

Analytix

Issue 3 • 2009



Sigma-Aldrich® Colour Chart

Sigma-Aldrich Color Chart



- Bee-toxic Pesticide Standards
- Microbiological Food Control
- High-purity Carboxylic Acids for Trace Analysis
- pH Buffers for Karl Fischer Titration
- Titer & Temperature in Volumetric Titration

Sigma-Aldrich® Colour Chart



Harald Agrinz
Supervisor, Quality Control

Dear Colleague,

What is the first thing you see when you open a container or bottle from Sigma-Aldrich? It's the appearance of the product, its form and colour – and we know how important first impressions can be.

As we all know too, colour is usually a very subjective impression. If you have ever had to choose the colour of your new curtains and discuss it with the people in your household, then you'll know what I am talking about. But you are not alone with this problem: for hundreds of years, artists, scientists and technicians have been trying to describe colours and find a common language for the communication of colours.

In the Feature Article of this Analytix about the Sigma-Aldrich Colour Chart, we would like to share our thoughts with you about this important topic.

Last but not least, we want to give our customers a tool so they can quickly and easily check this specification in the same way we do in our corporation: the Sigma-Aldrich Colour Chart.

With kind regards,

A handwritten signature in black ink that reads "Harald Agrinz". The signature is fluid and cursive, with the first letters of the first and last names being capitalized and prominent.

Harald Agrinz
Supervisor, Quality Control

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The Sigma-Aldrich® Colour Chart

Harald Agrinz, Supervisor Quality Control harald.agrinz@sial.com



“What colour is your product?”

“It’s brown. Well, not really brown, but sort of light brown.”

“You mean brownish-yellow? Like mustard?”

“Yes, but not that dark brownish-yellow, more olive, with a yellow tint ...”

Welcome to the wonderful (and sometimes confusing) world of colours.

What is colour?

A short question; but the answer would be very comprehensive. Isaac Newton, Johann Wolfgang von Goethe, Thomas Young, Hermann von Helmholtz and Ewald Hering are only a few names of people who have dealt with this topic. Already in ancient Greece, philosophers developed theories to show how colour perception is formed in the human brain via the eye, and by which parameters it is affected. It has to be mentioned, that not only the naming of colours has changed in the course of time: there are marked differences in the naming of colours in different languages and cultural areas, which makes global colour communication more difficult. Books on the subject could fill multiple libraries. So let’s try a more practical approach to this issue.

Again, what is colour?

Colour is an impression which an observer receives. This happens by the stimulation of photoreceptors in the human eye.

Why does an object appear coloured?

Light hits an object. Some wavelengths are absorbed, others are reflected by the object. The reflected portion of the light wavelengths reaches the human eye and is perceived as “colour”.

Today, as a result of past efforts, a multiplicity of descriptions exist for the naming, definition and characterisation of colour: colour tables for printer colours, colours for painting, computer screen colour tables ... some colour systems are compatible with each other, others are not.

It makes a difference too, if colours result from image-forming radiation (e.g. a computer monitor) or by reason of the reflection of light by a coloured surface. In the latter case, it makes a difference what type of light is reflected (natural daylight, light from fluorescent tubes ...), and even the strength of the light is an important factor.

As a worldwide operating manufacturer of chemicals, Sigma-Aldrich is affected by the question of “colour”, too. One of our most important specifications for

a product is its appearance, which means its form and colour. This specification led to many misunderstandings and enquiries of our external and internal customers. In a tremendous effort we are currently harmonising our systems, specifications and definitions worldwide in our corporation; one important part of that is the topic "colour".

What is necessary, to see colours?

Three things: a light source, a sample to look at and a detector.

- **The light source:** We use natural daylight or an artificial daylight lamp with defined colour temperature.
- **The sample:** It is important to have enough of the sample; too small quantities lead to a colour adaptation from the ambience.
- **The detector:** The human eye. Our employees who perform visual colour tests are checked for colour vision (no colour blindness) to ensure correct analytical results.

How should a colour testing system of a worldwide operating manufacturer of chemicals be designed?

Considering our huge portfolio of different chemical products, the common colour system should not only bring clarity in the jungle of colours but also be simple to apply in daily practice. Furthermore, it should be

based upon an accepted colour system; communicable, reproducible and applicable everywhere.

The choice of the colour system

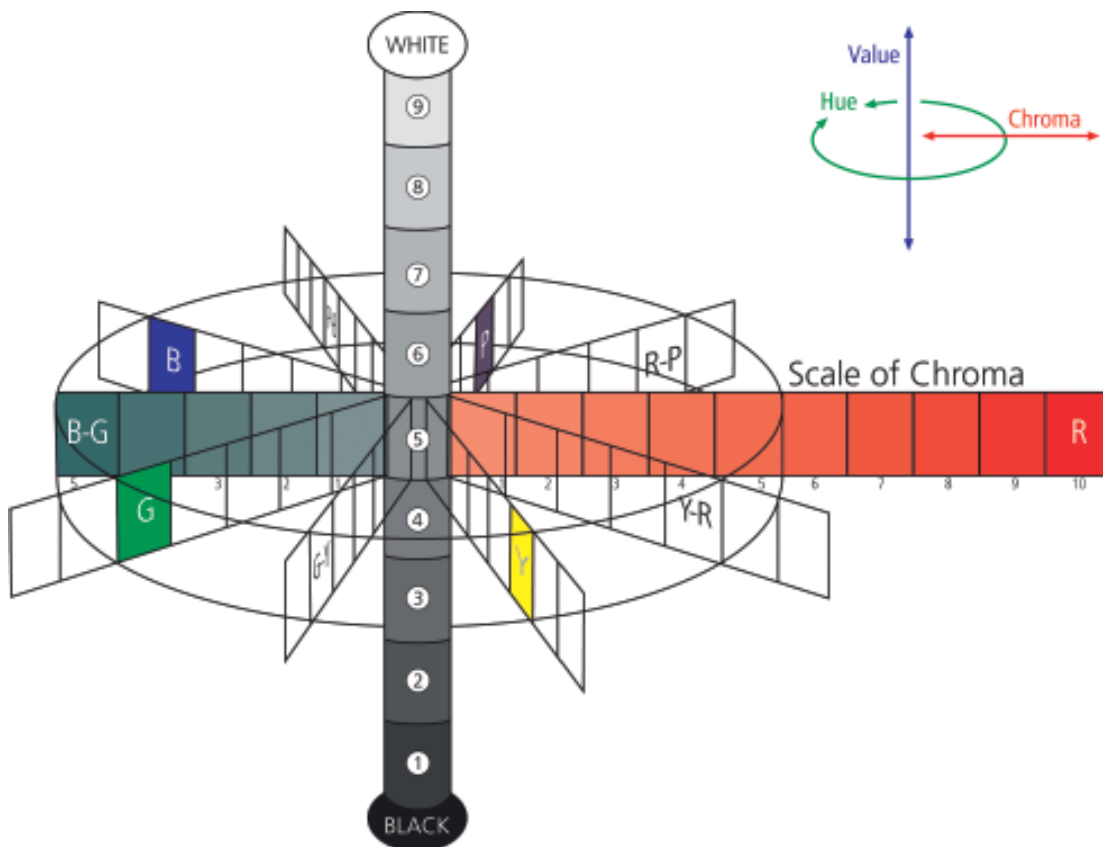
Many colour order systems use a quasi-spherical colour space, in which the position of each colour is described with three coordinates. We choose the Munsell colour order system, which fulfilled all our needs. Each colour is identified by the attributes of hue, value and chroma.

The three colour coordinates

Hue: Distinguishes red from green etc.: An attribute of colour perception. There are five principal hues: red, yellow, green, blue and purple. There are five intermediate hues: yellow-red, green-yellow, blue-green, purple-blue and red-blue. Black, white and grey are neutral colours with no hue.

Value: Indicates lightness on a scale from 0 (black) to 10 (white). This is the quality that distinguishes lighter colours from darker ones.

Chroma: Indicates the distance from a grey of the same value; correlates with the intensity of the colour. This is the quality that describes the vividness of a colour. It can also be described as the degree of difference from the neutral colour of the same "value".



Source: X-Rite Europe GmbH, www.xrite.com

(continued on page 6)

Munsell book of colour

The Munsell Book of Colour, based on the colour order system developed by Albert H. Munsell, contains thousands of colour chips which are defined according to the Munsell colour system. This large number of colours is much too big to handle in everyday practice. By practical tests we identified the colours most frequently occurring in our products; which colours often cause problems in colour communication; and which colours we frequently have to use paraphrases for when we want to explain them.



The result is a selection of colours from the Munsell colour order system; this selection contains 51 colour chips. These chips are applied to a white background on one page. No turning of pages is necessary, which facilitates and speeds up the daily use of the colour chart. Below the colour chips you find the short code plus the internally defined colour name. On the other page there is a table where you can see the hue, value and chroma for each of the colours according to the Munsell system.

The names

The hue/value/chroma notation describes an exact position of the colour in the Munsell colour space; but in practice you would have problems imagining the colour of a product just having these three coordinates. So we gave names to the colours in our chart, which, though subjective, was necessary to give us and our customers an impression of the actual colour. We avoided imprecise descriptions such as those you find everywhere nowadays: grass-green, sea-blue, desert-brown etc. are not useful for effective colour communication.

The Sigma-Aldrich® Colour Chart: a new tool for the colour determination of solid chemicals

The appearance of the product, the form and colour, is the first thing our customers see and check when they open a container. Usually, they don't use complicated measuring methods; a simple glance is enough to find out that a product has the wrong colour.

With the Sigma-Aldrich Colour Chart we now give our customers a fast and uncomplicated tool to check this important specification and the possibility to do this the same way we do it in our whole corporation.

The Sigma-Aldrich Colour Chart is available under the Fluka brand with product number 91711.

And now I come back to the question asked at the beginning of this article:

„What colour is your product?“

“It's BE8 (light beige) according to the Sigma-Aldrich Colour Chart!”

Easy, isn't it?

New Analytical Standards of Illicit Drugs

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



The availability of reliable analytical standards is crucial for the analysis of illegal drug substances in forensic and clinical laboratories.

Sigma-Aldrich® has an extensive portfolio of DEA drug standards to meet this need. However, the export of these standards outside the USA can be problematic due to US regulatory issues, resulting in high shipping fees and taxes as well as long lead times. To address

Amphetamine and its derivative DL-N-Ethylamphetamine. All these substances have been known for a century or even longer, and are still among the most widespread drugs of abuse.

We offer these five substances as analytical standards in the form of hydrochloride salt and, in the case of morphine, sulphate salt. In addition to neat standards, they are also available as solutions in methanol (except

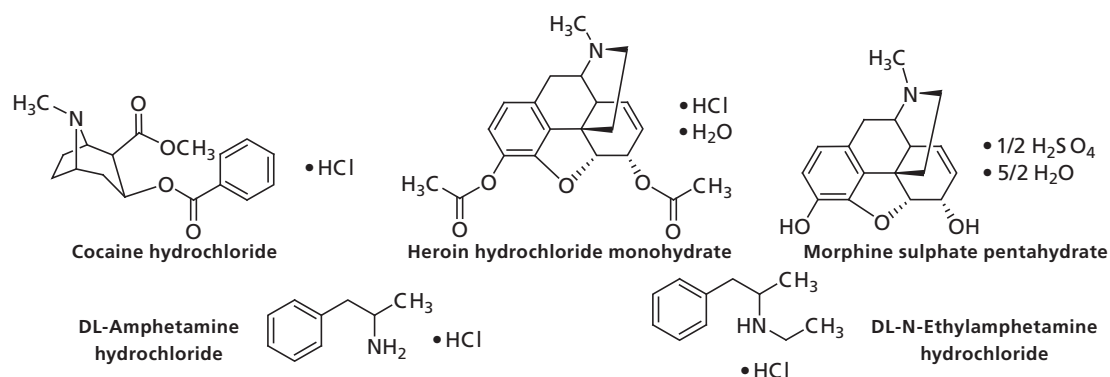


Figure 1 Molecular structures of the new drug standards

this problem, we introduced a series of European sourced drug standards under the Fluka brand. These standards are produced in Switzerland and can be exported into all EU countries without these restrictions.

The latest Sigma-Aldrich European product additions include the illicit drugs cocaine, heroin, morphine, DL-

for heroin, which is dissolved in acetonitrile). The ampoules contain 1.25 mL of a 1 mg/mL solution, allowing the withdrawal of exactly one mL of the solution.

For our complete portfolio of drug standards, please visit our website at sigma-aldrich.com/standards

Cat. No.	Brand	Description	Pack Size
19330	Fluka	Cocaine hydrochloride, solution in methanol	1 mL
51621	Fluka	Cocaine hydrochloride	10 mg
44508	Fluka	Heroin hydrochloride, solution in acetonitrile	1 mL
67357	Fluka	Heroin hydrochloride monohydrate	10 mg
94775	Fluka	Morphine sulphate solution, solution in methanol	1 mL
44179	Fluka	Morphine sulphate pentahydrate	10 mg
50772	Fluka	DL-Amphetamine hydrochloride, solution in methanol	1 mL
94777	Fluka	DL-Amphetamine hydrochloride	10 mg
40354	Fluka	DL-N-Ethylamphetamine hydrochloride, solution in methanol	1 mL
40916	Fluka	DL-N-Ethylamphetamine hydrochloride	10 mg

Table 1 New analytical standards of illicit drugs

Cat. No.	Brand	Description	Pack Size
C1528	Fluka	Cocaine hydrochloride, solution in methanol	1 mL
C5776	Sigma	Cocaine hydrochloride	5 g
H5144	Fluka	Heroin hydrochloride, solution in methanol (100 µg/mL)	1 mL
H159	Fluka	Heroin	25 mg
M9524	Fluka	Morphine sulphate solution, solution in methanol	1 mL
M8777	Fluka	Morphine sulphate pentahydrate	25 mg, 50 mg, 250 mg
610240	Fluka	DL-Amphetamine, solution in methanol	1 mL
A5880	Fluka	D-Amphetamine hemisulphate	1 g, 5 g

Table 2 Corresponding drug standards for the US market

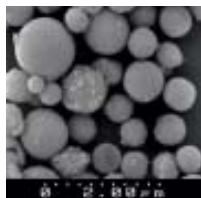
Certified Viscosity and Particle Size Standards

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

Sigma-Aldrich® offers a wide range of physical properties standards for various applications in the chemical, food and material science industries. These include standards for density, melting point, conductivity, molecular weight (MS markers), pH calibration, Redox, turbidimetry and X-Ray as well as colour reference standards (see page 4 to 6). To get an overview of the physical property standards available from Sigma-Aldrich, please visit our website at: sigma-aldrich.com/physicalproperties

Recently, new certified reference materials for particle size, as well as for viscosity, were added to our portfolio. These reference materials are manufactured and certified in the United Kingdom by internationally recognised specialists at Whitehouse Scientific Ltd. and Paragon Scientific Ltd. respectively.

Polydisperse Particle Size Standards and Sieve Standards from Whitehouse Scientific



Certified Polydisperse Particle Size Standards

These NIST and NPL traceable standards were commissioned by the Bureau of Certified References (BCR) and are measured by a large international team including the 20 top laboratories in the field of particle size. For the certification of the glass particles, several unambiguous primary methods such as microscopy, sieving, sedimentation and Coulter counter were used.

The standards are delivered in sets of 10 vials in quantities suitable for any method of analysis without further subdivision.

Cat. No.	Brand	Product Name	Pack Size
51358	Fluka	Polydisperse Particle Standard 0.1–1 µm	10 x 0.02 g
42459	Fluka	Polydisperse Particle Standard 1–10 µm	10 x 0.1 g
05724	Fluka	Polydisperse Particle Standard 3–30 µm	10 x 0.05 g
94078	Fluka	Polydisperse Particle Standard 3–30 µm	10 x 0.1 g
80847	Fluka	Polydisperse Particle Standard 10–100 µm	10 x 0.25 g
40579	Fluka	Polydisperse Particle Standard 10–100 µm	10 x 0.5 g
08718	Fluka	Polydisperse Particle Standard 10–100 µm	10 x 1 g
57563	Fluka	Polydisperse Particle Standard 50–350 µm	10 x 0.5 g
78456	Fluka	Polydisperse Particle Standard 50–350 µm	10 x 2.5 g
94773	Fluka	Polydisperse Particle Standard 150–650 µm	10 x 2.5g

Table 1 Polydisperse Particle Size Standards from Whitehouse Scientific

Certified Sieve Calibration Standards

The Sieve Calibration Standards from Whitehouse Scientific offer a unique method of calibrating sieves with traceability to NIST and NPL. The glass microspheres are delivered in sets of single shot bottles in order to eliminate operator-sampling errors.

The calibration requires only about two minutes, following a simple procedure: the glass microspheres are placed into the sieve, which is shaken either manually or mechanically, until an equilibrium has been reached and no more particles pass through the sieve.

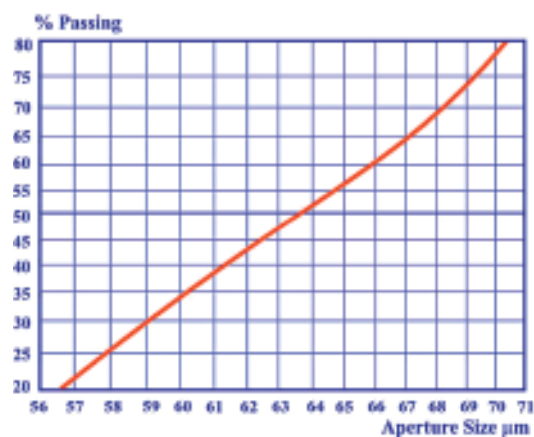


Figure 1 Calibration graph for the 63 µm sieve standard

By weighing the sieve and the single shot bottle before and after the calibration, the percentage of microspheres passing through the sieve can be calculated and the mean aperture size of the sieve can thus be determined using the calibration graph supplied with the test certificate. **Figure 1** shows an example of a calibration graph for the 63 µm sieve standard.

Cat. No.	Brand	Product Name	Mesh #	Calibration Range	Pack Size
12655	Fluka	Sieve Standard 45 µm	325	42.0 – 50.8	5 x 1.0 g
49500	Fluka	Sieve Standard 63 µm	230	56.6 – 70.4	5 x 1.0 g
42696	Fluka	Sieve Standard 75 µm	200	67.1 – 82.8	5 x 1.0 g
61847	Fluka	Sieve Standard 106 µm	140	91.4 – 117	5 x 1.0 g
18867	Fluka	Sieve Standard 125 µm	120	112 – 139	5 x 1.0 g
75446	Fluka	Sieve Standard 150 µm	100	134 – 167	5 x 1.5 g
43390	Fluka	Sieve Standard 180 µm	80	161 – 199	5 x 1.5 g
41544	Fluka	Sieve Standard 250 µm	60	226 – 281	5 x 2.5 g
67246	Fluka	Sieve Standard 300 µm	50	270 – 333	5 x 2.5 g
78900	Fluka	Sieve Standard 500 µm	35	440 – 557	5 x 2.5 g

Table 2 Sieve Calibration Standards from Whitehouse Scientific

Viscosity Standards from Paragon Scientific



Accurate viscosity measurements play an important role in various fields of industry, such as food and beverages, paint and coatings and the pharmaceutical and petroleum industries. For the calibration and verification

of viscosity measuring equipment, analytical standards with known viscosity are needed.

United Kingdom in an ISO 17025 certified laboratory. They are manufactured in strict accordance with ASTM D 2162 (Standard Practice for basic calibration of master viscosity standard oils) and are traceable to NIST.

In the certificate, the viscosity values and density are stated at various temperatures as well as the expiry date and uncertainties. The viscosity values are given as kinematic viscosity (mm²/s (cSt)) and as dynamic viscosity (mPa*s (cP)) for all temperatures.

We are pleased to present 26 viscosity standards from Paragon Scientific, which have been recently added to the Fluka portfolio. These standards are produced in the

The table below shows the Certified Viscosity and Density standards now available at Sigma-Aldrich®.

Cat. No.	Brand	Product Name	Kinematic Viscosity (mm ² /s (cSt)) at 25 °C	Pack Size
01446	Fluka	Viscosity and Density Standard N 0.4	0.4583	500 mL
67348	Fluka	Viscosity and Density Standard N 0.8	0.6205	500 mL
19044	Fluka	Viscosity and Density Standard N 1.0	1.189	500 mL
93835	Fluka	Viscosity and Density Standard N2	2.216	500 mL
41859	Fluka	Viscosity and Density Standard S 3	4.014	500 mL
49571	Fluka	Viscosity and Density Standard D5	6.086	500 mL
05867	Fluka	Viscosity and Density Standard S6	8.792	500 mL
05854	Fluka	Viscosity and Density Standard D10	12.42	500 mL
63484	Fluka	Viscosity and Density Standard N10	17.01	500 mL
05428	Fluka	Viscosity and Density Standard S20	34.11	500 mL
18964	Fluka	Viscosity and Density Standard N35	65.07	500 mL
05397	Fluka	Viscosity and Density Standard S60	118.7	500 mL
05395	Fluka	Viscosity and Density Standard N100	238.3	500 mL
01437	Fluka	Viscosity and Density Standard S200	456.2	500 mL
69443	Fluka	Viscosity and Density Standard D500	578.1	500 mL
04870	Fluka	Viscosity and Density Standard N350	851.2	500 mL
08670	Fluka	Viscosity and Density Standard D1000	1159	500 mL
08577	Fluka	Viscosity and Density Standard S600	1460	500 mL
19495	Fluka	Viscosity and Density Standard N1000	2981	500 mL
38177	Fluka	Viscosity and Density Standard S2000	5267	500 mL
73284	Fluka	Viscosity and Density Standard D5000	5644	500 mL
11492	Fluka	Viscosity and Density Standard D7500	8702	500 mL
09216	Fluka	Viscosity and Density Standard N4000	11627	500 mL
50989	Fluka	Viscosity and Density Standard S8000	22553	500 mL
13219	Fluka	Viscosity and Density Standard N15000	44855	500 mL
41272	Fluka	Viscosity and Density Standard S30000	79747	500 mL

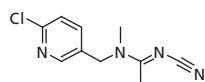
Table 3 Certified Viscosity and Density standards from Paragon

Neonicotinoids Pesticides and Metabolites

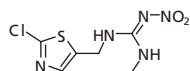
Ingrid Hayenga, Senior Chemist R&D Europe ingrid.hayenga@sial.com



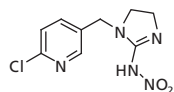
Active neonicotinoide



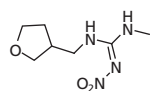
Acetamiprid



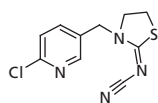
Clothianidin



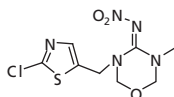
Imidacloprid



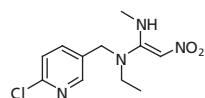
Dinotefuran



Thiacloprid



Thiamethoxam



Nitenpyram

Figure 1
Neonicotinoids

In spring 2008, a mass death of bees in Germany's Baden-Württemberg state was reported. The German Federal Office of Consumer Protection and Food Safety (BVL) acted promptly, ordering the immediate suspension of the approval for eight seed treatment products used in oilseed rape (canola) and sweetcorn, which contained the following neonicotinoide pesticides: imidacloprid, clothianidin, thiamethoxam and methiocarb. According to the German Federal Research Centre for Cultivated Plants, 29 out of the 30 dead bees it had examined had been killed by contact with clothianidin. This was tied to a failure to apply a "glue" agent that affixes the compound to the coats of seeds. The manufacturer maintains that without the fixative agent, the compound drifted into the environment from sown rapeseed and sweetcorn, thus affecting the honeybees.

Mode of action

Neonicotinoids are fairly new chemicals, but they have established themselves as key components in insecticides because of their unique selectivity. The mode of action of neonicotinoids is similar to the natural insecticide nicotine. They selectively bind and interact with the insect nicotinic acetylcholine receptor site. When neonicotinoids bind to the binding site of an insect, their electronegative tip, consisting of a nitro or cyano group, interacts with a unique cationic subsite of the insect's receptor. On the other hand, the action of protonated neonicotinoids requires a cationic interaction for binding to a mammal receptor. In insects, neonicotinoids cause paralysis which leads to death, often within a few hours; however, they are much less toxic to mammals, and under the WHO/EPA classification these compounds are placed toxicity class II or class III. Because the neonicotinoids block a specific neural pathway that is more abundant in insects than in warm-blooded animals, these insecticides are selectively more toxic to insects than mammals. This target site selectivity is a major factor in the favorable toxicological properties of neonicotinoids.

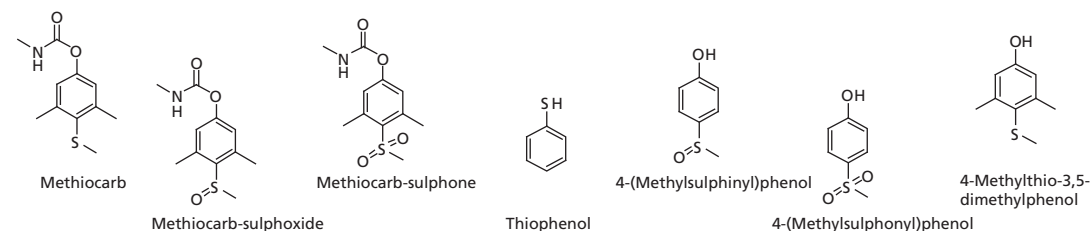


Figure 2 Structure of methiocarb and its main metabolites

Metabolism

Methiocarb

Methiocarb does not belong to the group of neonicotinoide but is one of the ingredients of the banned seed treatment products. It is mainly used as an acaricide, as seed treatment for control of fruit flies on maize, flea beetles on oilseed rape, a molluscicide and a bird repellent. It is a potent neurotoxin carbamate and acts as a cholinesterase inhibitor. The main metabolism pathways are cleaving of the carbonyl group and oxidation of the sulphur atom. If released to soil or water, methiocarb hydrolyses quite rapidly to 4-methylthio-3,5-dimethylphenol and methylcarbamic acid. The oxidation leads to methiocarb sulphone or methiocarb sulphoxide.

Imidacloprid

Because of its low toxicity to most animals, other than insects due to its specificity for this type of nicotinic acetylcholine receptor, imidacloprid allows for lower concentrations to be used for insect control than other neurotoxins (particularly organophosphates). Used as a systemic insecticide, it is taken up by plant roots and diffuses in the plant via the xylem; its systemic properties then rely on insects ingesting the insecticide.

In the body the main metabolites are 6-chloronicotinic acid, 6-Hydroxynicotinic acid and 6-Methylmercaptopyridinic acid.

Clothianidin

Clothianidin is one of the newest neonicotinoids and a systemic insecticide. It is mainly used as a seed treatment. The main metabolites are 1-Methyl-3-nitroguanidine (MNG), nitroguanidine (NTG), N-(2-chlorothiazol-5-ylmethyl)-N-nitroguanidine (TZNG), N-(2-chlorothiazol-5-ylmethyl)-N-methylurea (TZMU).

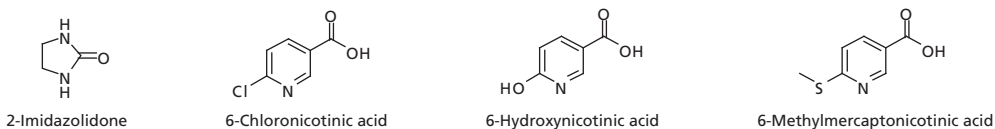


Figure 3 Main metabolites of imidacloprid

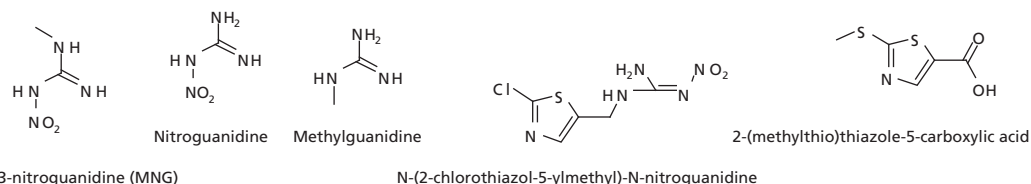
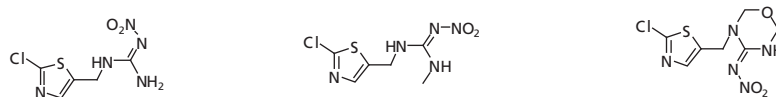


Figure 4 Main nonvolatile metabolites of clothianidin

Thiamethoxam

Thiamethoxam belongs to the new class of thianicotinyl compounds of the neonicotinoids (2nd generation). They possess a chlorothiazole heterocycle. Its mode of action in the plant is similar to the rest of this substance class. Its main metabolites are listed below.

Sigma-Aldrich® is glad to offer our customers diverse deuterated and non-deuterated neonicotinoids in standard quality as well as several main metabolites.



N-(2-chlorothiazol-5-ylmethyl)-N-nitroguanidine (CGA 265307)

Clothianidin (CGA 322704)

3-(2-Chloro-1,3-thiazol-5-ylmethyl)-1,3,5-oxadiazinan-4-ylidene(nitro)amine (CGA 330050, plasma metabolite)

Figure 5 Major metabolites of thiamethoxam

Cat. No.	Brand	Description	Purity	Pack Size
33674	Fluka	Acetamiprid		100 mg
39246	Fluka	d ₃ -Acetamiprid		50 mg
33589	Fluka	Clothianidin		100 mg
56816	Fluka	d ₃ -Clothianidin		50 mg
37894	Fluka	Imidacloprid		100 mg
46341	Fluka	Imidacloprid solution (100 ng/l in acetonitrile)		2 ml, 10 ml
34170	Fluka	d ₄ -Imidacloprid		10 mg
37905	Fluka	Thiacloprid		100 mg
33897	Fluka	Thiacloprid-amide		100 mg
37924	Fluka	Thiamethoxam		100 mg
38176	Fluka	d ₄ -Thiamethoxam		50 mg
46077	Fluka	Nitenpyram		100 mg
36152	Fluka	Methiocarb		100 mg
38135	Fluka	d ₃ -Methiocarb	> 97 %	50 mg
MET543A	Supelco	Methiocarb sulphone		50 mg
34177	Fluka	Methiocarb sulphoxide		100 mg
09286	Fluka	Thiophenol	> 99.5 %	5 ml, 25 ml
73351	Fluka	4-(Methylsulphonyl)phenol		50 mg
42221	Fluka	4-(Methylsulphonyl)phenol		50 mg
31534	Fluka	2-Imidazolidone		250 mg
68678	Fluka	6-Chloronicotinic acid	> 98 %	100 mg
19386	Fluka	6-Hydroxynicotinic acid	> 98 %	100 mg
69646	Fluka	6-Methylmercaptonicotinic acid	> 98 %	100 mg
342122	Aldrich	1-Methyl-3-nitroguanidine	contains ~25 % water	25 g, 100 g
N17351	Aldrich	Nitroguanidine	contains 25 % water	100 g, 500 g
222402	Aldrich	Methylguanidine hydrochloride	98 %	5 g
89404	Fluka	N-(2-chlorothiazol-5-ylmethyl)-N-nitroguanidine		50 mg
16947	Fluka	2-(methylthio)thiazole-5-carboxylic acid		50 mg
39013	Fluka	4-Methylthio-3,5-dimethylphenol		50 mg
73348	Fluka	3-(2-Chloro-1,3-thiazol-5-ylmethyl)-1,3,5-oxadiazinan-4-ylidene(nitro)amine		50 mg

Table 1 Neonicotinoids and their metabolites

New HybriScan® Kits – Microbiological Rapid Test Systems

Detection of *Campylobacter spp.* and *Enterobacter sakazakii* in food with a new test kit system

Manuela Fabienke, Scanbec GmbH; Jvo Siegrist, Product Manager Microbiology, Sigma-Aldrich ivo.siegrist@sial.com

Background

Development of novel methods for a rapid, sensitive and reliable detection and quantification of microorganisms and pathogens in food, beverages and water is receiving increasing attention. The sandwich hybridisation method used in the HybriScan® Test System is a suitable alternative for a sensitive and reliable detection and identification of microorganisms.

The HybriScan method is nearly independent of influences of sample matrices, and is able to distinguish between live and dead cells. Furthermore, the detection of non-culturable microbes is possible.

The HybriScan method is based on the detection of hybridisation events between two specific oligonucleotide probes and target nucleic acids. The capture probe is used to immobilise the target sequence on a solid support and the detection probe is labeled with a detectable marker (**Figure 1**). Sandwich hybridisation is relatively sensitive and can be performed with crude biological samples [1]. Sandwich hybridisation assays from crude cell samples or in connection to PCR have been extensively used in clinical diagnostics for detection of nucleic acids from bacteria [2, 3, 4] and viruses [5]. The sandwich hybridisation method is ideal for identification of specific rRNAs in bacterial cells and yeasts. The sensitivity of this RNA-based assay benefits from the typically high number of ribosomes in each cell. Compared to only a few copies of genomic DNA a single cell contains several thousand copies of rRNA. Although a direct detection of the ribosomal RNA does not match the sensitivity of a PCR-based DNA assay, it offers advantages like quantification, live/dead-discrimination, no additional amplification steps and simple assay protocols with standard laboratory equipment.

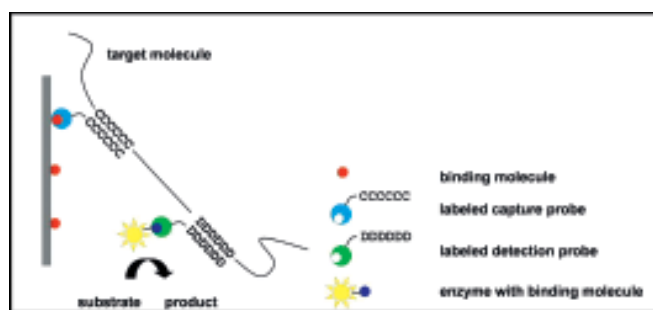


Figure 1 Principle of the HybriScan sandwich hybridisation assay

HybriScanD *Campylobacter*:

Detection of *Campylobacter spp.* in food

Bacteria of the species *Campylobacter* are zoonotic pathogens which annually infect 1 % of Western Europe's population. Some *Campylobacter* species are known to infect animals, especially infections of the reproductive tract.

Campylobacter are ubiquitous and often found in domestic animals. In this way, they are present frequently in the environment and on many raw foods of plant and animal origin. A very high concentration of *Campylobacter* can be found on raw poultry meat [6].

According to authorities in Germany, human *Campylobacteriosis* is the most common reported disease caused by bacteria besides Salmonellosis. In 2004, around 55,000 and in 2005, 60,000 *Campylobacter* infections were observed [7].

Therefore a rapid and reliable detection of *Campylobacter* is required to ensure microbiological safety and quality. In contrast to HybriScan, classical cultural methods for the detection of *Campylobacter spp.* are time consuming and well-trained laboratory personnel is required for each type of bacteria.

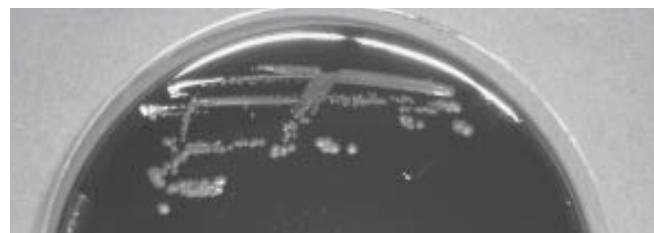


Figure 2 *Campylobacter jejunii* colonies grown on CCDA agar

HybriScanD *Campylobacter* is a rapid molecular test system for the detection of bacteria of the genus *Campylobacter* in different food matrices, including the detection of the most relevant species *C. jejunii* (**Figure 2**), *C. coli*, *C. lari* and *C. upsaliensis*.

HybriScanD *Campylobacter* enables a reliable and comprehensive control of suspicious results in the context of classical microbial diagnostics but makes detection more rapid, with results available after 48 hours. An overview of the validation results of HybriScan-*Campylobacter spp.* are presented in **Figure 3**. 108 food samples were analysed with HybriScanD *Campylobacter* and compared with a cultivation-based method according to § 64 LFGB. In total, five different food categories were tested. The results of the validation lead to a relative accuracy of 95.2 %, a relative specificity of 97.5 %, and relative sensitivity of 93 %, respectively.

Brand	Cat. No.	Name	Assays
Fluka	56917	HybriScanD <i>Campylobacter</i>	96

Table 1 Order information

HybriScanD *Enterobacter sakazakii*:

Detection of *Enterobacter sakazakii* in food

Enterobacter sakazakii is ubiquitous and frequently found on vegetables, meat and dairy products, and especially in baby food. Consumption of contaminated powdered infant formula milk (IFM) can result in life-threatening neonatal infections caused by the

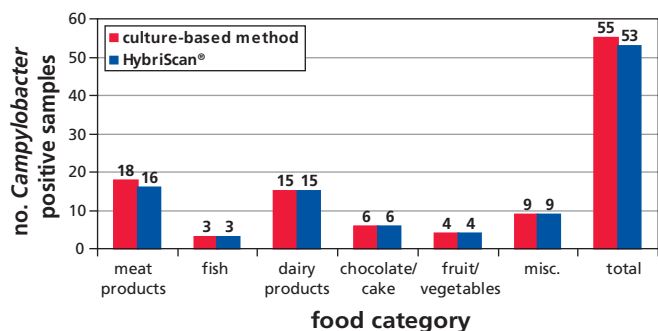


Figure 3 Overview of the validation results of HybriScan®D *Campylobacter*. 108 food samples were analysed with HybriScan®D *Campylobacter* and compared to a cultivation-based method according to § 64 LFGB. Numbers on the bars represent the number of analysed food samples in each food category. The validation was performed according to ISO 16140:2003 (ASU L00.00-22).

pathogen. Taxonomic studies have determined that *E. sakazakii* comprises a high genetic heterogeneity and should be reclassified as a novel genus, “Cronobacter” [8] (**Figure 4**).



Figure 4 *Enterobacter sakazakii* colonies grown on M1 agar

Accurate methods are required for the rapid detection and identification of *Enterobacter sakazakii*, since even low cell numbers have been reported to cause a disease. HybriScan®D *E. sakazakii* is a rapid molecular test system for detection of bacteria of the genus *E. sakazakii* in food, especially in dried infant formula milk and its production environment. **Figure 5** shows the specificity of HybriScan®D *E. sakazakii*. Different cell amounts and related *Enterobacteriaceae* were tested within a validation study. No signals were observed using 2.3×10^8 *Enterobacter cloacae* cells or 7×10^8 *Citrobacter freundii* cells per assay, whereas clear specific signals were detectable using 2.6×10^3 , 1.3×10^4 , and 2.6×10^4 cells of *E. sakazakii*, respectively. These results demonstrate that the HybriScan system is highly specific for *Enterobacter sakazakii*.

A validation study of HybriScan®D *E. sakazakii* was performed using two different enrichment procedures: (1) single-step enrichment for 24–26 hours at 37 °C in ESSB broth (AES Chemunex) and (2) two-step enrichment starting with a pre-enrichment for 18–20 hours at 37 °C in buffered peptone water and followed by a selective enrichment for 24–26 hours at 45 °C in mLST selective broth. The results of the above-mentioned validation study are presented in **Table 2**.

	Total Number of Samples		Number of Positive Samples	
	n	ISO/TS 22964	HybriScan	
Single-step enrichment	31	26	25	
Two-step enrichment	99	71	70	

Table 2 Results of a validation study of HybriScan®D *E. sakazakii*

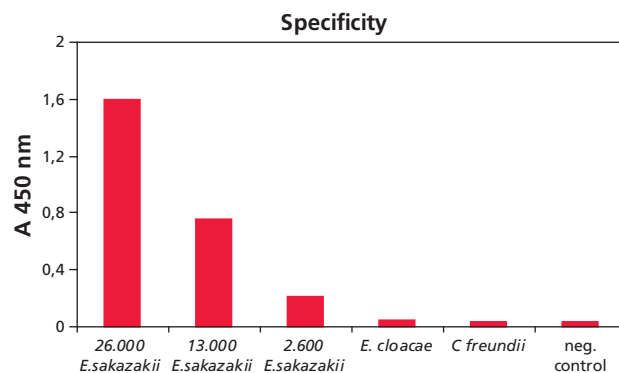


Figure 5 Specificity of HybriScan®D *E. sakazakii*. Different cell numbers of *E. sakazakii* and related *Enterobacteriaceae* like *E. cloacae* and *Citrobacter freundii* were tested. Measurement data for HybriScan analyses represent absorbance at 450 nm.

130 samples of powdered infant formula milk were analysed with HybriScan®D *E. sakazakii* and compared to two cultivation-based methods according to ISO/PFR TS 22964. In total, six products from different manufacturers were tested. Results of the validation study lead to a relative accuracy of 95 %, a relative specificity of 92.9 % and relative sensitivity of 95.7 %, respectively.

Brand	Cat. No.	Name	Assays
Fluka	12838	HybriScan®D <i>Enterobacter sakazakii</i>	96

Table 3 Order information

HybriScan®D *E. sakazakii* enables a reliable and comprehensive control of suspicious products in the context of classical diagnostic but makes detection more rapid with results available after 48 hours.

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New High-purity Carboxylic Acids for Trace-level Determinations

Sigma-Aldrich® offers new carboxylic acids used for trace analysis techniques such as ion-chromatography, AAS and polarography

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



Introduction

Organic acids are weak acids that don't dissociate completely in water. They are often used in trace analysis applications as buffer and complexing agents for improving the separation. Further on, ascorbic acid is a highly effective antioxidant and therefore used as a stabiliser in various analytical applications. The most important fields of application are in metallurgy, environmental analysis, food analysis, toxicology, and clinical analysis. Sigma-Aldrich has recently introduced three organic acids with extremely low levels of inorganic impurities.

For more information about our high-purity reagents, please have a look at sigma-aldrich.com/traceselect

Hydride Generation-Atomic Absorption Spectroscopy (HG-AAS)

Metalloids like antimony, arsenic and selenium are mainly analysed by AAS using the hydride generation technique, which is easily connectable to various detection systems. The detection limits using this technique are below 1.0 µg/L.

A mixture of potassium iodide and ascorbic acid is most often used for the prereduction of As(V) to As(III), whereas potassium is the reducing agent, and ascorbic acid the antioxidant for stabilising the potassium iodide solution. [1]

Several heavy metal ions, such as iron(III), chromium(VI) and copper(II), can suppress the arsenic or antimony hydride generation. These interferences can be eliminated by the addition of potassium iodide and ascorbic acid. [2]

Ion Chromatography (IC) applications

IC is a technique that is used to separate and quantify ppt-%-levels of common anions and cations in aqueous samples based on the charge properties of the ions.

Citric acid, oxalic acid and other carboxylic acids in dissociated form chelate metal ions. By using different organic acids, a competitive complex formation takes place.

Besides their complex forming properties, the carboxylic acids also act as buffer substances. Their strength depends on their pK_A values (see **Table 1**).

Depending on the separation problem, variation of the pH value, the use of a complexing agent and/or an increase in column temperature are powerful tools to broaden the scope of cation chromatography.

Organic acid	Formula	pK_A
Ascorbic (I)	$H_2C_6H_6O_6$	4.2
Ascorbic (II)	$HC_6H_5O_6^-$	11.6
Oxalic (I)	$H_2C_2O_4$	1.2
Oxalic (II)	$HC_2O_4^-$	4.2
Citric (I)	$H_3C_6H_5O_7$	3.2
Citric (II)	$H_2C_6H_4O_7^-$	4.8
Citric (III)	$HC_6H_4O_7^{2-}$	6.4

Table 1 Acid dissociation constants at 25 °C in water [3]

L-Ascorbic acid (vitamin C) is added to reduce Fe(III) to Fe(II) [4-7] for the determination of iron using cation chromatography. Further on, it can also be used in polarography to eliminate the interfering effect of iron(III) ions [8] or for the speciation analysis of Fe(III) and Fe(II) ions.

Citric acid and oxalic acid form strong complexes especially with di- and trivalent cations. **Tables 2** and **3** illustrate the effect of citric and oxalic acid on different analytes.

Eluent composition*	Lithium	Sodium	Potassium	Rubidium	Cesium	Ammonium	Magnesium	Calcium	Manganese	Cobalt	Nickel	Zinc	Methylamine	Dimethylamine	Trimethylamine	Ethanolamine	Diethanolamine	Triethanolamine
Nitric acid	-	-	-	-	-	-	+	++	++	+++	+++	+++	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nitric acid	+	+	+	+	+	+	+++	+++	++	++	++	++	+	+	++	+	+	+
Citric acid	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++	+	+	+++	+	+	+
Oxalic acid	+	+	+	+	+	+	+++	+++	+++	n.d.	n.d.	n.d.	+	+	++	+	+	+

- no remarkable effect on retention time
 + slight effect on retention time
 ++ strong effect on retention time

+++ very strong effect on retention time, n.d. not determined
 *the varied eluent component is printed in red

Table 2 "Effect of eluent composition on IC column retention times". The original work can be downloaded from <http://products.metrohm.com> (search for 8.000.6015EN)

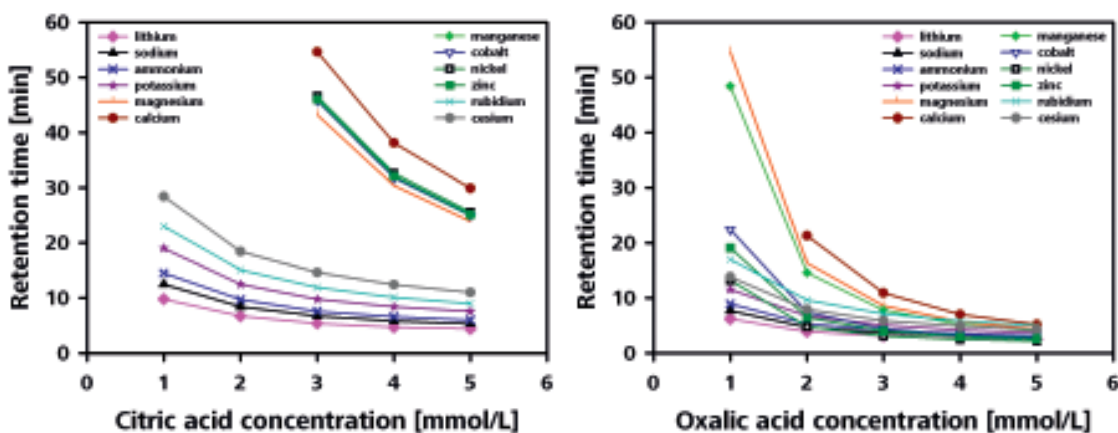


Table 3 Effect of different concentrations of citric acid and oxalic acid on the retention time of various cations using the Metrosep C 2 – 150 separation column

Part No.	Brand	Description	Pack Size
94068	Fluka	Citric acid monohydrate, TraceSELECT®	100 g
05878	Fluka	L-Ascorbic acid, TraceSELECT®	100 g
93722	Fluka	Oxalic acid dihydrate, TraceSELECT®	100 g

Product table for High-purity Carboxylic Acids

References

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- 3] Shriver, D.F.; Atkins, P.W. (1999). *Inorganic Chemistry* (3rd ed.). Oxford: Oxford University Press. ISBN 0198503318. Chapter 5: Acids and Bases.
- 4] Metrohm – IC Application Note No. C-54 "Potassium, iron, magnesium and calcium in clay".
- 5] Metrohm – IC Application Note No. C-79 "Nickel, zinc, cobalt, iron(II) and manganese in lithium bromide using post-column reaction".
- 6] Metrohm – IC Application Note No. C-63 "Five cations in lithium bromide using post-column reaction".
- 7] Metrohm – IC Application Note No. C-62 "Five cations including iron in monoethylene glycol (MEG)".
- 8] Metrohm – Polarography Application Bulletin No. 242/1 "Tungsten at Ultra Trace Graphite Electrode by anodic stripping voltammetry".

Ion Pair Chromatography

Hansjörg Tinner, Michael Kiselewsky michael.kiselewsky@sial.com

It was the Russian botanist Mikhail Tswett who discovered the principle of chromatography in 1901. More than 100 years later, chromatography is omnipresent in analytical research and far from being out of fashion. Even nowadays, it is part of the latest hi-tech development of instruments with extreme ranges of sensitivity and selectivity. Despite the many modern computer-assisted instruments currently available, several traditional variations of chromatography are still widely used. One such chromatographic variation is Ion Pair Chromatography (IPC).

In the past, the approach used to separate charged analytes was ionic suppression. By changing the pH value of the mobile phase, charged analytes become non-ionised. This approach of method development can be time consuming and is better suited for single analytes or simple mixtures where pK_a 's of the analytes are close together.

On the other hand, IPC is a more general and applicable approach that allows the separation of complex mixtures of very polar and ionic molecules. The mobile phase is supplemented with an ion-pairing reagent. Ion-pairing reagents consist of large ionic molecules having a charge opposite to the analyte of interest as well as a substantial hydrophobic region that allows interacting with the stationary phase, plus associated counter-ions. In total, IPC results in different retention of analytes, thus facilitating separation. IPC is an established and reliable technique that provides:

- Reduced separation times
- Highly reproducible results
- Sharper peak shapes
- Simultaneous separation of ionised and non-ionised analytes
- Wide choice of additives to improve separation

The more sensitive modern instruments become, the easier they will detect any impurity added by auxiliaries in addition to the analytes of interest. Therefore, the purity of any kind of eluent additives will influence performance and accuracy. Only products that have been tested for suitability and that have been carefully analysed for purity will guarantee the quality and performance for your application.

Figure 1 shows an example of polar compounds that have been successfully separated using IPC additives on a Supelco Ascentis® Express C18 column. The analytes are imidazolium and pyridinium derivatives. The column was subsequently rinsed with solvent in order to prevent ion pair reagent agglomeration.

Sigma-Aldrich® has a long tradition of offering superior-quality analytical reagents. We are proud to offer you a wide range of accurately tested IPC reagent products under our Fluka brand. For almost 20 years, Sigma-Aldrich has followed a well-established, reliable technique and can provide you with a broad range of products and application notes to help you to resolve your samples. Our reagents are of the highest purity and exhibit minimal extinction in the low UV. They have excellent transparency down to 200 nm, even at high concentrations. In addition, they are tested for the absence of insoluble matter. Non-absorbing impurities like redox-traces, which may interfere with the sample, are also tested. The suitability tests are carefully performed, using a very steep gradient.

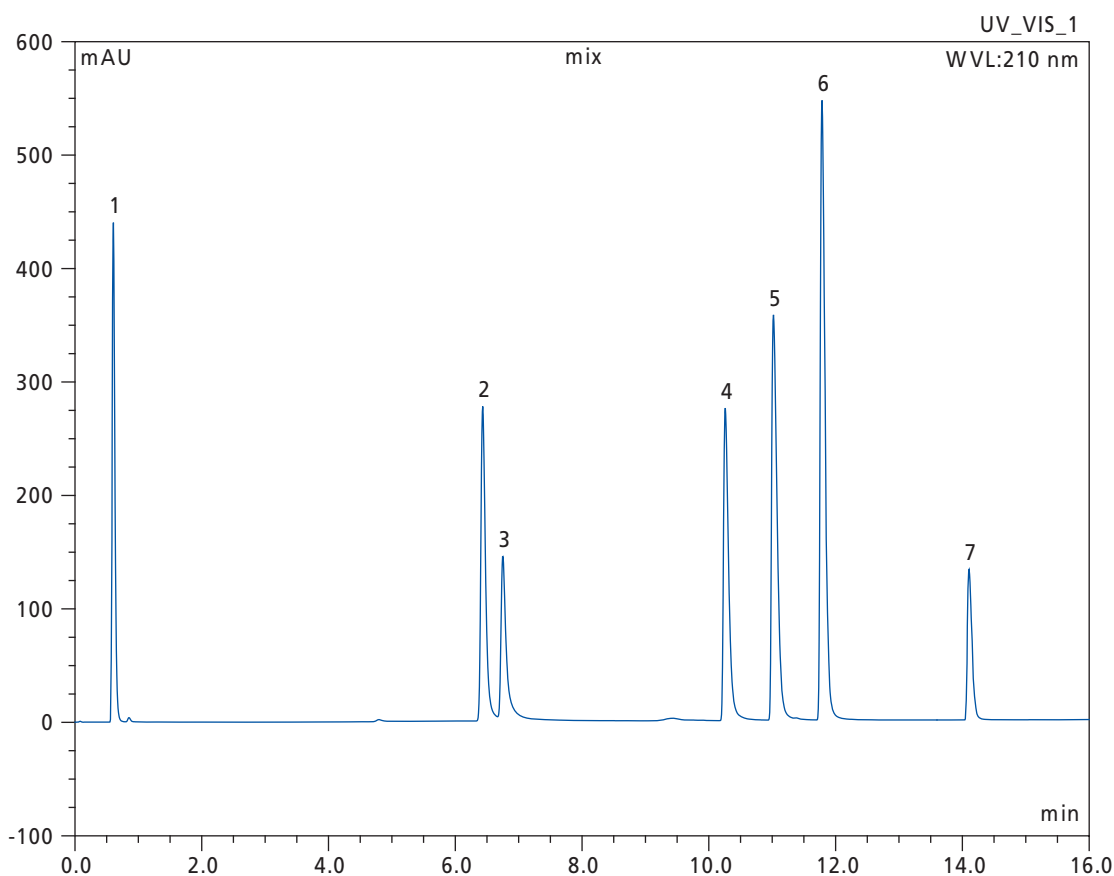


Figure 1 Separation of polar compounds on a Supelco Ascentis® Express C18, 2.7 μm , 7.5 cm x 4.6 mm I.D. (Supelco 53819-U). Acetonitrile was used as a gradient with a buffer of 1.1 g Sodium 1-heptanesulphonate monohydrate (Fluka 51832) and 700 μL Phosphoric acid 85 % (Fluka 79606) in 1 l Water. Sample volume 5 μL , flow rate 1.0 mL/min, T = 25 °C, UV detection = 210 nm, gradient: t = 0 2 % acetonitrile, t = 1 min to 10 min 2 – 20 % and t = 15 min 35 % acetonitrile. Analytes: anions (1), 1-(3-Cyanopropyl)-3-methylimidazolium dicyanamide (2), 1-Methyl-2-vinylpyridinium triflate (3), 1-Butyl-3-methylimidazolium bromide (4), 1-Butyl-2,3-dimethylimidazolium tetrafluoroborate (5), 1-Benzyl-3-methylimidazolium tetrafluoroborate (6), 1-Hexyl-3-methylimidazolium chloride (7)

Brand	Cat. No.	Separation	Name	Carbon Length
Fluka	76952	cationic	Sodium 1-pentanesulphonate monohydrate, for IPC	C5
Fluka	52862	cationic	Sodium 1-hexanesulphonate monohydrate, for IPC	C6
Fluka	51832	cationic	Sodium 1-heptanesulphonate monohydrate, for IPC	C7
Fluka	74882	cationic	Sodium 1-octanesulphonate monohydrate, for IPC	C8
Fluka	75073	cationic	Sodium octyl sulphate, for IPC	C8
Fluka	74316	cationic	Sodium 1-nonanesulphonate, for IPC	C9
Fluka	30631	cationic	Sodium 1-decanesulphonate, for IPC	C10
Fluka	71726	cationic	Sodium dodecyl sulphate, for IPC	C12

Brand	Cat. No.	Separation	Name	Carbon Length
Fluka	74202	anionic	Tetramethylammonium chloride, for IPC	C1
Fluka	88103	anionic	Tetrapropylammonium bromide, for IPC	C3
Fluka	86852	anionic	Tetrabutylammonium chloride, for IPC	C4
Fluka	86857	anionic	Tetrabutylammonium bromide, for IPC	C4
Fluka	86853	anionic	Tetrabutylammonium bisulphate, for IPC	C4
Fluka	87299	anionic	Tetrahexylammonium hydrogensulphate, for IPC	C6
Fluka	87296	anionic	Tetraheptylammonium bromide, for IPC	C7
Fluka	87578	anionic	Tetrakis(decyl)ammonium bromide, for IPC	C10
Fluka	52367	anionic	Hexadecyltrimethylammonium bromide, for IPC	C16

Table 1 Selection of anionic and cationic reagents for Ion Pair Chromatography (IPC). Please check out our website for all products and specifications at sigma-aldrich.com/hplc

Silica Gel Adsorbents

Broadest selection at economical price

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

Silica gels are used as an adsorbent in numerous applications, including chromatographic separations and removal of impurities through adsorption. In chromatography or column chromatography, the stationary phase is most often composed of silica gel, and due to its polarity, non-polar components elute before the polar components (normal phase chromatography). On the other hand, silica gels with C18 type hydrophobic groups attached to its surface allow elution of polar components first and are used in reversed-phase chromatography. Silica is also used in thin-layer chromatography. Silica gel exhibits strong desiccant properties and, therefore, is also frequently used in applications for control of relative humidity.

Sigma-Aldrich offers a broad selection of silica gels

- With a broad particle size range
- Products from leading manufacturers (Davisil® and Merck)
- Modified/bonded silica gels
- Generic silica gel for FLASH chromatography (for rapid preparative separations)
- Specialised silica gels for scavenging

These materials are useful for all types of low and medium pressures, and can be applied to cleanup and purification of a wide range of synthetic and natural compounds. Sigma-Aldrich offers a wide selection of silica gels and silicic acid for non-chromatographic purposes.

A wide-particle size range of silica gels is offered to meet your application requirements. Our products are especially economical for high-volume usage applications, while exhibiting quality comparable to products offered by other leading manufacturers. **Table 1** presents our

selected products grouped into two particle size ranges, along with comparable Merck and Davisil brands.

Table 2 lists the Davisil silica gels of different particle sizes that Sigma-Aldrich® offers to meet your performance criteria. These products are commonly used in preparative column chromatography applications. The silica gels are already processed to minimise or eliminate impurities, such as minor metallic oxides that can modify the surface and unpredictably alter the adsorption process. Davisil silica grade 12 is recommended for ASTM method D-2007 for rubber extender processing oils. Grade 923 meets ASTM-1319-70 specifications for hydrocarbon analysis. Its low metal oxide content minimises olefin polymerisation.

The Merck silica gels that we offer (see **Table 2**) are widely used for column chromatography. These adsorbents are pure (iron <0.02 % and chlorine <0.02 %), and demonstrate consistent particle size and defined pore structure. The most popular products are listed by Grade (*EM catalogue number*). For the proper Merck designation, simply insert Silica Gel or Kieselgel before the pore size – for example, Silica Gel 40 or Kieselgel 40.

Grade 7754 silica gel is an extra-pure material (each Cd, Cu, Pd, PO₃ and Zn is <0.0005 %, Cl <0.008 %, Fe <0.002 %, NO₃ <0.004 %, and SO₂ <0.003 %). Grade 9385 silica gel for Flash is widely used for purification of organic synthesis products by gas-pressurised liquid chromatography.

Sigma-Aldrich also offers Flash columns and a full line of other supplies needed for Flash chromatography. For additional product information, visit sigma-aldrich.com

Table 1 Silica gels from Sigma-Aldrich and comparable Davisil and Merck products

Grade	Brand	Particle Size	Pore Volume (cm ³ /g)	Pore Size (Å)	Surface Area (m ² /g)	Size	Cat. No.
Particle size range: 35 – 75 µm							
-	Sigma-Aldrich	200 – 425 mesh 35 – 75 µm	0.75	60	470-530	500 g 2.5 kg 5 kg 25 kg	645524-500G 645524-2.5KG 645524-5KG 645524-25KG
-	Sigma-Aldrich	200 – 425 mesh 35 – 75 µm	0.75	60	500	500 g 1 kg 10 kg	288594-500G 288594-1KG 288594-10KG
-	Sigma-Aldrich	200 – 425 mesh 35 – 75 µm	0.8	60	500	250 g 1 kg 25 kg	60738-250G 60738-1KG 60738-25KG

Grade	Brand	Particle Size	Pore Volume (cm ³ /g)	Pore Size (Å)	Surface Area (m ² /g)	Size	Cat. No.
-	Sigma-Aldrich	200–425 mesh 35–75 µm	0.8	60	530	500 g	60737-500G
						2.5 kg	60737-2.5KG
633	Davisil	200–425 mesh 35–75 µm	0.75	60	480	100 g	236772-100G
						1 kg	236772-1KG
9385	Merck	230–400 mesh 40–63 µm	~0.8	60	550	100 g	227196-100G
						1 kg	227196-1KG
						5 kg	227196-5KG
						25 kg	227196-25KG
Particle size range: 63–200 µm							
-	Sigma-Aldrich	70–230 mesh 63–200 µm	0.75	60	~500	250 g	288624-250G
						1 kg	288624-1KG
						5 kg	288624-5KG
High purity	Sigma-Aldrich	70–230 mesh 63–200 µm	0.8	60	500	500 g	60740-500G
-	Sigma-Aldrich	70–230 mesh 63–200 µm	0.8	60	500	1 kg	60741-1KG
						6x1 kg	60741-6x1KG
						25 kg	60741-25KG
Fluorescence indicator	Sigma-Aldrich	70–230 mesh 63–200 µm	0.8	60	500	1 kg	60743-1KG
634	Davisil	100–200 mesh 75–150 µm	0.75	60	480	100 g	236780-100G
						1 kg	236780-1KG
7734	Merck	70–230 mesh 63–200 µm	~0.8	60	550	100 g	391484-100G
						1 kg	391484-1KG
						5 kg	391484-5KG
						25 kg	391484-25KG
7754	Merck	70–230 mesh 63–200 µm	~0.8	60	~500	25 g	403598-25G
						100 g	403598-100G
						500 g	403598-500G

Table 2 Other offered Davisil and Merck silica gels

Grade	Brand	Particle Size	Pore Volume (cm ³ /g)	Pore Size (Å)	Surface Area (m ² /g)	Size	Cat. No.
Grade 12	Davisil	28–200 mesh 75–650 µm	0.43	22	800	250 g	214396-250G
						1 kg	214396-1KG
						5 kg	214396-5KG
Grade 62	Davisil	60–200 mesh 75–250 µm	1.15	150	300	100 g	243981-100G
						500 g	243981-500G
						2.5 kg	243981-2.5KG
						1 kg	236799-1KG
Grade 635	Davisil	60–100 mesh 150–250 µm	0.75	60	480	1 kg	236799-1KG
						100 g	236802-100G
						1 kg	236802-1KG
Grade 636	Davisil	250–500 mesh 35–60 µm	0.75	60	480	25 kg	236802-25KG
						100 g	236810-100G
						1 kg	236810-1KG
Grade 643	Davisil	200–425 mesh 35–70 µm	1.15	150	300	100 g	236829-100G
						1 kg	236829-1KG
Grade 644	Davisil	100–200 mesh 75–150 µm	1.15	150	300	100 kg	236837-100G
						1 kg	236837-1KG
Grade 645	Davisil	60–100 mesh 150–250 µm	1.15	150	300	100 g	236845-100G
						1 kg	236845-1KG
Grade 646	Davisil	250–500 mesh 35–60 µm	1.15	150	300	100 g	236845-100G
						1 kg	236845-1KG
						100 g	214477-50G
Grade 923	Davisil	100–200 mesh 75–150 µm	0.4	30	75	250 g	214477-250G
						1 kg	214477-1KG
						100 g	13660
Grade 653XWP	Davisil	230–400 mesh 35–70 µm	1.1–1.2	300	150–170	100 g	13660
Grade 663XWP	Davisil	230–400 mesh 35–70 µm	1.1–1.2	500	78–85	100g	13662

(continued on page 20)

Grade	Brand	Particle Size	Pore Volume (cm ³ /g)	Pore Size (Å)	Surface Area (m ² /g)	Size	Cat. No.
Grade 10180	Merck	63–200 µm	~ 0.68	40 Å	750	100 g	403563-100G
						1 kg	403563-1KG
						5 kg	403563-5KG
Grade 10181	Merck	200–500 µm	~ 0.68	40 Å	675	100 g	242179-100G
						500 g	242179-500G
						2 kg	242179-2KG
						100 g	403598-100G
						500 g	403598-500G
Grade 10184	Merck	70–230 mesh	-	100 Å	300	100 kg	403601-100G
						1 kg	403601-1KG
						5 kg	403601-5KG
Grade 15111	Merck	15–40 µm	~ 0.8	60 Å	550	100 g	S9258-100G

Water Content Determination of pH-influencing Samples

HYDRANAL[®] Reagents for Karl Fischer titration

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The optimum pH range for the Karl Fischer reaction that ensures a rapid and stoichiometric course of the reaction lies between pH 5–7. The pH balance can be disturbed by the introduction of an excess strong acid or base. Adding an acidic sample to the titration cell decreases the pH value in the working medium and reduces the titration reaction rate significantly. On the other hand, addition of a strongly basic sample can increase the pH of the KF system if its basicity exceeds the buffering capacity of the reagent solution. As a result, the titration endpoint will not be reached.

For a KF titration, **Figure 1** demonstrates the change in the reaction rate constant, log K, as a function of the solution pH. The pH of 5–7 appears as the optimum pH range, since the reaction rate exhibits no significant change here.

HYDRANAL[®] reagents contain imidazole as the base that neutralises the acid formed in the KF reaction and thus buffers the titration system; it is stabilised around the ideal pH value. When using a two-component KF technique with HYDRANAL-Solvent (Fluka cat. no. 34800) as a working medium, some buffer capacity is already provided: 1 mL HYDRANAL-Solvent can buffer approximately 0.6 mmol of acid. With 20 mL HYDRANAL-

Solvent as working medium, a maximum of 12 mmol of an acid can be added without interfering with the reaction rate. However, for strongly acidic or basic samples, the working medium must be neutralised by the addition of an appropriate weak base or acid, or a suitable buffer solution.

Neutralisation of acidic samples

An acidic sample must be neutralised prior to starting the titration by directly adding a suitable base to the working medium in the titration cell. However, it is important that the addition of a base does not raise the pH level excessively (outside the optimum range) that can prevent reaching an endpoint in the pre-titration. We recommend the use of imidazole or, preferably, the liquid HYDRANAL-Buffer Acid to neutralise a larger quantity of an acidic sample. HYDRANAL-Buffer Acid has a buffer capacity of ~5 mmol acid/mL. Certain carboxylic acids that tend to esterify are neutralised by HYDRANAL-Buffer Acid without esterification.

Highly concentrated acids like sulphuric acid and hydrochloric acid esterify very easily with methanol and, therefore, must be neutralised before the titration in an alcohol-free medium.

Neutralisation of basic samples

Strong bases must be neutralised prior to starting the titration by addition of an adequate amount of an acid. For this purpose, a ready-to-use buffer solution, liquid HYDRANAL®-Buffer Base, can be used for water determination in basic samples. It contains salicylic acid and has an approximate buffer capacity of 1 mmol base/mL. Other acids like benzoic or propionic acid can also be used.

Benzoic acid is typically used because it is easy to handle and has no unpleasant odour. Salicylic acid is a stronger acid and can reduce the pH to between 4 and 5, which is outside the optimal pH range and, therefore, can slow down the course of the titration. It is especially useful when determining the water content of phenols. Propionic acid has acidification properties similar to benzoic acid. It is easier to handle due to its liquid form but it has an unpleasant odour. Acetic acid is not recommended as it leads to esterification.

Both Hydranal-Buffer Acid and Base are liquid and can be added to the working medium in a suitable amount.

Many pharmaceutical compounds contain nitrogen in their chemical structure. Like other basic components, they must be titrated in HYDRANAL-Buffer Base or one of the above-mentioned acids for accurate titration results. Weak basic compounds generally do not influence the pH-value of the KF medium and can be determined following the standard procedures.

For coulometric Karl Fischer determination, we recommend addition of benzoic acid for basic samples and imidazole for acidic samples to the analyte solution.

Determination of pH value in the KF medium

When sluggish or vanishing endpoints appear, the pH of the system may not be in the optimal range. In such a case, the pH value of the working medium plus the sample should be measured. A glass pH electrode, calibrated in an aqueous solution, can be used. The pH of the system should not be measured directly in the titration cell, as the pH electrode can introduce water into the system. Instead, the pH should be measured in a portion of the fluid outside the titration cell.

To obtain application reports, our HYDRANAL Manual with many detailed procedures or answers to any other questions regarding KF titration, please visit our website sigma-aldrich.com/hydranal

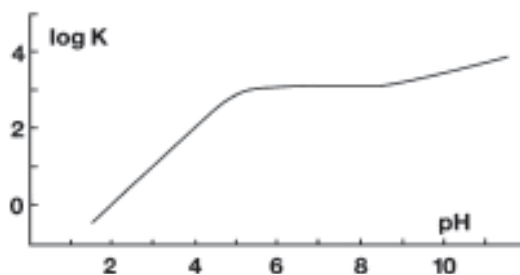


Figure 1 Dependence of the reaction rate constant K on the pH (Verhoef, J.C. and Barendrecht, E.; Mechanism and Reaction Rate of the Karl-Fischer Titration Reaction. *J. Electroanal. Chem.* 1976, 71, 305–315)

Substance	Application No.
Pyrogallol	L004
Sulphuric acid, conc.	L049
Amoxicillin	L352
Trifluoroacetic acid	L380
Folic acid	L383
Maleic acid / Acrylic acid copolymer	L391
Perchloric acid 70 %	L406
Benserazid hydrochloride	L416
Hydrazine hydrate solution	L435
Potassium clavulanate	L437
Bisaminophenoxyphenylpropane PAPP	L451
3-Chloro-4-fluoroaniline	L464
Aminophylline	L468
Whey powder	L493
2-Dimethylaminomethylcyclohexanone	L518
L-Lysine monohydrate	L528
5-Hydroxy-1-methylpyrazole	L540

Table 1 List of selected Hydranal application reports for acidic and basic substances

Fluka Cat. No.	Description	Pack Size
34804	HYDRANAL-Buffer Acid (Buffer cap. 5 mmol acid/mL)	500 mL
37859	HYDRANAL-Buffer Base (Buffer cap. 1 mmol base/mL)	1 L
37864	HYDRANAL-Imidazole	500 g
32035	HYDRANAL-Benzoic acid	500 g
37865	HYDRANAL-Salicylic acid	500 g

Table 2 HYDRANAL products for pH adjustment

References

Eugen Scholz "Karl-Fischer Titration" Springer-Verlag, Berlin, Heidelberg, New York, Tokyo (1984).

Titer Precision and Temperature Correction in Volumetric Titration

Volumetric solutions and concentrates for precise and accurate results

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Calculation of factor and titer

When carrying out a titration using ready-to-use solutions or concentrates, it is important to pay attention to the factor given on the label of each package. This factor must be used to calculate the titer of the solution by multiplying it with the nominal concentration. For example, for hydrochloric acid with nominal concentration of 0.1 mol/L and a factor of 1.001, the actual concentration is $0.1 \times 1.001 = 0.1001$ mol/L.

The titer t is the ratio of the actual concentration of a standard solution $c(X)_{ACTUAL}$ (actual value) and the desired concentration of the same solution $c(X)_{NOMINAL}$ (nominal value):

$$t = \frac{c(X)_{ACTUAL}}{c(X)_{NOMINAL}}$$

The titer, which can be determined using the certified reference materials, therefore, equals a factor for designation of the concentration.

As an example, for a standard solution of sulphuric acid of a nominal concentration $c(\text{H}_2\text{SO}_4)_{NOMINAL} = 0.1000$ mol/L and an actual concentration $c(\text{H}_2\text{SO}_4)_{ACTUAL} = 0.1027$ mol/L, the titer t is calculated as follows:

$$t = \frac{0.1027 \text{ mol/L}}{0.1000 \text{ mol/L}} = 1.027$$

In order to obtain the consumption of a solution of concentration $c(\text{H}_2\text{SO}_4) = 0.1000$ mol/L, the volume of consumed sulphuric acid standard solution in the titration has to be multiplied by the titer $t = 1.027$.

Titer precision of ready-to-use solutions vs. concentrates

The measured titer (factor) of the ready-to-use solutions is displayed on the certificate of analysis. However, for FIXANAL® concentrates, the titer is adjusted to 1.000 (to a precision of ± 0.2 %) during the filling process.

With the titer so determined, the weight of the volume of liquid corresponding to exactly 0.1 mol of the specified analyte is calculated. This exact volume of liquid is added to every ampoule in the lot, using high-precision

filling equipment. The variation of the resulting weight is < 0.1 %; the typical standard deviation is lower than 0.04 %.

The **concentration** of the liquid inside each FIXANAL ampoule may vary slightly from lot to lot, but the **amount** of the specified material in each ampoule is exactly the same, for example 0.1 mol HCl (not mol/L).

Ready-to-use solutions

Contain exact amount in terms of concentration (e.g. 1 mol/L)

Titer precision 1.000 ± 0.1 %

FIXANAL® concentrates

Contain exact amount of substance (e.g. 1 mol)

Titer precision 1.000 ± 0.2 %

Customised use of ampoule concentrates to fulfil your titration needs

Every FIXANAL ampoule contains a precise given amount of a concentrate. This can be diluted to a final volume desired by the user. Usually, the concentrates are diluted to 1 L but other dilutions can also be prepared.

Amount of substance in the ampoule: 0.1 mol	Final volume			
	500 mL	1 L	2 L	10 L
Resulting concentration of solution	0.2 mol/L	0.1 mol/L	0.05 mol/L	0.01 mol/L

The water used in dilution should be distilled and degassed, otherwise sluggish endpoints or changes of titer can occur.

Influence of temperature

The volume of a solution depends on temperature. Therefore temperature is an important condition for all volumetric determinations.

The influence of temperature on aqueous solution is ten times more than that on the glass, and averages ~ 0.02 % per degree Celsius. For example, when an aqueous standard solution prepared at 20 °C is used at 25 °C, its volume should be corrected using a factor of 0.999 if striving for an error considerably below 0.1 %.

Table 1 shows the temperature-related dependences of:

- Density of water
- Volume of 1000 mL water (measured at 20 °C); as an approximation also valid for aqueous standard solutions
- Correction factors for standard solutions prepared at 20 °C (valid for Fluka ready-to-use standard solutions)

- Correction factors for standard solutions prepared at 25 °C (e.g. standard solutions prepared at 25 °C by use of FIXANAL® concentrates) are used at the temperature equivalent to that indicated for fill-up time.

Note: No temperature correction is required when standard solutions made from FIXANAL concentrates

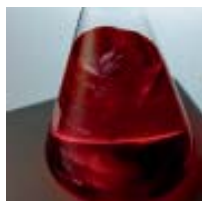
For further information about volumetric titration and to take advantage of our extensive portfolio of titration products, please visit our website sigma-aldrich.com/titration

T/°C	Water Density (g/mL)	Volume (mL) of 1 L water at 20 °C	Factor (solution prepared at 20 °C)	Factor (solution prepared at 25 °C)
10	0.999699	998.50	1.0015	1.0027
11	0.999604	998.60	1.0014	1.0026
12	0.999497	998.71	1.0013	1.0025
13	0.999376	998.83	1.0012	1.0023
14	0.999243	998.96	1.0010	1.0022
15	0.999099	999.10	1.0009	1.0021
16	0.998942	999.26	1.0007	1.0019
17	0.998773	999.43	1.0006	1.0017
18	0.998595	999.61	1.0004	1.0016
19	0.998403	999.80	1.0002	1.0014
20	0.998203	1000.00	1.0000	1.0012
21	0.997991	1000.21	0.9998	1.0010
22	0.997769	1000.43	0.9996	1.0007
23	0.997537	1000.67	0.9993	1.0005
24	0.997295	1000.91	0.9991	1.0003
25	0.997043	1001.16	0.9988	1.0000
26	0.996782	1001.43	0.9986	0.9997
27	0.996531	1001.68	0.9983	0.9995
28	0.996231	1001.98	0.9980	0.9992
29	0.995943	1002.27	0.9977	0.9989
30	0.995645	1002.57	0.9974	0.9986
31	0.995339	1002.88	0.9971	0.9983
32	0.995024	1003.19	0.9968	0.9980
33	0.994701	1003.52	0.9965	0.9977
34	0.994370	1003.85	0.9962	0.9973

Table 1 Correction of temperature for volumetric solutions

New Silver Nitrate Standard Solution FIXANAL®, 0.01 M Volumetric Concentrate

Andrea Felgner, Product Manager Analytical Reagents andrea.felgner@sial.com



One of the most common precipitation titration methods is the quantitative determination of halides and pseudo halides with silver nitrate, also known as argentometric titration. Different titration procedures can be used:

The Mohr method uses direct titration of halide-containing solutions with silver nitrate solution; potassium dichromate indicator forms red silver chromate as a precipitate at the equivalent point.

The Fajans method also uses this direct titration with eosin or fluorescein as indicators that exhibit colour change at the equivalent point.

The Volhard method is an indirect (back) titration for halides, cyanides and thiocyanates; after addition of excess silver nitrate solution, silver halide precipitates and the back titration of remaining silver nitrate is performed with a thiocyanate solution.

To meet these titration needs, Fluka offers a new 0.01 mol ampoule concentrate in addition to our established 0.1 mol and 0.5 mol silver nitrate ampoule concentrates.

Cat. No.	Brand	Description
38001	Fluka	Silver nitrate solution FIXANAL, pkg of 0.01 mol (1.699 g AgNO ₃) NEW
38310	Fluka	Silver nitrate solution FIXANAL, pkg of 0.1 mol (16.988 g AgNO ₃)
38311	Fluka	Silver nitrate solution FIXANAL, pkg of 0.5 mol (84.94 g AgNO ₃)

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