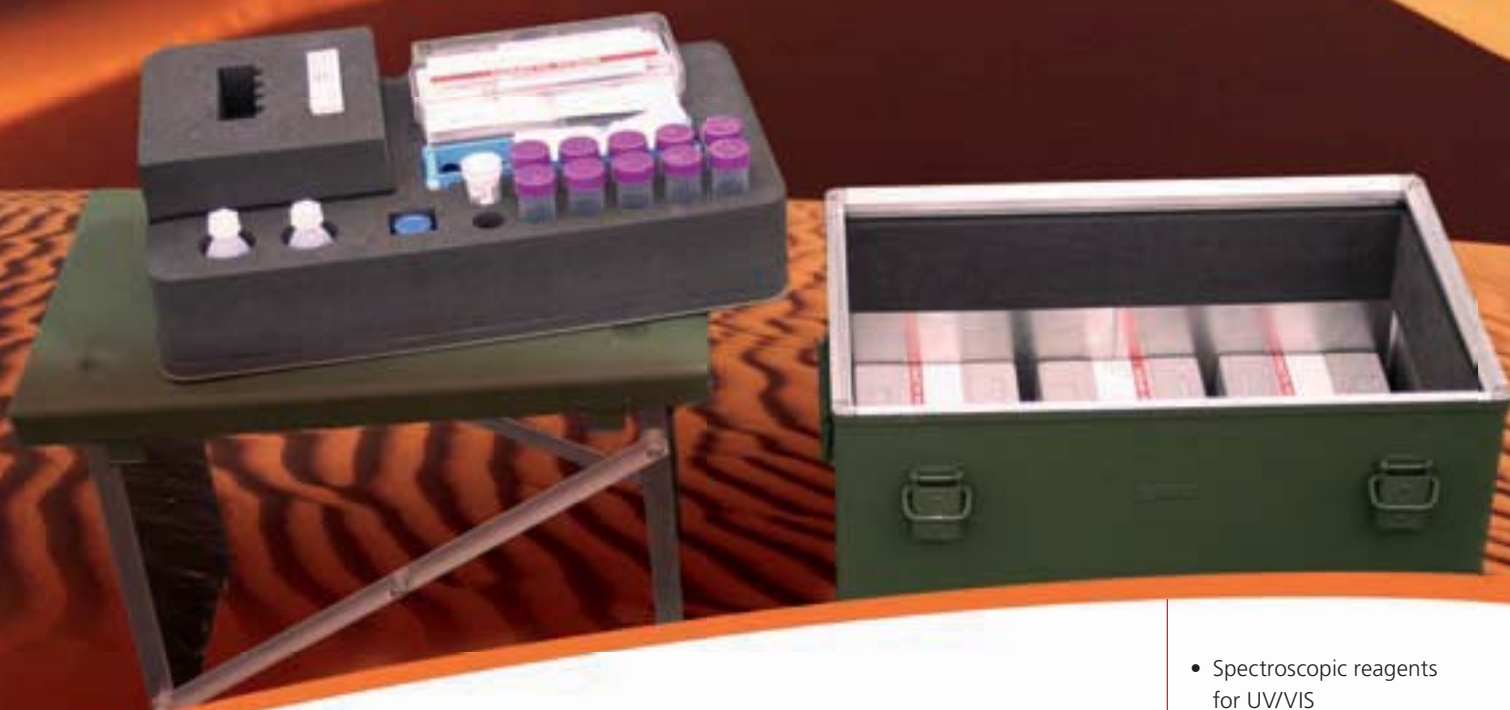


Analytix

Issue 4 • 2009



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Gerd Hayenga
Project Manager
Research Specialities

Dear Colleague,

You may be surprised to find obvious military equipment on the front cover of this *Analytix*. Hopefully this will give you an indication of Sigma-Aldrich's broad range of capabilities in analytical testing. Our know-how does not end at the lab door. Analytical information about the identity and quantity of impurities in our environment are the basis of decisions at every possible level. We must be able to obtain analytical data in every situation, be it in the city or in the desert; be it food, water or any other matrix. Sigma-Aldrich provides analytical reagents, high-purity solvents, standards, and even ready-to-use kits for almost every analytical problem.

In this issue of *Analytix* you will find examples of our broad range of analytical products. The cover story is about the exciting development of a "Water testing kit – NBC defence" for the German Armed Forces. For an army in the field, the quality of the water supply is a critical issue. This is why the German Army searched for a method to test water under field conditions. We developed equipment with all the necessary properties in terms of transport stability, ease of handling, analytical robustness etc. to be useful in the field. The equipment developed has two parts. A potable water module which serves to control standard water parameters. This comprises of photometric tests for different cations and anions, pH and conductivity. A second part, the "Chemical Warfare Agents (CWA) Module", adds the capability to detect different CW agents in water. The German Armed forces have used this equipment since 2006, together with their mobile water treatment equipment, to

control the quality of raw water before treatment and to test the quality of the potable water after treatment, in field situations. This is a success story that we would like to share with you. Maybe you have onsite analytical problems or parameters which you would like to detect in your special situation or under your special conditions. Or do you need to calibrate an instrument?

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With kind regards,

Gerd Hayenga
Project Manager
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Water Testing Kit – NBC Defence: An Example of Sigma-Aldrich® Analytical Kit Capabilities

Gerd Hayenga, Project Manager Research Specialities gerd.hayenga@sial.com



Picture 1

Sigma-Aldrich was approached by the German Armed Forces to develop a water testing kit for use in the field. Our expert and dedicated custom applications team were able to meet all the stringent military requirements and a successful kit is now in routine use. This is a specific example of our analytical kit capabilities to illustrate what Sigma-Aldrich may be able to do for you.

One of the major problems for an army on manoeuvres is the lack of infrastructure in the theatre of operation. Before any campaign can be entered into, factors like securing the reliable supply of safe drinking water are paramount. Mobile water treatment plants are available but they do require raw water of acceptable quality in order to work properly. If the raw water has too many impurities or impurities, present at too high a concentration, then the treatment plants are unable to produce drinking water of sufficient quality. There is also a requirement to test the purified water in order to be assured that the critical parameters are below the legally

acceptable limits for potable water. In addition, the water needs to be tested for different chemical warfare agents according to limits defined by NATO regulation (STANAG 2136 med).

The specifications for the water testing kit were therefore as follows:

1. To determine that concentrations of critical parameters are below the maximum permissible limit in raw water (see **Table 1**)
2. To determine that concentrations of critical parameters are below the legal limit for potable water according to drinking water regulations (see **Table 1**)
3. To determine that concentrations of chemical warfare agents are below the maximum permissible limit in raw water (see **Table 2**)
4. To determine that concentrations of chemical warfare agents are below the legal limit for potable water according to NATO regulations (see **Table 2**)

Key to the development of an analytical testing kit for use by the Armed Forces was ensuring the kit was sufficiently robust. All the components of the kit are stored in strong aluminium boxes, which have been thoroughly tested by the German Army to make sure that they can withstand rigorous transport and handling. All reagents and materials were developed to withstand a wide range of climatic conditions, from the cold arctic to the hot desert, as well as a wide range of relative humidity. All the analytical tests have been designed to be able to be carried out by any soldier with a minimum of training.

The “Water Testing Kit – NBC Defence” consists of two modules. The “Potable Water Module”, housed in two separate aluminium boxes, is illustrated in **Picture 1** and **2**. One box contains equipment such as a photometer, a pH meter, pipettes etc. The second box contains all the reagents required. It was essential that everything required for analysing the water sample was included in the kit. The parameters that can be tested with this module are shown in **Table 1**.

Ammonium	Water hardness
Chlorine	pH
Cyanide	Conductivity
Iron	Nitrite
Nitrate	Sulphate

Table 1 The parameters tested in the Potable Water Module

The analysis of anions and cations is done by barcode-assisted photometric tests. Water hardness is carried out by means of a test-strip method. Conductivity and pH are measured using easy-to-handle equipment. This module can be operated independently of the second “Chemical Warfare Agents (CWA) Module”.

The CWA Module, illustrated in **Picture 3**, includes the capability to test for four classes of chemical warfare agents shown in **Table 2**. Arsenic is tested by a method that reduces any arsenic compounds to gaseous arsine. An arsenic test stick is inserted through a slit in the lid of the test bottle, ensuring maximum sensitivity. Cyanide and Lost Agents are analysed in colourimetric tests,



Picture 2

(continued on page 6)

which can be further quantified using the photometry equipment from the Potable Water Module. The Nerve Agent test is based on Acetylcholine esterase (ACE). This required the development of an enzyme stable for at least 30 days at 40 °C with a sensitivity of 0.02 ppm nerve agent in water.

CWA containing Arsenic (Lewisites)
Cyanide containing compounds
Lost Agents (Mustard Agents)
Nerve Agents (ACE-inhibiting agents)

Table 2 The parameters testing in the Chemical Warfare Agents Module

A short instruction manual accompanies each kit, where each test is described step by step. With no chemical knowledge or laboratory experience, soldiers in the field are able to achieve reliable results.

A useful design feature of the boxes is that the lid can be used as a table. The lid contains legs that can be unfolded and secured to give a stable working area (see **Picture 4**). All boxes have two shelf levels; the top level can be removed and placed on the “table lid”. The kit also contains heating pads to warm the test vials, for reactions that are only fast enough above 15 °C.

Sigma-Aldrich® is proud of the completeness of this kit, which contains everything required to test water in any environmental condition. The German Army has extensively evaluated the kit under field conditions and has been very positive about the comprehensive solution to their water testing requirements.

There are many situations in the civilian world where testing needs to take place outside the laboratory or utilising minimally trained staff. There are many elements of the “Water Testing Kit – NBC Defence” that would be useful in manufacturing and research. If this article has inspired you to seek a solution to a previously unresolved problem, please get in touch with the Sigma-Aldrich custom applications team at customware@sial.com



Picture 3



Picture 4

Selective Media for Bifidobacteria

Sigma-Aldrich® supplies media for the selective isolation, identification and enumeration of bifidobacteria like *Bifidobacterium longum*, *infantis* and *brevi* which are used for quality control in the manufacture of dairy products.

Bifidobacteria are Gram-positive, non-motile, rod-shaped and often branched anaerobic bacteria. They have a positive effect on the immune system and control intestinal pH. Bifidobacteria produce bacteriocins and bacteriocin-like inhibitory compounds which inhibit the growth of other bacteria.

B. longum is the best-characterised species in the genus *Bifidobacterium*. It is able to utilise a broad range of substrates for energy, such as plant polymers, glycoproteins and glyconjugates, as well as having specialised proteins for the catabolism of oligosaccharides.

Bifidobacteria also have a unique hexose metabolism called the bifid shunt. The key enzyme, fructose-6-phosphate phosphoketolase, is not found in any other gram-positive intestinal bacteria and therefore provides an ideal target for a diagnostic test.

In adult's intestine, only 3–6 % of the faecal flora is composed of bifidobacteria while in breast-fed infants bifidobacteria can be up to 90 %. With increasing age the number of bifidobacteria decrease. It was observed that babies and adults with lower numbers of bifidobacteria have a higher risk for diarrhoea and allergies. For this reason bifidobacterium are added as a probiotic supplement to infant formulas, drinks, yoghurts and a lot of other products.

Because of the wide use of bifidus, Fluka has developed Bifidobacteria Selective Media (BSM), available as an agar or a broth, for standard for quality control. This allows for easy and fast quality control of yoghurt made with bifidus and can be used to control the count of bifidus bacteria.

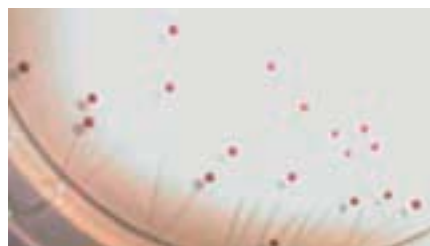
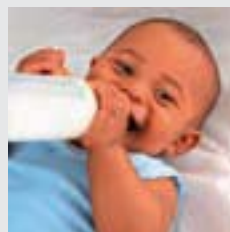


Figure 1 Yoghurt sample cultured on BSM Agar. Bifidobacteria appears as purple-brown colonies.

Bifidobacteria grow very well on this medium whilst *Lactobacillus* and *Streptococcus* strains are inhibited. *Bifidobacterium* colonies grow within 24–48 hours (occasionally up to three days because of the highly selective conditions). The *Bifidobacterium* colonies are purple-brown and therefore easy to differentiate from other organisms.

Jvo Siegrist, Product Manager Microbiology ivo.siegrist@sial.com

Did you know?



Using an infant formula with probiotics reduces the risk of diarrhoea by nearly half when the baby has been prescribed an antibiotic.

In a Swiss governmental evaluation study for the enumeration of bifidobacteria in sour milk products, the traditional method was compared to Wilkins-Chalgren Agar with 100 mg/l mupirocine and BSM Agar. The traditional method gave significant differences while Wilkins-Chalgren Agar and BSM Agar showed similar results without any significant differences. The study remarked: "On the BSM Agar the bifidobacteria forms purple-brown colonies which made the enumeration easy".

Organisms (ATCC)	Growth	Colony appearance
<i>Bifidobacterium longum</i> (15707)	+++	red-brown (maroon)
<i>Bifidobacterium infantis</i> (15697)	+++	red-brown (maroon)
<i>Streptococcus thermophilus</i> (14486)	-	-
<i>Lactobacillus acidophilus</i> (314)	-	-
<i>Lactobacillus bulgaricus</i> (11842)	-	-

Table 1 *Bifidobacterium* sp. cultural characteristics on BSM Agar

References

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- [3] Grand M., Küffer M. and Baumgartner A.: Quantitative analysis and molecular identification of bifidobacteria strains in probiotic milk products. *Eur. Food Res. Technol.* 217, 90–92 (2003).
- [4] Rada V. and Koc J.: The use of mupirocin for selective enumeration of bifidobacteria in fermented milk products. *Milchwissenschaft.* 55, 65–67 (2000).
- [5] Bundesamt für Gesundheit: Schweizerisches Lebensmittelbuch (SLMB): Kapitel 56, «Mikrobiologie» Neuausgabe 2000, Stand 2004.
- [6] IUPAC: Protocol for the design, conduct and interpretation of method-performance studies.

Name	Brand	Cat. No.	Pack Size
BSM Agar	Fluka	88517	500 g
BSM Broth	Fluka	90273	500 g
BSM Supplement	Fluka	83055	5 g
Wilkins Chalgren Anaerobic Agar	Fluka	W1761	500 g
Mupirocin Li salt	Sigma	07188	100 mg 1 g

Table 2 Selective medium for *Bifidobacterium*

Specific and Early Detection of Fungal Hyphae on Bread by Fluorescently Labelled Lectins

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Bernhard Schoenenberger, Supervisor R&D; Jakob Zbaeren, Inselspital Bern, Switzerland



Food quality assurance and control have gained importance as factors of concern to public health. Perishable foods are of particular focus for routine food analysis. One of the most critical issues is the contamination of breads and fruits by fungi and moulds, as these food items are especially susceptible to mould development. Specific fungal species and the mycotoxins produced can adversely affect human and animal health, and early identification of negative fungi can allow contaminated products to be removed before entering the food chain. Here, we demonstrate a highly specific technique for early identification of fungi in bread via direct fluorescence detection, using fluorescently labelled lectins.

Lectins are ubiquitous proteins or glycoproteins that can be isolated from plant and animal sources and can bind to specific carbohydrate moieties. Due to their high affinity to sugar residues, lectins have become important tools for sensitive detection of cellular carbohydrates, revealing subtle alteration in glycosylation between otherwise indistinguishable cells. This allows identification of cellular surface structures, e.g. cell surface, cytoplasm, and nuclear structures. Furthermore, lectin affinity binding allows for the detection of fungi in food, such as bread or fruit.

The recently developed Atto-dye labelled lectins have many applications, including carbohydrate, mitogenic, and histochemical studies and food analysis techniques.

Atto-dyes have very bright fluorescent signals and high photo stability, which enable a direct one-step sample-binding protocol. Time-consuming multi-stage amplification procedures are not required with Atto-dye lectin conjugates (see **Figure 1**).

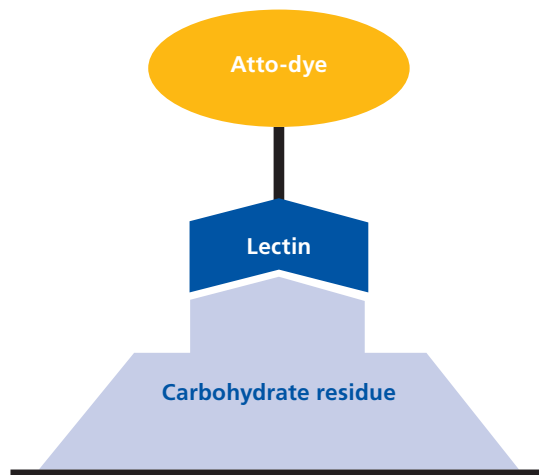


Figure 1 Direct one-step binding of fluorescently labelled Lectins

Lectin binding was performed using a smear preparation of bread containing fungi. The lectin conjugate used was *Phytolacca americana*-Atto 488 conjugate (**Cat. No. 39905**). The conjugate was diluted 100x in PBS buffer (pH 7.4) before incubating with each specimen for 30 min. After washing to remove any unbound lectin, the samples were examined using a microscope equipped for epifluorescence with a 450–490 nm excitation band-pass filter and a 520–560 nm barrier (emission) filter.

The images obtained show a very specific labelling of fungi that are present in the bread samples (see **Figure 2**). The image demonstrates clear and fine filaments of the fungi with typical *mycelium*, and individual fungi cells are visible. The separating cross-walls (*septa*) structures show a slightly brighter fluorescence, which is due to a higher concentration of target carbohydrate. No background is observed.

Fungal cell walls contain chitin, a polymer of β -(1 \rightarrow 4) linked N-acetyl-D-glucosamine, while animal and plant cells do not synthesise chitin. The lectin *Phytolacca americana* targets the fungal carbohydrate fragment chitotriose [$(\beta$ -N-acetyl-D-glucosamine) $_3$, (GlcNAc) $_3$] shown in green (λ_{ex} 485 nm). Due to the lack of the target carbohydrate chitotriose in the bread carbohydrates, no specific interaction between the lectin *Phytolacca americana* and the bread residue is observed. The bright and stable fluorescence properties of the Atto 488 dye provide a strong fluorescent signal without requiring additional amplification steps. The presence of surface fungi can be detected long before visible surface mould develops. These results confirm this approach to be a successful and reliable way to detect mould on bread. This fascinating application may encourage scientists to investigate specific binding of lectins to other carbohydrate targets for food analysis.

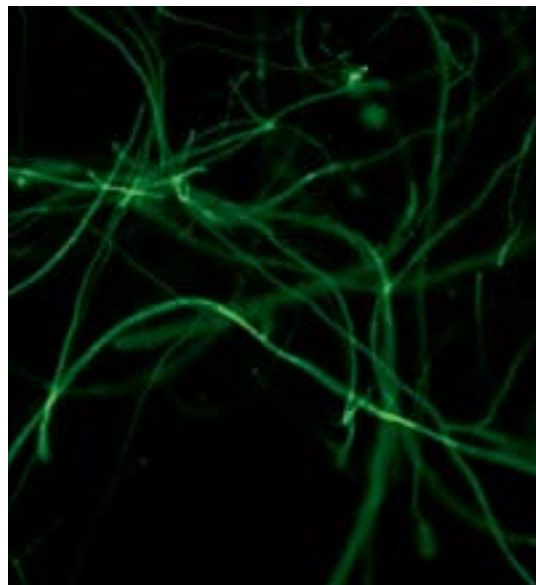


Figure 2 Fluorescence microscopy of a smear preparation of bread containing mould. The target carbohydrate subunit chitotriose [(GlcNAc) $_3$] of the fungi are specifically bound to lectin from *Phytolacca americana* Atto 488 conjugate (green). Image by J. Zbaeren, Inselspital Bern, Switzerland.

The following Atto-dye lectin conjugates are now available from Sigma® Life Science. Additional lectins and lectin conjugates available from Sigma Life Science may be found at sigma-aldrich.com/enzymeexplorer

Description	$\lambda_{ex}/\lambda_{em}$ (nm)	Carbohydrate Specificity	Cat. No.	Pack Size
Concanavalin A-Atto 565-conjugate	563/592 in PBS	α -Mannose, α -Glucose	69535	1 mg
Lectin from <i>Artocarpus integrifolia</i> -Atto 594 conjugate	601/632 in PBS	O-Methyl- α -Galactose	76158	1 mg
Lectin from <i>Ulex europaeus</i> -Atto 488 conjugate	501/523 in PBS	α -L-Fucose	19337	0.5 mg
Lectin from <i>Ulex europaeus</i> -Atto 550 conjugate	554/576 in PBS	α -L-Fucose	94165	0.5 mg
Lectin from <i>Ulex europaeus</i> -Atto 594 conjugate	601/ 632 in PBS	α -L-Fucose	73873	0.5 mg
Lectin from <i>Phaseolus vulgaris</i> -Atto 488 conjugate (Leucoagglutinin)	501/523 in PBS	GlcNAc-Man	75319	1 mg
Lectin from <i>Phaseolus vulgaris</i> -Atto 550 conjugate (Leucoagglutinin)	554/576 in PBS	GlcNAc-Man	90852	1 mg
Lectin from <i>Phaseolus vulgaris</i> -Atto 647N conjugate (Leucoagglutinin)	644/669 in PBS	GlcNAc-Man	77363	1 mg
Lectin from <i>Phytolacca americana</i> -Atto 488 conjugate	501/523 in PBS	(GlcNAc) $_3$	39905	1 mg
Lectin from <i>Phytolacca americana</i> -Atto 550 conjugate	554/576 in PBS	(GlcNAc) $_3$	94816	1 mg
Lectin from <i>Phytolacca americana</i> -Atto 647N conjugate	644/669 in PBS	(GlcNAc) $_3$	03065	1 mg
Lectin from <i>Tritium vulgaris</i> -Atto 488 conjugate	501/523 in PBS	(GlcNAc) $_2$, α -N-acetylneuraminic acid	16441	1 mg
Lectin from <i>Tritium vulgaris</i> -Atto 532 conjugate	532/558 in PBS	(GlcNAc) $_2$, α -N-acetylneuraminic acid	68917	1 mg

New Spectroscopic Reagents for UV/VIS by Sigma-Aldrich®

Michael Jeitziner, Market Segment Manager Analytical Reagents & Standards michael.jeitziner@sial.com



Introduction

UV/VIS spectroscopy is routinely used in the quantitative determination of solutions for cations, anions and highly conjugated organic compounds.

Sigma-Aldrich offers an extensive range of chemicals for the quantitative photometric analysis of ions and molecules by UV/VIS spectroscopy. In order to be suitable for this application, our reagents are guaranteed to have a homogeneous appearance, no extraneous colour and to be of reliable quality.

Quantitative Analysis of cyanide [1]

Cyanides are extensively used in many industrial processes; they frequently occur in toxic waste and wastewater of diverse origin and in streams that receive uncontrolled and unpurified industrial effluents [2]. The detection limit for the sensor is $1,0 \times 10^{-6}$ M, fully meeting the U.S. EPA water quality criterion of sensitivity below 0.2 mg/L.

The C-O bond of the oxazine at the spiro centre cleaves when titrated with nucleophilic cyanide anions (Figure 1), resulting in obvious changes in colour (Figure 2).

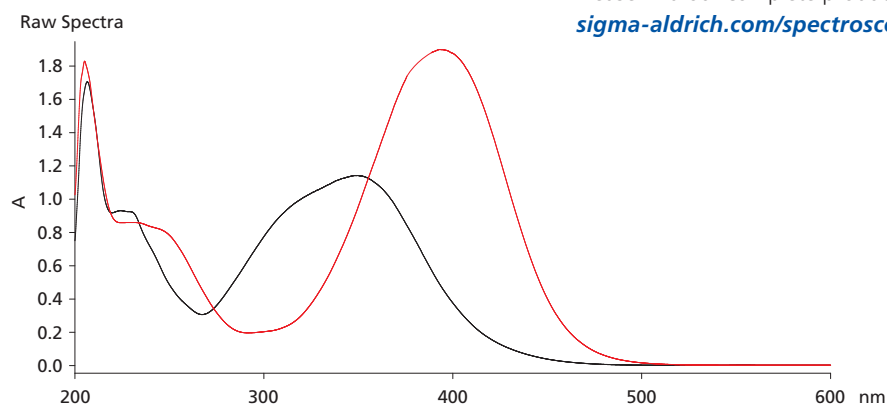


Figure 2 Absorption spectra of chemosensor oxazine (0.1 mM, 298 K in methanol) without NaCN (black curve) and with 1 mM NaCN (red curve)

Brand	Prod. No.	Description	Analyte	CAS No.	Pack Sizes
FLUKA	07670	<i>N,N</i> -Diethyl- <i>p</i> -phenylenediamine sulphate salt	S ²⁻ , Cl ₂	6283-63-2	25 g, 100 g
FLUKA	08751	4-Amino-3-hydroxy-1-naphthalenesulphonic acid	Si	116-63-2	25 g, 100 g
SIGMA	11635	Azomethine-H monosodium salt hydrate	B	206752-32-1	5 g, 25 g
FLUKA	11880	Bathophenanthroline	Fe	1662-01-7	500 mg, 1 g, 5 g
FLUKA	15100	Bismuthiol I	Bi, Cu, Pb, Sb	1072-71-5	10 g
FLUKA	32750	3,3'-Diaminobenzidine tetrahydrochloride hydrate	Se, Te	868272-85-9	1 g, 5 g, 25 g
FLUKA	94979	2,8-Dinitro-5a,6,6-trimethyl-5a,6-dihydro-12H-indolo[2,1-b][1,3]benzoxazine	CN ⁻	1023640-20-1	100 mg
FLUKA	87748	3,3',5,5'-Tetramethylbenzidine	Cl ₂	54827-17-7	1 g, 5 g, 25 g

Product table (selection)

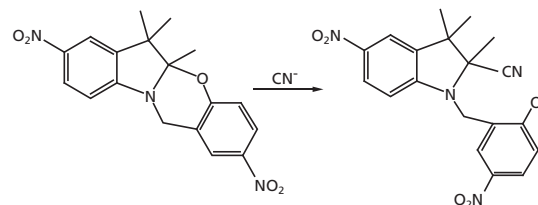


Figure 1 Reaction of oxazine derivative with cyanide

The chemosensor exhibits a linear working range from 10^{-6} to 10^{-5} M and is therefore sufficient to monitor micromolar concentrations of cyanide. It shows a very fast response within 30s and can also be detected by the naked eye.

Oxazine is available from Sigma-Aldrich (94979) along with a variety of chemosensors for other analytes, see table below.

References

- [1] J. Ren, et al. A highly sensitive and selective chemosensor for cyanide. *Talanta* **75** (2008) 760–764.
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Please find our complete product list at sigma-aldrich.com/spectroscopy

Synthetic Cannabinoids – New Standards, Methods and Suitable Sample Utilities for Forensic Toxicology

Rudolf Köhling, Namtso Reichlin, R&D LC/MS Applications rudolf.koehling@sial.com

In most countries, the ownership and trade of products from the plant *Cannabis sativa*, like hashish or marijuana, is illegal, and driving under the influence of cannabinoids can result in prosecution. As a consequence, new cannabinoid-like compounds originating from pharmaceutical developments, e.g. for cancer therapy, were used as additives in hashish-like mixtures of herbs and other plants to bypass these legal restrictions. A large number of these substances interact in the same way with the cannabinoid receptors as tetrahydrocannabinol (THC), but their effect is much stronger and the potential of becoming addicted is assumed to be comparable to THC. For a short while, these synthetic cannabinoids in herbal blends, e.g. "Spice", were widely available, as there was no information about the identity of the ingredients in the blends. V. Auwärter and his group identified several compounds including one unknown CP 47,947 homologue, which led to the prohibition of several cannabinomimetics and homologous compounds [1].

Sigma-Aldrich® offers a large variety of cannabinoid receptor agonists as drug standards for analytical or pharmacological purposes. The new compounds are added to this list and further will follow. Furthermore, development of applications for the analysis of synthetic cannabinoids with LC/MS are in progress, and first results of HPLC method with the new Supelco Ascentis® Express RP-Amide column are presented in this article. The method used separate compounds in a methanolic extract of "Spice Diamond" and identifies the main active pharmacological ingredient with mass spectrometry. Finally, the issue of sample loss in glass vials after storage is demonstrated for THC, which typically shows this phenomenon in standard glass vials. Therefore, pre-treated silanised vials are presented as an alternative, to prevent this kind of sample degradation.

Ingredients of "Spice" and other potentially misused synthetic cannabinoids

The table opposite shows a list of synthetic and natural cannabinoids used for the method development. JWH-018 is one of the compounds found in "Spice". Interestingly, the JWH compounds have no structural relation to typical cannabinoids like THC.

Ascentis Express RP-Amide Method

Several separation methods for cannabinoids using different chromatographic techniques were developed in the past. Predominantly GC/MS methods with derivatisation are installed in most laboratories as they guarantee LOQs of 1 ng/mL. The introduction of LC/MS systems, especially the robust orthogonal sprayer geometry, opened new ways for a more efficient sample preparation without derivatisation reactions [2-3]. Small particles and the development of new key technologies like Fused-Core™ improved the resolution and sensitivity of HPLC significantly. Besides sensitivity, the selectivity of the surface chemistry also determines significantly the quality

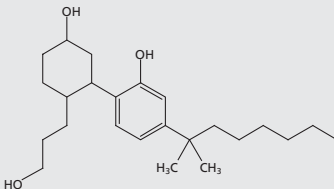
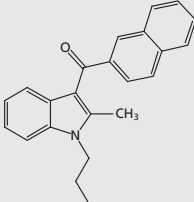
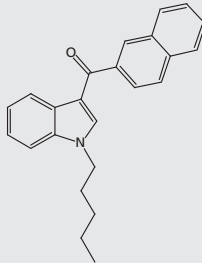
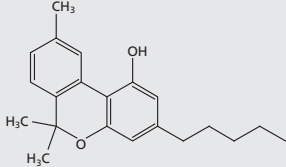
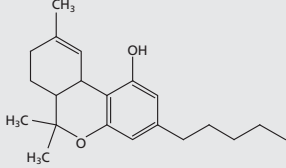
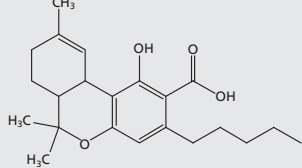
No.	Name	Structure	Order No.
1	CP 55,940		C1112
2	JWH-015		J4252
3	JWH-018		55653
4	Cannabinol		C6888
5	THC		56296
6	THC-COOH		39382

Table 1 Compound list used in the method development process. THC and cannabinol as very similar molecules serve as a benchmark for the selectivity of the HPLC column.

of the chromatography for a given set of analytes. For cannabinoids, superior results can be achieved with the new Supelco Ascentis Express RP-Amide stationary phase. This combines both a high resolution of the peaks and a selective stationary phase to separate very similar molecules, like cannabinol and

(continued on page 12)

Δ^9 -tetrahydrocannabinol, as well as very different molecules, like the more polar internal standard digoxine (IS) or the metabolite Δ^9 -tetrahydrocannabinolic acid.

Figure 1 shows the chromatograms of 6 different cannabinoid and cannabinomimetic standards detected in the positive and negative ESI mode. The compounds show different sensitivity depending on their ionisation energy in the related ESI mode. But one can see that the right HPLC column provides baseline separation for compound 4 (cannabinol) and 5 (Δ^9 -tetrahydrocannabinol) and a very good symmetry of all peaks.

After testing the suitability of the chromatographic system, a real “Spice”-Mixture (“Spice Diamond”) was extracted with methanol for 1 h. The sample was vortex-mixed and sonicated several times. 5 μ L of the sample solution was injected into the HPLC system. The total ion chromatogram of the “Spice” extract in **Figure 2** shows a large number of different compounds and a high noise signal from the matrix. But with the knowledge of the mass and the retention time, one can easily identify the compound JWH-018 (inset) in this very complex mixture. Even under these conditions, the peak shape and peak position remain absolutely comparable to the matrix-free standards solution.

Sample Loss during Storage

After the preparation of samples and storage of 1 or more days, one can frequently observe the loss of signal intensity depending on the compounds, concentrations, solvents or sample matrices. Cannabinoids, particularly, show this effect. This sample loss is shown in the decrease of calibration curve slopes in **Figure 3** and is due to the irreversible adsorption of the analyte on the surfaces of the sample container.

In order to prevent sample loss and to guarantee a stable compound concentration, the use of pre-configured silanised vials or the silanisation of the vials with suitable agents is recommended. In the case of the cannabinoid samples, Supelco-silanised clear glass vials (27060-U) were used and resulted in repeatable calibration curves even after a 2-day storage at room temperature. Additionally, a better linearity up to a concentration of 1000 ng/mL was observed (**Figure 4**).

Method Parameters

Column:	Supelco Ascentis Express RP-Amide (53911-U), 5 cm x 2.1 mm I.D., 2.7 μ m
Temperature:	40 °C
Flow rate:	0.3 ml/min
MS:	ESI(+/-), SPS target 315 m/z, stability 100 %, trap lvl. 100 %, optimise normal, range 100-1000 m/z, nebuliser 35 psi, dry gas 9 L/min, dry temp. 365 °C
Injection volume:	5 μ L
Run time:	25 min (5 min post time)
Solvents:	(A) Water v. 0.1 % formic acid (B) Acetonitrile
Gradient	0.0 min 40 % (B) 0.25 min 40 % 5.0 min 95 % 15.0 min 95 % 17.0 min 40 %

Table 2 HPLC method for separation of the 7 dyes listed in Table 1 after the optimisation

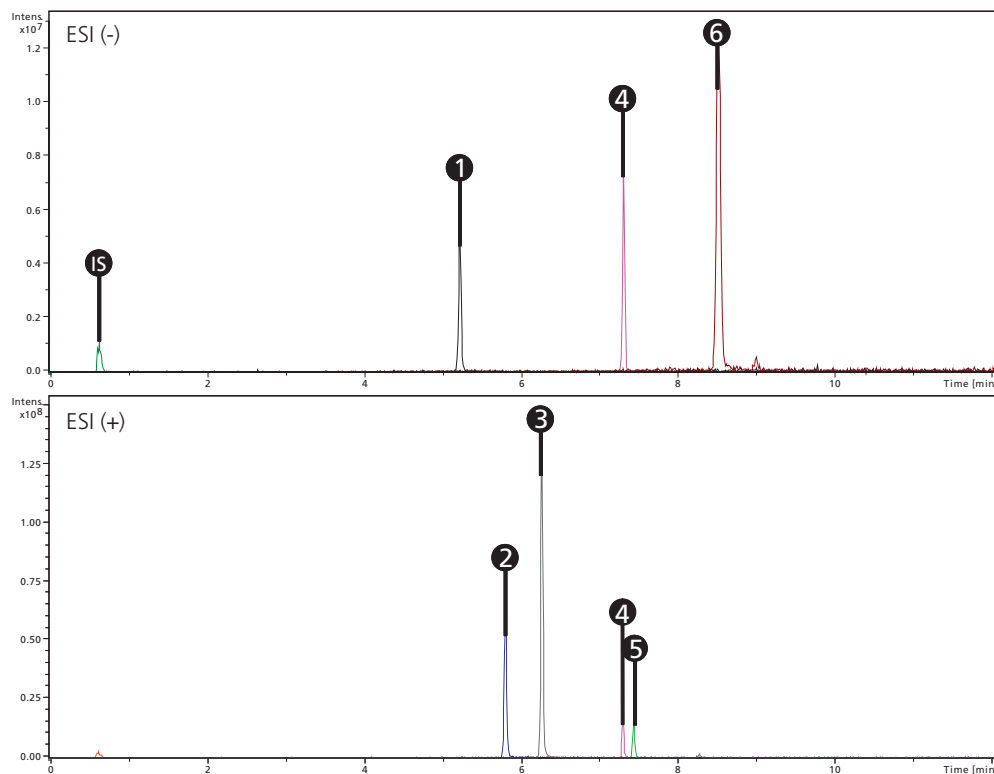


Figure 1 Extracted ion chromatograms (EIC) of the cannabinoid test mixture containing 6 different natural and synthetic cannabinoids and digoxin as internal standard (IS)

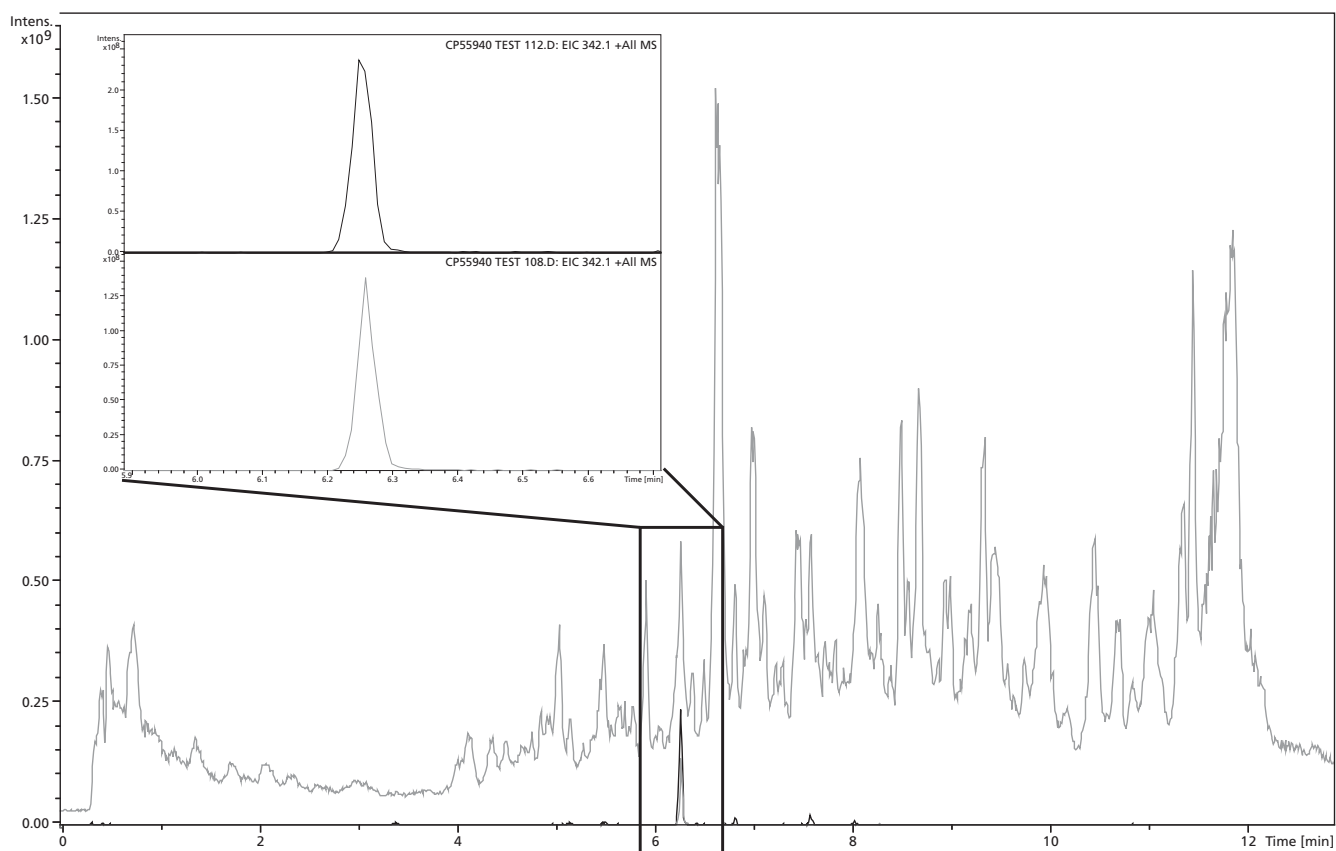


Figure 2 Total ion chromatogram (TIC) of the “Spice” extract in methanol. The inset shows the extracted ion chromatogram of mass 342.1 m/z compared to the EIC of the control sample. The observed mass by the ion trap is 342.12 m/z and the calculated mass of $C_{24}H_{23}NO$ is 342.19 m/z, which identifies the compound as JWH-018 – a typical ingredient of “Spice”.

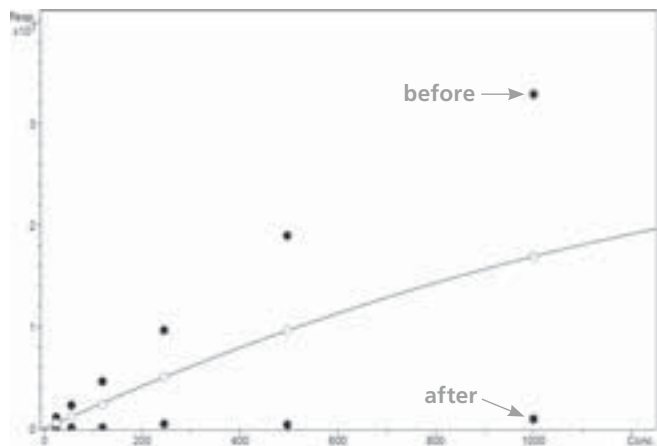


Figure 3 Calibration curves of THC before and after storage in non-silicised sample vials. The adsorption of the analyte leads to a significant decrease of the response compared to the original response.

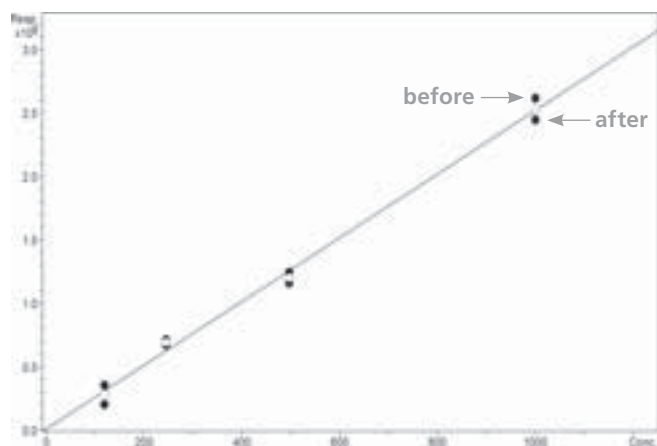


Figure 4 Calibration curves of THC before and after storage in silicised vials (Supelco)

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Carbon Adsorbents

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Shyam Verma, Market Segment Manager, Reagents & Chemicals shyam.verma@sial.com

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- non-porous graphitised carbon blacks (GCB)
- carbon molecular sieves
- highly porous activated carbons and charcoals

Graphitised Carbon Black (GCB)

Generally, these are non-porous and non-specific adsorbents that exhibit high surface homogeneity. The high purity of these adsorbents ensures effective desorption of the analyte of interest. These adsorbents are suitable for gas-solid chromatography, even for eluting polar compounds. GCBs exhibit hydrophobic surface characteristics and, therefore, can be effectively used in trapping organic compounds in humid streams. Their hydrophobic nature minimises sample displacement by water, so accurate samples can be obtained despite levels of high levels of humidity. Trapped compounds can be desorbed by a solvent or thermal desorption at 100 % desorption efficiency. These adsorbents offer excellent thermal stability, ensure minimal bleed at thermal desorption temperatures, and prevent high pressure-drop.

Carbotrap B, -C, and -F trap a wide range of airborne organics (C4-C5 hydrocarbons) to polychlorinated biphenyl and other large molecules

- Carbotrap B adsorbent: surface area = 100 m²/g, useful in monitoring airborne C5-C12 compounds.
- Carbotrap C and -F adsorbents: surface area = 10 and 5 m²/g, respectively, useful for trapping and efficiently releasing larger molecules (C9-C30).

Description	Size	Qty.	Cat. No.
Carbotrap	20/40 mesh	10 g	20287
Carbopack B	60/80 mesh	10 g	20273
Carbotrap C	20/40 mesh	10 g	20309
	20/40 mesh	500 g	11047-U
Carbopack C	60/80 mesh	10 g	10257
Carbotrap X	20/40 mesh	10 g	10435-U
Carbopack X	40/60 mesh	10 g	10436
	60/80 mesh	10 g	10437-U
Carbotrap Y	20/40 mesh	10 g	10460-U
Carbopack Y	40/60 mesh	10 g	10461-U
	60/80 mesh	10 g	10462
Carbopack Z	60/80 mesh	10 g	11051-U

Table 1 Product information

Carbon Molecular Sieve (CMS)

A carbon molecular sieve (CMS) also called molecular sieve carbon, is a specialised carbon that has a tailored porosity for selective adsorption. These materials are primarily used for collecting very small molecular-sized compounds (C2-C5). The size and shape of the analyte molecule, and the size and shape of the pore entrances in the CMS particle, determine how well the analyte is adsorbed and desorbed.

As our CMSs are prepared from high-purity polymers, resulting material is a high-purity carbon that allows effective adsorption of an analyte, and its efficient desorption for quantification. Carbosieve S-III and Carboxen CMS have upper temperature limits of at least 400 °C.

- Carbosieve S-II (mostly used in GC columns) is recommended for analysing mixtures of permanent gases (H₂, O₂, Ar, CO and CO₂) and C1-C2 hydrocarbons (methane, ethane, ethylene, acetylene). Maximum operating temperature is 225 °C with oxygen-free carrier gas.
- Carbosieve S-III is a spherical adsorbent with a surface area of ~ 820 m²/g and pore size of 15–40 Å. It is an excellent adsorbent for trapping small airborne molecules, such as chloromethane. The pure carbon framework permits thermal desorption of analyte molecules without loss.
- Carbosieve G (mostly used in GC columns) is recommended for analysing C1-C3 hydrocarbons. Maximum operating temperature is < 200 °C with oxygen-free carrier gas.

Description	Size	Qty.	Cat. No.
Carbosieve S-II	60/80 mesh	10 g	10189
Carbosieve S-III	60/80 mesh	10 g	10184
Carbosieve G	40/60 mesh	5 g	10197
	60/80 mesh	5 g	10198

Table 2 Product information

Carboxen

These CMS materials are hydrophobic in nature, thus ensuring accurate sampling at high humidity levels.

Carboxen-563 and Carboxen-564:

- our preferred versions of Ambersorb® XE-340 and Ambersorb XE-347.
- show higher capacity (breakthrough volume) for many volatile organic compounds (VOCs).
- useful for analysing water quality or adsorbing airborne compounds.
- Carboxen-564 is effective for monitoring many C2-C5 VOCs.

Carboxen-569:

- 20/45-mesh material with no Ambersorb equivalent.
- high hydrophobicity and higher capacity for organic molecules.

Carboxen-572: most efficient for purge & trap, and air sampling applications.

Carboxen-1000:

- large surface area enables effective and efficient adsorption and desorption.
- suitable for low volume sampling of very volatile compounds, such as vinyl chloride.
- primarily used in narrow bore tubes that are desorbed directly into the chromatographic column.

Carboxen-100: trap and retain small compounds.

Carboxen-1003: large surface area for efficient adsorption/desorption and good hydrophobicity.

Carboxen-1012: highly-activated with large micropores; effective for aqueous phase adsorption of organic compounds, or for air sampling of C4-C6 compounds.

Carboxen-1016: analyte group of permanent gases.

Carboxen-1018: narrow (~6–7 Å) micropores for adsorption/desorption of small analytes such as, ethane, acetylene, ethylene and the C3 hydrocarbons. Hydrophobic.

Carboxen-1021: for air sampling of small molecules.

Description	Size	Qty.	Cat. No.
Carboxen-563	20/45 mesh	10 g	10263
Carboxen-564	20/45 mesh	10 g	10264
Carboxen-569	20/45 mesh	10 g	10269
	20/45 mesh	500 g	11048-U
NEW! Carboxen-572	20/45 mesh	10 g	11072-U
Carboxen-1000	40/60 mesh	50 g	10477-U
	60/80 mesh	10 g	10478-U
Carboxen-1003	40/60 mesh	10 g	10471
Carboxen-1016	60/80 mesh	10 g	11021-U

Table 3 Product information

Activated Carbons and Charcoals

Activated coconut charcoal has been used extensively as a general purpose adsorbent due to its ability to adsorb/desorb a wide range of volatile analytes.

Description	Size	Qty.	Cat. No.
Activated Charcoal	20/40 mesh	10 g	10275
High-purity powder (Supelco)	powder	250 g, 750 g	31616
Darco G60	-100 mesh	1 Kg	242276
Desorex CG – pure granular	12-35 mesh (500-1600 µm)	500 g, 1 Kg, 2.5 Kg 6x500 g, 6x1 Kg	18002

Table 4 Product information

Carbon Adsorbent Sampler Kits

Choosing the right adsorbent or combination of adsorbents can often be difficult. Selecting a suitable adsorbent means an adsorbent that can retain a specific analyte, or group of analytes, for a specific sample volume, and also able to release the analyte(s) during the desorption process.



E000926

By using one of the Supelco Carbon Adsorbent Kits, the method developer obtains a cost-effective process to evaluate several carbon adsorbents when designing adsorbent-based applications and products. Once the appropriate material has been identified, we will work with you to produce larger quantities to your specifications.

Description	Cat. No.
Graphitised Carbon Black Kit, 20/40 Mesh 5 g each of Carbotrap F, Carbotrap C, Carbotrap Y, Carbotrap B, Carbotrap X	13027-U
Graphitised Carbon Black Kit, 60/80 Mesh 5 g each of Carbopack F, Carbopack C, Carbopack Y, Carbopack B, Carbopack X, Carbopack Z	13026-U
Carbon Molecular Sieve Kit 5 g each of Carboxen-569, Carboxen-1000, Carboxen-1003, Carboxen-1012, Carboxen-1016, Carboxen-1018, Carboxen-1021, Carbosieve G, Carbosieve S-III	13028-U

Table 5 Product information

Carbon Adsorbent Selection Guide

For multi-bed tubes, use the weaker adsorbent in front of the stronger adsorbent. For example, use Carbopack C in front of Carbopack B.

Relative Analyte Sizes	Recommended Materials (listed weakest to strongest)
>C20	Carbotrap F, Carbopack F
C12-C20	Carbotrap C, Carbopack C, Carbotrap Y, Carbopack Y
C5-C12	Carbotrap B, Carbopack B
C3-C9	Carboxen 1016, Carbotrap X, Carbopack X, Carbopack Z
C2-C5	Carboxen 569, Carbosieve G, Carboxen 1000, Carbosieve S-III, Carboxen 1021, Carboxen 1018, Carboxen 1003, Carboxen 1012

Note: Analyte size relative to n-Alkanes. Consider all atoms, not just Carbon. For example, even though 1,2-Dichloroethane is a C2, the two Chlorine atoms give it a relative size between C4 and C5.

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Several of our unique carbons were used in tests on several NASA space missions: 1995 Galileo Mission to Jupiter, 2005 Cassini-Huygens Mission to Titan, and 2007 Mars Mission.

Rapid Separation of Anthocyanins and Flavonol Glycosides Utilising Discovery® DSC-MCAX Solid Phase Extraction

Contributed Article

Neel M. Shah and James M. Chapman, Rockhurst University, Kansas City, MO, USA james.chapman@rockhurst.edu

Introduction

Flavonols and anthocyanins are water-soluble vacuolar flavonoid compounds that are synthesized by organisms of the plant kingdom. Flavonoids are widely distributed in plants fulfilling many functions including pigmentation and protection from UV light and attack by microbes and insects [1].

The chemical structure of the anthocyanidins is based upon the flavonoid family of molecules that is in turn based on the C6-C3-C6 configuration in the flavan nucleus [2]. **Figure 1** illustrates the structure of the flavylium ion which makes up the backbone of the anthocyanidins. The chemical structure of the flavonols is also based upon the flavonoid family of molecules where flavonols use the 3-hydroxyflavone backbone. Anthocyanins are anthocyanidins linked with one or more sugars that are sometimes acylated. Flavonols and flavones are glycosylated and acylated similarly to anthocyanins [1].

Since 1992 more than 277 anthocyanins have been reported and more recent literature estimates the total number of identified anthocyanins at 550 [1]. Consumers and food manufacturers have become interested in flavonoids for their medicinal properties, especially their potential role in the prevention of cancers and cardiovascular disease [3,4]. The beneficial effects of fruit, vegetables, and tea or even red wine have been attributed to flavonoid compounds rather than to known nutrients and vitamins [5].

Both anthocyanins and flavonols are typically characterized through the utilisation of reversed-phase HPLC with UV-VIS detection coupled to electrospray ionisation

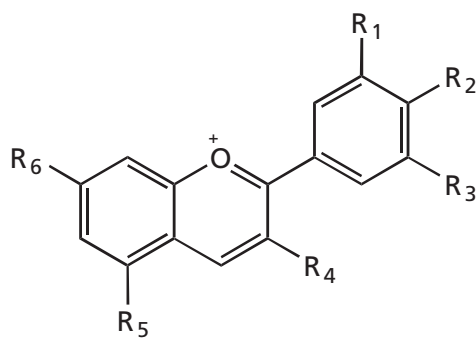


Figure 1 Flavylium ion backbone of anthocyanidins & anthocyanins

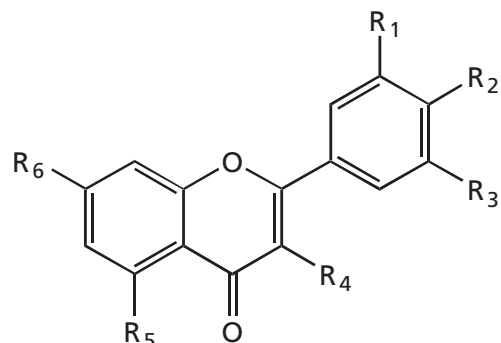


Figure 2 3-Hydroxyflavone backbone of flavonols & flavonol glycosides

mass spectrometry (ESI-MS). Due to the complexity of the flavonol and anthocyanin isomeric structures that may exist in plant tissues, the separation of these molecules by RP-HPLC can lead to co-eluting compounds. The separation of the flavonols and anthocyanins prior to analysis by LC-ESI-MS would greatly facilitate the identification.

The DSC-MCAX mixed-mode cation SPE cartridges were used in the separation of anthocyanin-O-glycosides (positively charged) and flavonol-O-glycosides (neutral) in red tulip flower petals.

Extraction of Anthocyanins from Tulip

Red tulip blooms (*Tulipa darwin hybrid 'Apeldoorn'*) were treated with 50/50 methanol/water with 0.1 % formic acid, ground with a glass stirring rod and placed in sonicator for several minutes to extract the anthocyanins and flavonols. The mixture was filtered to remove large particles.

SPE Fractionation of Flavonol Glycosides and Anthocyanins

A DSC-MCAX SPE cartridge, 100 mg/3 mL (52783-U), was conditioned with 1.5 mL of methanol and equilibrated with 1.5 mL of water containing 0.1 % formic acid. A small aliquot of tulip extract was diluted with an equal volume of water with 0.1 % formic acid and loaded onto the cartridge. The cartridge was rinsed with 1.5 mL water with 0.1 % formic acid solution (0.5 mL x 3). The flavonol glycosides were eluted with 1.5 mL methanol (0.5 mL x 3). The anthocyanins were eluted with 1.5 mL (0.5 mL x 3) of a 50/50 solution of potassium phosphate buffer pH 6.0 and methanol.

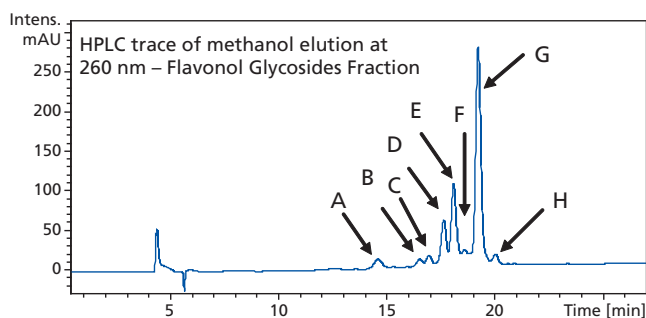


Figure 3 HPLC Trace of Tulip Extract Before and After SPE Fractionation

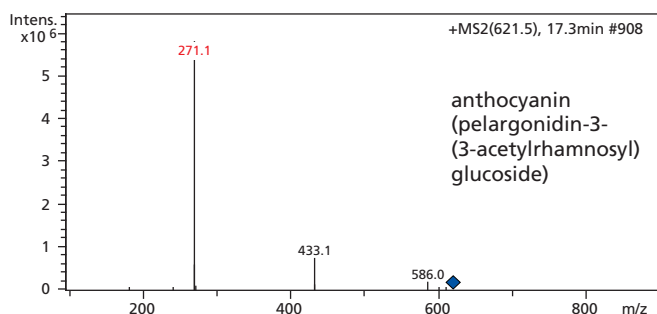
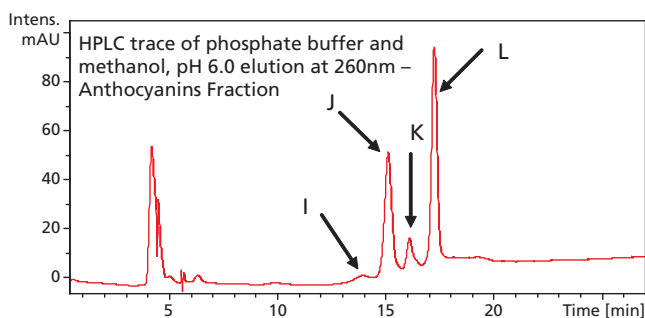
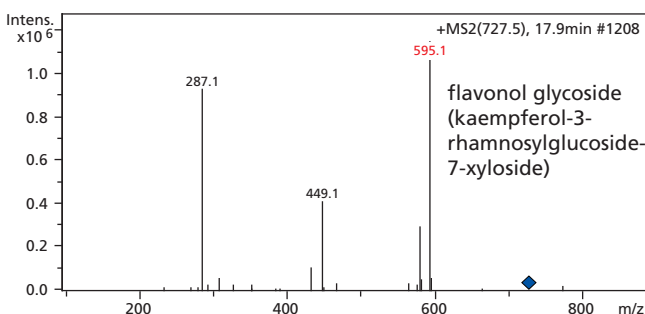


Figure 4 Mass spectrum of anthocyanin & flavonol glycoside



Instrumentation – HPLC/DAD/ESI-MS/MS Analyses

LC/ESI-MS/MS experiments were performed on an Agilent MSD XCT ion trap mass spectrometer (Palo Alto, CA) equipped with an electrospray ionisation (ESI) interface, 1100 HPLC, a DAD detector, and Chemstation software. The column used was a 150 x 0.5 mm i.d., C18 phase with 5 μ m particle size. Solvents were (A) 0.1 % formic acid/ 99.9 % water (v/v) and (B) 0.1 % formic acid/ 99.9 % acetonitrile (v/v). Solvent gradient was 0–20 min, 10–50 % B; 20–31 min, 50 % B; and 31–35 min, 50–10 % B. Flow rate was 0.6 mL/min, injection volume was 0.5 μ L, and column temperature was 25 $^{\circ}$ C. The ion trap mass spectrometer was operated in positive ion mode scanning from m/z 100 to m/z 2200 at a scan resolution of 13000 amu/s. Representative chromatograms, PDA UV Spectrums, and MS results are depicted in **Figures 3–4**. Using this data, identities were proposed for the flavonol glycoside and anthocyanin extracts (**Table 1**).

Conclusion


Flavonol glycosides and anthocyanins are compounds that are found in plants, flowers and fruits. These compounds are involved in the protection of the plant from UV light and aid in pollination by producing brilliant colours to attract insects and animals. Because the structures of the flavonols and anthocyanins are very similar, it is extremely difficult to successfully separate and identify the compounds in plant extracts. The method described can be used as a simple and rapid separation of the flavonol glycosides and anthocyanins. By exploiting the different ionisation characteristics between the two compound classes, DSC-MCAX (mixed-mode cation exchange) was used as a fractionation tool prior to analysis. Once separated, the characterisation of both classes of compounds is made much easier and their potential uses can be more effectively evaluated.

	RT	Proposed Identity
Flavonols		
A	14.6	Unknown flavonol
B	16.5	Apigenin/genistein acetylramnosyl glucoside
C	17.0	Quercetin di-glucoside
D	17.7	Apigenin/genistein acetylramnosyl glucoside (isomer)
E	18.1	Kampferol rhamnosyl glucoside xyloside
F	18.6	Unknown isorhametin derivative
G	19.2	Luteolin rutinoside
H	20.1	Luteolin rutinoside (isomer)
Anthocyanins		
I	13.9	Cyanidin rutinoside
J	15.2	Pelargonidin rutinoside
K	16.1	Pelargonidin acetylramnosyl glucoside
L	17.3	Cyanidin acetylramnosyl glucoside

Table 1 Proposed identities of flavonol & anthocyanin constituents

References

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Water Determination in Pharmaceutical Compounds

Karl Fischer Titration with HYDRANAL® Reagents

Helga Hoffmann, Technical Service HYDRANAL Manager helga.hoffmann@sial.com
 Andrea Felgner, Product Manager Analytical Reagents andrea.felgner@sial.com



Water content determination is mandatory for many materials used in the manufacturing of medicines. Karl Fischer (KF) titration is the long-standing standard method for this analysis prescribed by the leading Pharmacopoeias, like the European (Ph.Eur.), the United States (USP) and the Japanese (JP).

Pharmaceutical compounds like ethosuximide, used for epilepsy treatment, (application L510) or the cytostatic drug cyclophosphamide (application L463) can be analysed according to this standard procedure without interference; these titrations can also be carried out in our non-toxic reagents, the HYDRANAL E-types.

European Pharmacopoeia requirements for Karl Fischer titration

Ph.Eur. specifies KF titration to measure the water content of many solvents, chemicals and other substances. In Ph.Eur., beginning with edition 5.7, chapter 2.5.12 "Water: Semi-Micro Determination" describes in Method A the direct titration of water and in Method B the indirect method of back titration. In practice, the direct method A is easier to carry out and widely used since the development of the highly reactive HYDRANAL reagents:

"Method A. Introduce into the titration vessel methanol R, or the solvent indicated in the monograph or recommended by the supplier of the titrant. Where applicable for the apparatus used, eliminate residual water from the measurement cell or carry out a pre-titration. Introduce the substance to be examined rapidly and carry out the titration, stirring for the necessary extraction time."

On our HYDRANAL website sigma-aldrich.com/hydranal you will find more information about the Ph. Eur. requirements for KF titration. Our HYDRANAL technical service team has carried out suitability tests for a range of substances, which are available on request.

Volumetric Karl Fischer technique

The volumetric KF technique can be used for samples with high water content, i.e. 1–100 mg. The sample is added to the titration vessel containing a suitable working medium, like HYDRANAL-Methanol dry or HYDRANAL-Solvent (E). With the corresponding iodine-containing titrating agent, HYDRANAL-Composite or HYDRANAL-Titrant (E), the water content of the sample is determined by titration. Endpoint determination is carried out by applying a constant current and measuring the voltage via double platinum electrode; or vice versa, applying a constant voltage and measuring the current. The water content is calculated from sample weight, consumption of titrating agent, and water equivalent (so-called titer) of the titrating agent.

Coulometric Karl Fischer technique

Samples with a lower water content (10 µg-10 mg) can be analysed using the coulometric KF method. The iodine required is generated electrochemically in the titration vessel by anodic oxidation of iodide, which is contained in the HYDRANAL-Coulomat reagents. The water content is calculated from the amount of consumed electric charge (which equals iodine consumption) over time.

Karl Fischer Oven technique

Insoluble samples, samples that undergo side reactions with the KF reagents, or samples that release their water only at high temperatures, may be analysed using the KF oven. The water of the sample is driven out at a variable temperature and transferred by a suitable carrier gas into the KF titration vessel. There it is determined according standard procedures.

5-Aminolevulinic acid-HCl (lyophilisate) shows a strong side reaction in methanol; an endpoint cannot be reached with direct KF titration methods. For this compound, we recommend determination with the KF oven in combination with the coulometric titration technique, due to the low water content of this substance. Suitable application parameters are: an evaporation temperature of 80 °C (decomposition of the sample starts at 120 °C), a determination time of 600 seconds and HYDRANAL-Coulomat AG or HYDRANAL-Coulomat AG-Oven and HYDRANAL-Coulomat CG as reagent (application L506).

Side reactions and pH-influencing samples

Substances containing nitrogen compounds may cause interference in the pH value of the working medium. Side reactions occur during the KF titration, possibly leading to coated electrodes, fading endpoints or no endpoints at all, and erroneous results. These side-reactions can be suppressed through the addition of suitable buffer substances to the working medium in the titration vessel.

(continued on page 20)

Benserazide hydrochloride, an active ingredient used in the treatment of Parkinson's disease, is an example of these nitrogen-containing substances; for correct determination of its water content, HYDRANAL®-Buffer Base or a mixture of HYDRANAL-Methanol dry and HYDRANAL-Salicylic acid should be used as a working medium, and then be titrated with HYDRANAL-Composite 2 (application L416).

Proflavine hemisulphate, a topical antiseptic, also increases the pH value of the working medium so that no titration endpoint is achieved. HYDRANAL-Buffer Base or a mixture of HYDRANAL-Methanol dry and HYDRANAL-Benzoic acid can be used to lower the pH value and prevent the side reaction; titrate with HYDRANAL-Composite 5 (application L354).

Penicillins are a group of fungal metabolites used to treat infections caused by bacteria. Water content determination in penicillins can be disturbed by pH-influences; a side reaction occurs: penicillin derivatives such as penicilloic acid and other hydrolysis products are oxidised by iodine. By conducting the titration in weakly acidic conditions, this side reaction can be suppressed. The KF one-component technique with HYDRANAL-Methanol dry or HYDRANAL-Methanol Rapid and HYDRANAL-Composite 2 gives a pH value of approx. 5 in the titration vessel; this is sufficient for titrating the water content in penicillins without any side reactions.



If an endpoint is not easily reached, an addition of HYDRANAL-Salicylic acid to the working medium (before pre-titration) is recommended. Titration of penicillins with the KF two-component technique is also possible; a mixture of HYDRANAL-Solvent and HYDRANAL-Salicylic acid is recommended as working medium and can be titrated with HYDRANAL-Titrant (application L166).

Enhancement of sample solubility

For samples that are only poorly soluble in the KF working medium, HYDRANAL-Buffer Base or a mixture of HYDRANAL-Methanol dry and HYDRANAL-Salicylic acid can be recommended as working medium to enhance solubility and therefore yield correct determination results (titrating agent HYDRANAL-Composite). Examples are the beta-lactam antibiotics amoxicillin-3-hydrate (application L352) or ampicillin (application L422).

Cat. No.	Brand	Description	Pack Size
Reagents for volumetric titration (one-component technique)			
34734	Fluka	HYDRANAL-CompoSolver E	1 L, 2.5 L
34827	Fluka	HYDRANAL-Composite 1	500 mL, 1 L, 2.5 L
34806	Fluka	HYDRANAL-Composite 2	500 mL, 1 L, 2.5 L
34805	Fluka	HYDRANAL-Composite 5	500 mL, 1 L, 2.5 L
34741	Fluka	HYDRANAL-Methanol dry	1 L, 2.5 L
37817	Fluka	HYDRANAL-Methanol Rapid	1 L, 2.5 L
Reagents for volumetric titration (two-component technique)			
34723	Fluka	HYDRANAL-Titrant 2 E	1 L
34732	Fluka	HYDRANAL-Titrant 5 E	500 mL, 1 L, 2.5 L
34811	Fluka	HYDRANAL-Titrant 2	500 mL, 1 L, 2.5 L
34801	Fluka	HYDRANAL-Titrant 5	500 mL, 1 L, 2.5 L
34730	Fluka	HYDRANAL-Solvent E	500 mL, 1 L, 2.5 L
34800	Fluka	HYDRANAL-Solvent	1 L, 2.5 L
Reagents for coulometric titration			
34739	Fluka	HYDRANAL-Coulomat AG Oven	500 mL
34836	Fluka	HYDRANAL-Coulomat AG	500 mL, 1 L
34840	Fluka	HYDRANAL-Coulomat CG	50 mL (10 * 5 mL)
Auxiliary Reagents (pH buffer substances and solubilising agents)			
37859	Fluka	HYDRANAL-Buffer Base	1 L
37865	Fluka	HYDRANAL-Salicylic acid	500 g
32035	Fluka	HYDRANAL-Benzoic acid	500 g
34724	Fluka	HYDRANAL-Formamide dry	1 L

Table Selected HYDRANAL Karl Fischer reagents

However, other substances like riboflavin phosphate sodium (biochemical cofactor; also used as food dye) prove insoluble in the alcohol-based KF media. As a suitable solubiliser for this compound, HYDRANAL®-Formamide is recommended; it can be added to the working medium (volumetric KF technique) in a ratio of 1:1 (application L495).

Laboratory Applications

The application reports stated in this article can be obtained from our HYDRANAL technical service teams; please contact our HYDRANAL-Laboratories. Also, visit our website sigma-aldrich.com/hydranal for more application reports, complete product listings and more information on Karl Fischer titration.

Take advantage of our expertise gained from over twenty-five years' experience and our extensive applications database on Karl Fischer titration. For any questions, help or feedback, please visit our website or contact our HYDRANAL specialists:

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Brand	Cat. No.	Description
FLUKA	35245	Sodium thiosulphate solution, Reag. Ph.Eur., 0.1 M
FLUKA	35418	Perchloric acid solution, 0.1 M in acetic acid
FLUKA	35328	Hydrochloric acid solution, Reag. Ph.Eur., 1 M
FLUKA	35329	Hydrochloric acid solution, Reag. Ph.Eur., 0.5 M
FLUKA	35335	Hydrochloric acid solution, Reag. Ph.Eur., 0.1 M
FLUKA	35354	Sulphuric acid solution, Reag. Ph.Eur., 0.5 M
FLUKA	35256	Sodium hydroxide solution, Reag. Ph.Eur., 1 M
FLUKA	35257	Sodium hydroxide solution, 0.5 M
FLUKA	35263	Sodium hydroxide solution, Reag. Ph.Eur., 0.1 M
FLUKA	35115	Potassium hydroxide solution, 0.5 M denat. Ethanol with Toluene
FLUKA	35127	Potassium hydroxide solution, 0.1 M denat. Ethanol with Toluene

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New Alkylphenol Standards for Water Analysis

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



In Analytix 5/2008 we reported on an inter-laboratory proficiency testing trial of the analysis of alkylphenols and its ethoxylates in surface and waste water according to ISO/CD 18857-2. In order to complement our portfolio of alkylphenol standards, we recently added

different Nonylphenol (the technical mixture of isotopes) and 4-tert-Octylphenol standards, and the corresponding mono- and diethoxylates, to the OEKANAL® product line.

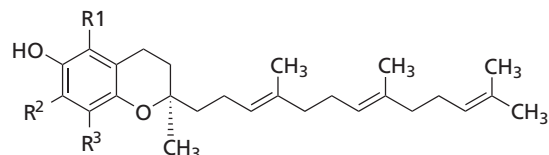
Brand	Description	Conc.	Part No.	Pack Size	Conc.	Part No.	Pack Size
Fluka	Nonylphenol solution in Acetone	5 µg/mL	32889	10 mL	50 µg/mL	32888	1 mL
Fluka	Nonylphenol-monoethoxylate solution in Acetone	5 µg/mL	32895	10 mL	50 µg/mL	32894	1 mL
Fluka	Nonylphenol-diethoxylate solution in Acetone	5 µg/mL	32899	10 mL	50 µg/mL	32898	1 mL
Fluka	4-tert.-Octylphenol solution in Acetone	1 µg/mL	32881	10 mL	10 µg/mL	32879	1 mL
Fluka	4-tert.-Octylphenol-monoethoxylate solution in Acetone	1 µg/mL	32883	10 mL			
Fluka	4-tert.-Octylphenol-diethoxylate solution in Acetone	1 µg/mL	32887	10 mL			

Tocotrienol Standards

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

Tocotrienols are natural compounds that form, together with the tocopherols, the vitamin E family. The tocotrienols differ from the tocopherols by the three double bonds in the farnesyl tail.

They have applications in the cosmetics as well as in the food industry due to their properties as antioxidants.



The four recently introduced tocotrienol standards have purities of at least 97 % and are all isolated from natural sources (plant material).

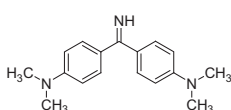
Part No.	Description	Brand	Package Size	R1	R2	R3
07205	α-Tocotrienol	Fluka	100 mg	Methyl	Methyl	Methyl
05644	β-Tocotrienol	Fluka	100 mg	Methyl	H	Methyl
49634	γ-Tocotrienol	Fluka	100 mg	H	Methyl	Methyl
69745	δ-Tocotrienol	Fluka	100 mg	H	H	Methyl

New Standards for Illegal Food Dyes

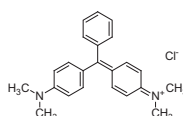
Matthias Nold, Product Manager Analytical Standards matthias.nold@sial.com

In issues 04/2008 and 01/2009 of Analytix, we presented a series of analytical standards for dyes which, although banned worldwide for use as food additives, are still sometimes used for this purpose. We have recently added some further illicit food dyes to complement this portfolio.

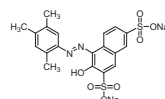
Part No.	Description	Brand	Pack Size
51362	Auramin	Fluka	25 mg
38800	Malachite Green Chloride	Fluka	25 mg
49904	Ponceau 3R	Fluka	25 mg
79285	Oil Orange SS	Fluka	25 mg



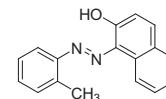
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Ponceau 3R 49904



Oil Orange SS 79285

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