

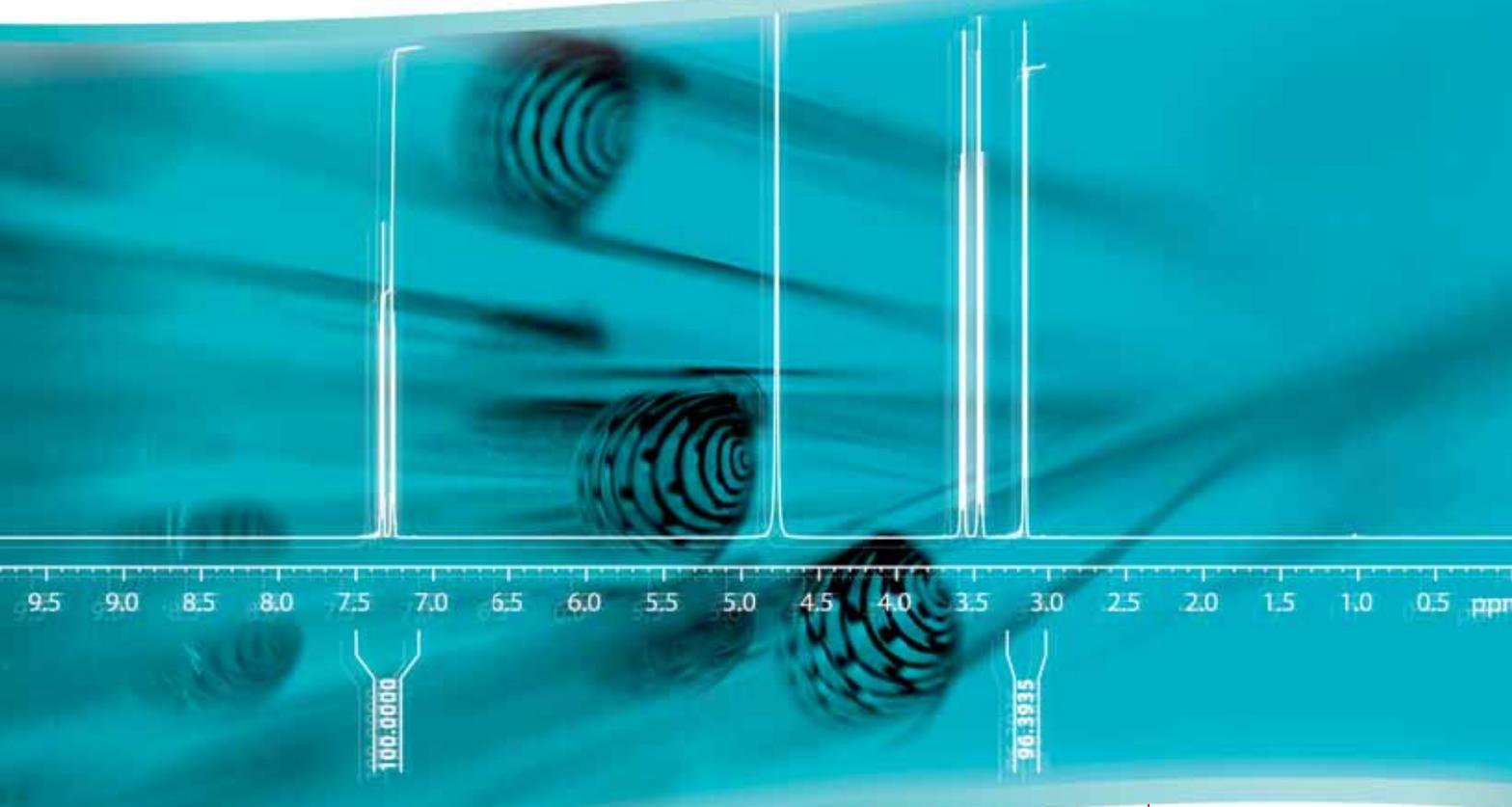
# Analytix

Issue 3 • 2010

 **Fluka**  
Analytical

 Riedel-deHaën

## New Generation of Organic CRM



- Organic CRMs
- Chromogenic Media for MiBi
- Inorganic Custom Standards
- Derivatization reagents for Pharmaceuticals
- Bioethanol analysis
- ISO/IEC 17025 accreditation of HYDRANAL® service lab

**SIGMA-ALDRICH®**

## New Generation of Organic CRM



Michael Weber  
Manager R&D/Innovation Europe

### Dear Colleague,

It has now been sixty years since Fluka® was founded in 1950 in the middle of St. Gallen, Switzerland. After the addition of new facilities in Buchs in the Rhine Valley, Fluka established itself as one of the leading producers and suppliers of research chemicals, reagents and standards. In 1989, Fluka joined the Sigma-Aldrich Corporation and eventually changed its name to Sigma-Aldrich Switzerland. Today, within the impressive Sigma-Aldrich product portfolio, Fluka is the brand representing high-quality reagents and standards for analytical chemistry.

Hence, it is a pleasure to present a new service tool as well as a new class of certified standards which are now launched under the Fluka brand. With the introduction of the new web-based dynamic Custom Standards Platform ([sigma-aldrich.com/csp](http://sigma-aldrich.com/csp)) we provide the capability to compose your individual inorganic custom standard within minutes. You can actually choose between multi-elemental standards for spectroscopy as well as anion and cation mixtures for ion chromatography. The whole process from specifying the composition of your individual standard to the submission of your request is a fast and easy online experience. And of course, all these custom standards are produced and certified under double accreditation following both ISO/IEC 17025 and ISO Guide 34. Try it!

As a second highlight, we are introducing the first series of a new generation of organic Certified Reference Materials for calibration and validation of chromatography or for other analytical applications. After the implementation of high-precision quantification with <sup>1</sup>H-NMR

in Buchs, our lab received ISO/IEC 17025 accreditation for qNMR analysis. As a relative primary method, qNMR offers outstanding opportunities for the exact determination of the purity of organic substances. The new class of organic **TraceCERT**® standards is traceable to NIST references and will be characterized not only by qNMR, but also by additional analytical techniques including HPLC, GC and other methods. Amino acids and PAHs have been selected to be the first CRM which are certified by this multi-step approach, but many more substances of high relevance to the analytical community will follow soon.

Happy birthday, Fluka brand, with 60 years of exceptional service, meeting the needs of the world-wide scientific community!

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## Feature Article

- 4 **Launch of a New Generation of Organic Certified Reference Materials**  
First Series of Products Includes 21 Amino Acids

## Microbiology

- 7 **Overview of Chromogenic Media**  
Chromogenic media offer a range of benefits for the enumeration, detection, and identification of microorganisms.

## Standards

- 10 **New Dynamic Platform for Inorganic Custom Standards**
- 12 **Certified Reference Materials for Spectroscopy**

## Chromatography

- 13 **Enantiomeric Purities of Pharmaceuticals Using Carbohydrate-based Isothiocyanates**
- 18 **Analysis of Bioethanol – Detection of Chloride and Sulfate with Dication and Trication Solutions**

## Titration

- 20 **Increasing Reliability and Value of Analytical Results**  
ISO/IEC 17025 accreditation of HYDRANAL® service lab

## Monthly Savings Program

- 22 **35% Off – Volumetric Titration Reagents**

## New Product Corner

- 23 **Analytical Standards of Alkylresorcinols**
- 23 **New Standards for Regulated Food Dyes**

## Launch of a New Generation of Organic Certified Reference Materials

First Series of Products Includes 21 Amino Acids

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We are pleased to present a first series of the new product line of organic **TraceCERT®** certified standards. These certified reference materials (CRMs) are produced and certified in a double accredited laboratory fulfilling both ISO/IEC 17025 and ISO Guide 34, using quantitative NMR for content determination with direct traceability to NIST references and the SI base unit kg. The double accreditation represents the highest achievable level of reliability and therefore earns the label of the “gold standard” among CRM producers.

The new product class of organic CRMs for use as internal standards for qNMR has been presented in the previous issue of Analytix. Here, we introduce our first product line of organic CRMs for use as chromatography standards.

Since the use of certified reference materials is becoming increasingly important, especially in the areas of food & beverage analysis as well as in environmental analysis, the first two series of CRMs include amino acids (which are presented here) and polyaromatic hydrocarbons (PAH) (which we will present in the next issue of Analytix).

### Why certified reference materials?

To ensure the reliability of analytical results, the quality of the reference material used for calibration of the instruments is crucial. Certified reference materials are most trustworthy since they fulfil several requirements:

- They must be traceable to an internationally accepted reference (e.g. from NIST, BAM or SI)
- The certified value must have a properly calculated uncertainty
- They must be shown to be sufficiently homogeneous
- The stability of the material must be carefully determined

If a manufacturer is accredited to ISO Guide 34 (“General requirements for the competence of reference material producers”) and also according to ISO/IEC 17025 (“General requirements for the competence of testing and calibration laboratories”), then the fulfillment of all these requirements is guaranteed.

For more than two years now, the Buchs site of Sigma-Aldrich in Switzerland has held this “gold standard” for the production of inorganic **TraceCERT** standard solutions ([sigma-aldrich.com/inorganiccrm](http://sigma-aldrich.com/inorganiccrm)) for AAS, ICP and IC. While for inorganic materials internationally

accepted reference materials are readily available (e.g. from NIST and other metrological institutes), it is a significant challenge to establish traceability for organic reference materials.

Conventionally, the purity of a substance is determined by means of GC or HPLC and the content is calculated by subtracting water content, residue solvents and inorganic impurities from the measured chromatographic purity. However, since every chemical substance has its own characteristic UV-absorption behaviour, direct traceability is only possible if an internationally accepted reference standard is available for the compound in question. This is not necessary if a relative primary analytical method such as quantitative NMR (qNMR) is used. With qNMR technology, traceability between completely different organic compounds can be achieved since the NMR signal intensity is in direct relation to the numbers of protons involved.



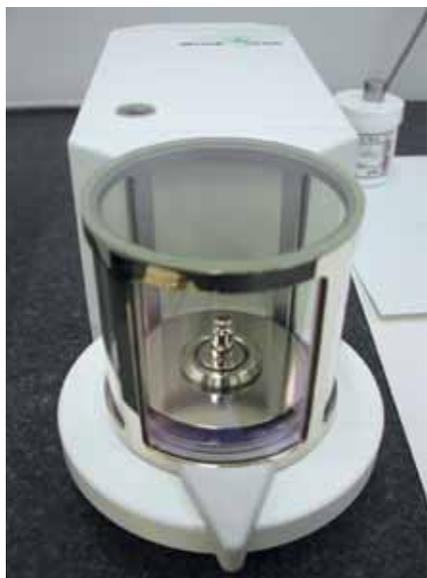
**STS 490**  
**ISO 17025**



**SRMS 001**  
**ISO Guide 34**

### Double accreditation for CRM production using qNMR

In the R&D lab of Sigma-Aldrich Buchs (Switzerland) a new Bruker Avance III 600 MHz Ultrashield NMR spectrometer was recently installed and the instrument performance was optimized for the high-resolution quantification of organic substances. By the end of 2009, the new NMR lab was fully accredited under both ISO/IEC 17025 and ISO Guide 34 for the certification of organic reference materials using <sup>1</sup>H qNMR. Quantitative NMR is a non-destructive technique that demonstrates many advantages over other analytical methods: it requires easy sample preparation; analysis is fast; it provides full structural information; data about impurities or isomers are available; and it is less time consuming than many other analytical techniques, since it requires no equilibration time.



$^1\text{H}$  qNMR is marked by its linearity, specificity and robustness regarding most parameter settings. The most outstanding attribute of  $^1\text{H}$  qNMR is that it may be considered a primary ratio method because it does not need a standard reference of the same material. The NMR spectrum of a solution shows all soluble organic substances present, with the peak area being directly proportional to the amount of nuclei being measured.

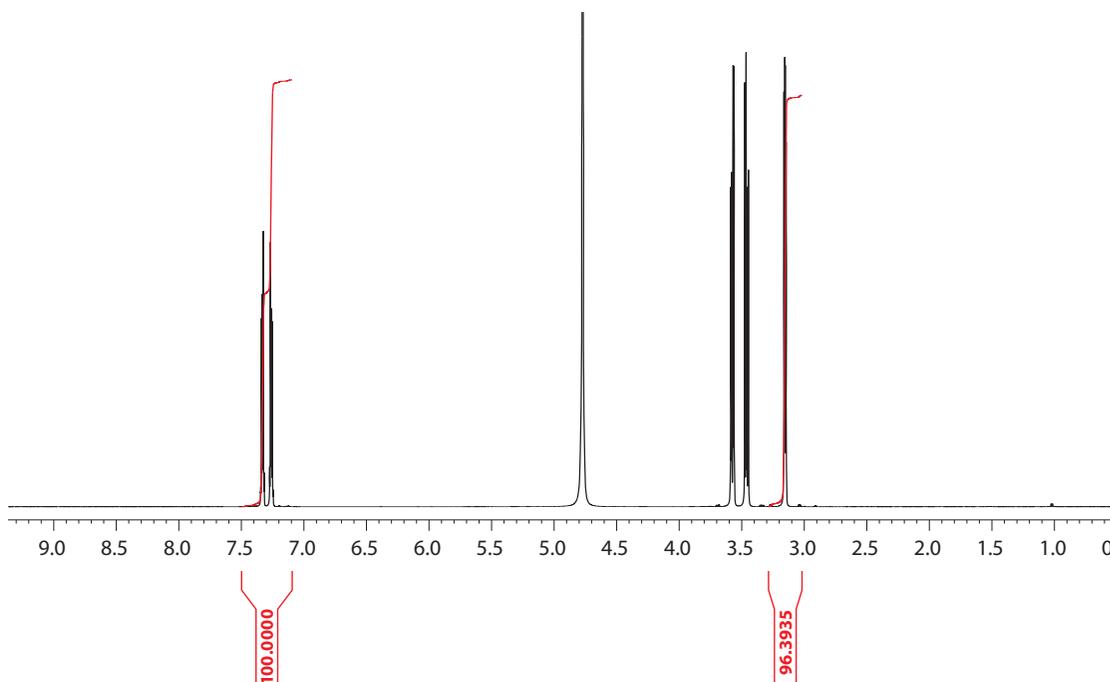
Hence, the signal ratio of two different protons can be measured with tremendous precision and the only significant contribution to the measurement uncertainty

is the integration of the signals. In other words, the direct response of a qNMR experiment is of the highest trueness. In the previous issue of *Analytix*, we already presented CRMs which have been tailor-made as qNMR calibrants. In the following, we will describe the certification process, exemplified by amino acids, our first series of organic CRMs.

#### Certification of amino acids by qNMR

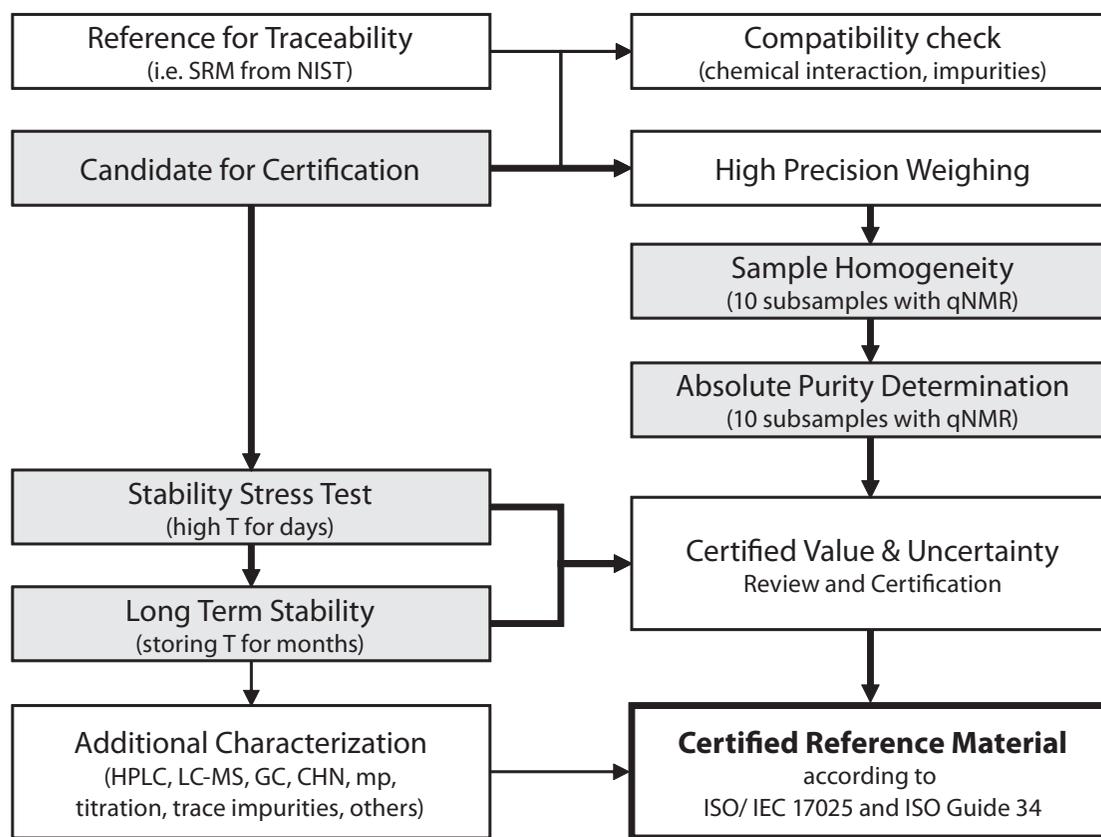
In the certification process, the following tests have been performed for any candidate substance: hygroscopy/volatility; choice of the appropriate deuterated solvent; chemical stability; 2D-NMR (H-H COSY) to detect potential underlying impurity signals and determination of the  $^1\text{H}$  relaxation time.

Once having selected a suitable starting material, a defined number of subsamples are taken from different positions within the bulk material. From those samples, some are stored at room temperature for periodical stability tests in order to get data on aging of the material. Other samples are stored at higher temperature for stress tests, helping to ensure stability at even extreme transportation conditions. Further samples go into quality control for chromatography, LC-MS or other standard techniques if required. However, 10 subsamples are taken for homogeneity assessment and content determination by qNMR. Therefore, it is necessary that a reference standard has certain required properties in order to be suitable for the quantification. Since the reference standard and the amino acid are weighed together into the same vial, both substances have to be soluble in the same deuterated solvent, but must not react with each other or with the solvent. Each of the substances must show at least one  $^1\text{H}$ -NMR-signal that is not overlapped, and the content of the reference standard must be traceable to NIST or SI. The weighing procedure is performed on an ultra-micro balance in such amounts that equal NMR signal intensities will result. Taking into account air buoyancy, the integrals of 10 subsamples contribute to the resulting content value. This is followed by a comprehensive uncertainty evaluation, resulting finally in a certificate associated with each amino acid which conforms with ISO Guide 31. A complete series of the 20 proteinogenic amino acids, along with Cystin, have been quantified by qNMR using potassium hydrogen phthalate (KHP) or maleic acid, respectively, as the internal reference standard. **Figure 1** shows the qNMR-spectrum of L-Serine with the internal standard KHP as an example. An illustration of the certification process is shown in **Figure 2**.



**Figure 1**  $^1\text{H}$  qNMR spectrum for the quantification of L-Serine (at 3 ppm) with KHP (at 7.2 ppm)

(continued on page 6)



**Figure 2** Multi-component certification approach

**Table 1** lists the organic *TraceCERT*<sup>®</sup> products for the proteinogenic amino acids and cystine. The products are supplied with a printed certificate containing certified value, comprehensive documentation, proper uncertainty calculation, lot-specific values, expiration date and storage information. On our website, customers can download complete example certificates as pdfs.

The amino acid CRMs described in this article are the first products launched by this innovative approach to high-accuracy qNMR quantification, with an exciting expanded portfolio to follow. As a next group we will launch CRMs of polyaromatic hydrocarbons (PAHs). Please visit our website at [sigma-aldrich.com/organicCRM](http://sigma-aldrich.com/organicCRM) for an up-to-date product listing.

Cat. No.	Description	Pack Size
44526	L-Alanine	1 g
90538	L-Arginine hydrochloride	1 g
51363	L-Asparagine	1 g
51572	L-Aspartic Acid	1 g
95437	L-Cysteine	1 g
95436	L-Glutamic acid	1 g
76523	L-Glutamine	1 g
76524	Glycine	1 g
73767	L-Histidine	1 g
56241	L-Isoleucine	1 g
76526	L-Leucine	1 g
67448	L-Lysine hydrochloride	1 g
39496	L-Methionine	1 g
40541	L-Phenylalanine	1 g
93693	L-Proline	1 g
54763	L-Serine	1 g
61506	L-Threonine	1 g
51145	L-Tryptophan	1 g
91515	L-Tyrosin	1 g
50848	L-Valine	1 g
49603	L-Cystine	1 g

**Table 1** Fluka labelled certified amino acid *TraceCERT* standards

## Overview of Chromogenic Media

Chromogenic media offer a range of benefits for the enumeration, detection, and identification of microorganisms.

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The use of traditional versus improved media formulation containing chromogenic substrate is currently an important topic in the field of microbiology. The focus behind such developments was to produce media that would make the detection and identification of microorganisms more rapid and more reliable. Chromogenic substrates such as ONPG, X-Gal, or X-Glu, together with a specified selectivity of the medium, is the simple principle behind chromogenic media. The target organisms are characterized by enzyme systems that metabolize the substrates to release the chromogen. The chromogen can then be visually detected by direct observation of a distinct colour change in the medium. Direct confirmation of the target organism without further testing is sometimes possible.

### Did you know?

**Sigma-Aldrich provides thirty-seven different chromogenic media.**



Figure 1 HiCrome™ Rapid Coliform Broth

Over the past 15 years, we have continued to develop and add new media to our ever expanding product range designed to meet customer needs

Species	Enzyme	Substrate	Selective Agents
<i>Bacillus cereus</i>	$\beta$ -glucosidase, Phosphatidylinositol-specific Phospholipase C	indoxyl- $\beta$ -glucopyranoside, indoxyl-myo-inositol-1-phosphate	polymyxin B
<i>Campylobacter</i>	na	na	deoxycholate, cefoperazone, amphotericin B
<i>Candida</i>	$\beta$ -acetylgalactosaminidase, alkaline phosphatase	indoxyl-N-acetyl- $\beta$ -D-glucosaminide, indoxyl-phosphate	Chloramphenicol, Gentamicin
<i>Clostridium perfringens</i>	$\beta$ -glucosidase (plus sucrose fermentation)	indoxyl- $\beta$ -D-glucoside	D-cycloserine, polymyxin B
Coliforms/ <i>E. coli</i>	$\beta$ -glucuronidase, $\beta$ -galactosidase	indoxyl- $\beta$ -glucuronide, Indoxyl- $\beta$ -galactoside	bile salts, tergitol 7 <sup>®</sup> , SDS, novobiocin, cefsulodin
<i>Cronobacter (E. sakazakii)</i>	$\alpha$ -glucosidase	indoxyl- $\alpha$ -D-glucoside	deoxycholate, crystal violet, sodium thiosulfate
<i>E. coli</i> O157	$\beta$ -glucosidase, $\alpha$ -galactosidase	indoxyl- $\beta$ -D-glucuronide, indoxyl- $\alpha$ -galactoside	bile salts, SDS, crystal violet, potassium tellurite, novobiocin, cefixime
Enterococci	$\beta$ -D-glucosidase	indoxyl- $\beta$ -glucoside	sodium azide, polysorbate 80
Extended Spectrum $\beta$ -Lactamase Enterobacteria (ESBL)	$\beta$ -D-glucosidase	indoxyl- $\beta$ -glucoside	cefepodoxime, cefotaxime, ceftazidime
<i>Klebsiella</i>	$\beta$ -D-ribofuranosidase, $\beta$ -D-glucosidase	indoxyl- $\beta$ -D-ribofuranoside, indoxyl- $\beta$ -D-glucoside	bile salts, SDS, carbenicillin
<i>Listeria spp.</i>	$\beta$ -glucosidase	indoxyl- $\beta$ -glucoside	lithium chloride, ceftazidime, amphotericin B, nalidixic acid, polymyxin B
<i>L. monocitogenes</i>	Phosphatidylinositol-specific Phospholipase C, $\beta$ -glucosidase,	indoxyl- $\beta$ -glucoside, indoxyl-myo-inositol-1-phosphate	lithium chloride, ceftazidime, amphotericin B, nalidixic acid, polymyxin B
<i>Pseudomonas</i>	$\beta$ -Alanil arylamidase	7-Amido-1-pentyl-phenoxazin-3-one	ceftiridime
<i>Salmonella</i>	$\alpha$ -galactosidase, lipase	indoxyl- $\alpha$ -galactoside, indoxyl-fatty acid ester	sodium deoxycholate
MRSA (Methicillin Resistant <i>Staphylococcus aureus</i> )	$\alpha$ -glucosidase	indoxyl- $\alpha$ -D-glucopyranoside	methicillin, high concentration of sodium chloride
<i>Staphylococcus aureus</i>	$\alpha$ -glucosidase, phosphatase, deoxyribonuclease	indoxyl- $\alpha$ -D-glucoside, phenolphthalein phosphate, indoxyl-phosphate	tellurite, lithium chloride
Streptococci	$\beta$ -glucuronidase	indoxyl- $\beta$ -glucuronide	sodium azide
UTI (Urinary Tract Infections)	$\beta$ -glucosidase, $\beta$ -galactosidase	indoxyl- $\beta$ -glucopyranoside, indoxyl- $\beta$ -galactoside	-
<i>Vibrio</i>	$\beta$ -glucosidase, $\beta$ -galactosidase	indoxyl- $\beta$ -glucoside, indoxyl- $\beta$ -galactoside, indoxyl- $\beta$ -galactoside	high concentration of sodium chloride, sodium thiosulphate, sodium citrate, sodium cholate
VRE (Vancomycin Resistant Enterococci)	$\alpha$ -glucosidase, $\beta$ -glucosidase, $\beta$ -galactosidase	indoxyl- $\alpha$ -glucopyranoside, indoxyl- $\beta$ -glucopyranoside, indoxyl- $\beta$ -galactoside	vancomycin
Yeasts and Moulds	$\beta$ -N-acetylgalactosaminidase, $\beta$ -xylosidase	indoxyl-N-acetyl- $\beta$ -D-glucosaminide, indoxyl- $\beta$ -D-xyloside	oxytetracycline

Table 1 Summary of possible enzyme activities, chromogenic substrates and selectivity system for microorganisms (continued on page 8)

**Advantage of chromogenic media:**

- Faster results (compared to traditional method)
- Reliable visual detection (often no further testing required)
- Additional testing possible directly from the media

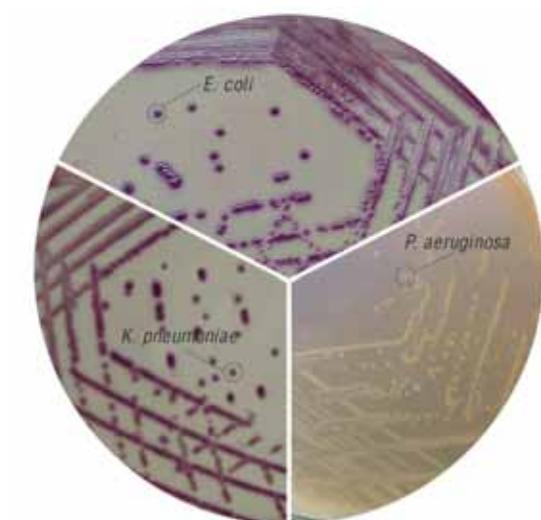
Within recent years, great strides have been taken in the sector of chromogenic media. Initial research concentrated on the use of synthetic substrates for the detection of enzymatic microbial activities. Nitrophenol and nitroaniline compounds were used at this time, producing a yellow colouration. The colour of nitrophenol, however, is influenced by a pH-change, making it difficult to use reliably in microbiology. Later developments included the use of naphthol or naphthylamine. Today, while diverse modern chromogenic substrates are available, most of the modern substrates are based on the indoxyl-substrate. The use of different chromophore and metabolite derivatives then makes it possible to detect diverse enzyme

activities all in one assay. The color of the indoxyl-substrates can be as follows: blue (5-bromo-4-chloro-3-indoxyl- = X, 3-indoxyl- =Y), magenta (5-bromo-6-chloro-3-indoxyl-), salmon (6-chloro-3-indoxyl-), purple (5-iodo-3-indoxyl-) and green (N-methylindoxyl-). One of the major advantages of the indoxyl-substrate and these chromophores is that they remain in the cell, making the characterization of a single cell possible (no diffusion into the media).

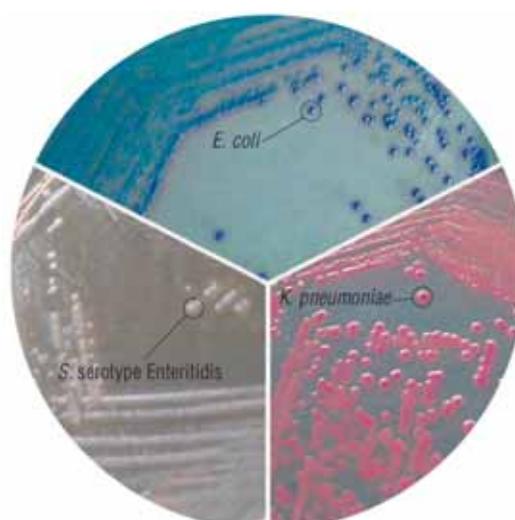
Additional advancements in the knowledge about enzyme and species specificity have also occurred within the past year. These recent gains in the development of selective agents and diverse chromogenic substrates have led toward an impressive range of chromogenic media available to meet our customers' unique analytical emphases (See **Tables 1 and 2**).

Organisms	Media
<i>Bacillus cereus</i>	Fluka® 92325 HiCrome™ Bacillus Agar
<i>Candida albicans</i>	Fluka 94382 <i>Candida Ident</i> Agar, modified
<i>Cl. perfringens</i>	Fluka 12398 CP ChromoSelect Agar
	Fluka 75605 m-CP Agar
<i>E. coli</i>	Fluka 70722 HiCrome™ <i>E. coli</i> Agar B
	Fluka 09142 HiCrome™ ECD Agar with MUG
	Fluka 92435 TBX Agar
<i>E. coli</i> & Coliforms	Fluka 81938 HiCrome™ Coliform Agar
	Fluka 73009 HiCrome™ ECC Agar
	Fluka 85927 HiCrome™ ECC Selective Agar
	Fluka 51489 HiCrome™ Rapid Coliform Broth
	Fluka 39734 Membrane Lactose Glucuronide Agar
<i>E. coli</i> O157:H7	Fluka 39894 HiCrome™ EC O157 Agar
	Fluka 72557 HiCrome™ EC O157:H7 Selective Agar, Base
	Fluka 80330 HiCrome™ Enrichment Broth Base for EC O157:H7
	Fluka 83339 HiCrome™ Mac Conkey Sorbitol Agar
Thermotolerant <i>E. coli</i>	Fluka 90924 HiCrome™ m-TEC Agar
<i>Enterobacter sakazakii</i> ( <i>Cronobacter</i> spp.)	Fluka 92324 HiCrome™ <i>Cronobacter</i> spp. Agar
	Fluka 14703 HiCrome™ <i>Cronobacter</i> spp. Agar, modified
Enterococci	Fluka 52441 HiCrome™ Enterococci Broth
	Fluka 51759 HiCrome™ Rapid Enterococci Agar
<i>Enterococcus faecium</i>	Fluka 90919 HiCrome™ <i>Enterococcus faecium</i> Agar Base
<i>Klebsiella</i>	Fluka 90925 HiCrome™ <i>Klebsiella</i> Selective Agar Base
<i>Listeria</i>	Fluka 53707 HiCrome™ <i>Listeria</i> Agar Base, modified
	Fluka 77408 <i>Listeria</i> mono Differential Agar (Base)
<i>Proteus</i> , enteropathogenic gram-positive organisms	Fluka 16636 HiCrome™ UTI Agar, modified
<i>Salmonella</i>	Fluka 00563 HiCrome™ MM Agar
	Fluka 90918 HiCrome™ RajHans Medium, Modified
	Fluka 78419 HiCrome™ <i>Salmonella</i> Agar
	Fluka 05538 HiCrome™ <i>Salmonella</i> Agar, Improved
	Fluka 84369 <i>Salmonella</i> Chromogen Agar
<i>Staphylococcus aureus</i>	Fluka 05662 HiCrome™ <i>Aureus</i> Agar Base
	Fluka 68879 Phenolphthalein Phosphate Agar
Methicillin-resistant <i>Staph. aureus</i>	Fluka 90923 HiCrome™ MeReSa Agar Base
<i>Vibrio</i>	Fluka 92323 HiCrome™ <i>Vibrio</i> Agar
Yeasts and fungi	Fluka 66481 HiCrome™ OGYE Agar Base

**Table 2** Sigma-Aldrich's product line of chromogenic media according to organisms detected (Fluorogenic media are not listed. Complete product listings are available at [sigma-aldrich.com/microbiology](http://sigma-aldrich.com/microbiology))



**Figure 2** HiCrome™ ECC Agar (Fluka 73009)



**Figure 3** HiCrome™ Coliform Agar (Fluka 81938)

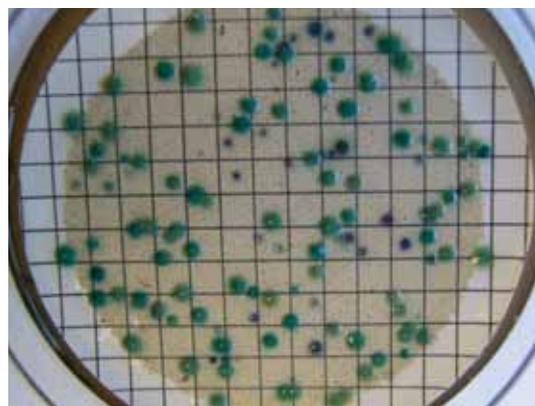
### The NEW CP ChromoSelect Agar

The European Directive on drinking water quality recommends mCP agar in the reference method for recovering *C. perfringens*. In the present study, three media (mCP, TSCF and CP ChromoSelect Agar) were evaluated for recovery of *C. perfringens* in different water samples. Out of 139 water samples tested, using a membrane filtration technique, 131 (94.2%) of the samples analyzed were found to be presumptively positive for *C. perfringens* on at least one of the culture media.

Green-colored colonies on CP ChromoSelect agar (CCP agar) were counted as presumptive *C. perfringens* isolates. Out of 483 green colonies on CCP agar, 96.9% (465 colonies, indole negative) were identified as *C. perfringens*, 15 colonies (3.1%) were indole positive and were identified as *C. sordelli*, *C. bifermentans* or *C. tetani*. Only 3 strains (0.6%) gave false positive results and were identified as *C. fallax*, *C. botulinum*, and *C. tertium*. Variance analysis of the data showed no statistically significant differences in the counts obtained between various media employed in this work.

The mCP method is very onerous for routine screening, and bacterial colonies could not be used for further biochemical testing. Conversely, the colonies on CCP and TSC were easy to count and subculture for confirmation tests. TSCF detects all sulfite-reducing clostridia, not only *C. perfringens*; however, in some cases, excessive blackening of the agar frustrated counting of the lower dilutions. If the contamination was too high, TSCF did not consistently produce black colonies and, as a consequence, the colonies' white color provided false negative results.

The identification of typical and atypical colonies isolated from all media demonstrated that CCP agar was the most specific medium for *C. perfringens* recovery in water samples.



**Figure 4** CP ChromoSelect Agar (*Cl. Perfringens* appears as green colonies, Fluka 12398)

## New Dynamic Platform for Inorganic Custom Standards

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Under the label **TraceCERT®** Sigma-Aldrich offers a wide range of Certified Reference Materials which are tailor-made for many common procedures and analytical techniques. However, every laboratory has its unique expertise, leading to many different applications. Customers who cannot find a suitable standard in our product portfolio can now use our new dynamic internet-platform for customized standard solutions.

### Reliable quality and documentation

All certified custom standards mixes are certified according to **ISO/IEC 17025** and **ISO Guide 34**. This double accreditation is the highest attestation of quality and is also called the "Gold Standard for CRM Producers". The certified values are traceable to internationally accepted references (SI unit, NIST SRM or other). Customers will get a printed lot-specific certificate according to ISO Guide 31. An example of the packaging and documentation is shown here. With our special packaging using pre-cleaned HDPE bottles in coated aluminum bags, we can ensure maximum shelf life and low uncertainties of 0.2% to 0.6% relative, depending on element composition and concentration levels. The shelf life is normally between 6 months and 3 years.



Example

### Customer benefits and workflow

One of the key benefits of customized standard solutions is time savings: making individual multi analyte blends from single stock solutions or other raw materials is time consuming and costly.

A major advantage that our new dynamic web service provides for our customers is that, while clicking on the desired elements, the customer can directly see if this combination is chemically possible or if the analyte is stable in the chosen matrix.

After examining the technical feasibility and calculation of the production, customers will get a quotation within a short time, usually within two days. The target delivery time for a custom standard mix is less than four weeks.

The order volume is generally free from minimum requirements. Nevertheless, we recommend ordering more than

one bottle of a custom standard in order to take advantage of the lower manufacturing cost per bottle. Especially for mixtures with longer shelf life, this can be a good opportunity to save money. From our long-standing expertise with the HDPE-bottles (100 mL, 250 mL or 500 mL), we can ensure the printed expiration date for each lot, since we know exactly the leaching and storage behavior of these containers.

### There are sometimes limitations

Some aspects can lead to limitations regarding the analyte concentrations in certain mixtures.

When high concentrations of some of the analytes are required (more than 1000 mg/L per analyte), their solubility in the mixture can become a limitation. On the other hand, very low concentration levels can also cause difficulties. Particularly at the ppb level, many analytes are not stable in solution for an extended time.

Another serious issue often occurs when a very high concentration of one of the analytes is required in a mixture while all the other analytes should be present in a very low concentration. In this case, the impurity profile of the highly concentrated analyte imposes certain limits. For example, consider when a mix with 5000 mg/L calcium is required together with 10 mg/L strontium and 10 mg/L sodium. Assuming that the starting material is calcium carbonate and the maximum impurities for strontium and sodium shall not exceed 1% of the target concentration (10 mg/L), the maximum allowable strontium and sodium impurity levels in the calcium carbonate are then 8 ppm. Often it is not possible to get a calcium carbonate with such a high purity. As a consequence, the realization of custom standards with high variances of analyte concentrations in one mixture is sometimes not possible and/or the analyte concentrations have high uncertainties.

### Using the platform is easy

On the landing page [sigma-aldrich.com/csp](http://sigma-aldrich.com/csp) we offer the possibility to choose between elemental standards (for AAS, ICP) and ion standards (for IC). Clicking on the corresponding links leads to an interactive periodic table (in case of the elemental standards) or to a table of anions and cations (in case of the ions standards).

### Step 1: Defining matrix

Before the elements of the standard can be chosen, the matrix has to be defined by choosing between HCl and HNO<sub>3</sub> (**Figure 1**). It is necessary to choose the matrix first, because some analytes are not compatible with both matrices.

Custom Standards Platform

Please choose your analytes

Matrix: hydrochloric acid

Analytes: Concentration mg/kg or mg/L

Iron:

Palladium:

Platinum:

Figure 2 Picking components for the multi-element standards from the periodic table of elements.

Please choose your matrix

HCl

HNO3

Figure 1 Customers can select from two different matrixes for multi-element standards

### Step 2: Defining elements or ions

The allowed elements for a specific matrix will then be marked green and the components for the standard can be picked by clicking on the green squares. The selected elements are then immediately marked in blue and a box for the definition of the concentrations appears on the right side of the periodic table (Figure 2).

For the multi-ion standards it works in the same way as for elements. One exception is that no pre-definition of the matrix is required since the matrix is automatically defined by the chosen analytes. For the anion standards, the matrix is water, whereas for cation standards, nitric acid (<0.1%) is used. With the first component defined, it is automatically defined whether a multi-anion or a multi-cation standard is composed. Thus, all cations will be disabled if you choose an anion and vice versa (Figure 3).

Bromide	Chloride	Chromate	Cyanide	Fluoride
Iodide	Nitrate	Nitrite	Phosphate	Sulfate
Chlorite	Chlorate	Perchlorate	Bromate	Thiocyanate
Thiosulfate	Sulfite			
Ammonium	Barium	Calcium	Cadmium	Cobalt
Copper	Lead	Lithium	Magnesium	Manganese
Nickel	Potassium	Sodium	Strontium	Zinc
Cesium				

Figure 3 List of anions and cations (in green) that can be chosen. This portfolio will be expanded continuously.

### Step 3: Defining concentrations and package size

After all components are chosen for the custom standard, the concentrations for all of the components must be entered before clicking the "continue" button. After this, the user can specify whether the custom standard should be produced in mass per mass (mg/kg) or mass per volume (mg/L) units. Finally, the number of required bottles and/or the total volume can be entered. The choices for container volume are 100 mL, 250 mL, or 500 mL HDPL packages, or a freely selected bulk quantity (Figure 4).

Matrix: hydrochloric acid

Analytes: Concentration mg/kg or mg/L

Iron:

Palladium:

Platinum:

Unit:  mg/kg  mg/L

Volume:  x 100 mL  
 x 250 mL  
 x 500 mL  
 mL Bulk

Figure 4 Required information for a custom standard request

After all parameters have been defined, the request can be submitted online and a summary of the request can be printed out before sending. With the new Custom Standards Platform, Sigma-Aldrich is proud to offer an easy and fast way for the design of certified custom standards. Try it!

## Certified Reference Materials for Spectroscopy

Matthias Nold, Product Manager Analytical Standards [matthias.nold@sial.com](mailto:matthias.nold@sial.com)

Optical Emission (OES) and X-Ray Fluorescence (XRF) Spectroscopy are efficient and fast techniques to determine the composition of metals, including trace elements as well as the major alloying ingredients. These techniques are therefore widely used for compositional control in the metal industry. The principle of OES is based on spark-discharge excitation of the solid sample, with measurement of the emitted light; this allows for quantification of the elements present. The principle for XRF is similar, although the excitation is provided by X-rays.

To ensure accurate measurement values, the instruments should be calibrated and monitored using one or more appropriate reference materials.

Sigma-Aldrich is launching a new product line of certified reference materials (CRMs) for spectroscopy. Certified values are given for the major alloy ingredients and the most rele-

vant impurities for each corresponding metal or alloy. The CRM samples (in the form of solid discs) are produced in the United Kingdom by MBH Analytical Ltd, a recognized manufacturer in the field of reference materials for spectroscopic analysis.

MBH CRM discs are made by casting metals that have been prepared to special formulations, usually including a range of (undesirable) trace elements. Products are checked for homogeneity, and then traceable analysis is performed by an international panel of analysts mostly operating within the terms of ISO/IEC 17025. All stages of processing, analysis and certification are in accordance with ISO guidelines for the preparation of CRMs, and each disc is supplied with a certificate showing its composition and details of the manufacturing and analytical techniques involved in its preparation.

Cat. No.	Description	Certified Values %																								
		C	Si	S	P	Mn	Ni	Cr	Mo	Cu	Sn	Al	V	W	As	Co	Nb	Ti	Pb	Sb	B	Ta	N			
97593	Steel (low Alloy)	0.261	0.753	0.0476	0.043	1.19	0.886	2.09	0.788	0.130	0.047	1.136	0.296	0.358	....	0.470	0.322	0.260				0.26	....			
05602	Duplex Steel	C	Si	S	P	Mn	Ni	Cr	Mo	Cu	Al	V	W	Co	Nb	N										
		1.492	1.89	0.0098	10.97	10.97	3.03	24.71	0.086	0.114	(0.028)	0.126	....	....	2.47	0.478										
51870	Copper-Nickel Alloy	C	Si	Mn	Cu	Fe	Cr	Mo	Co	Ti	Al	Mg	S	P	Pb	Nb	Cd	Ni								
		0.0051	0.293	1.00	30.48	1.24	0.073	0.023	0.029	(0.063)	0.086	0.035	0.0069	0.0054	0.0134	0.156	....	66.58								
51479	Leaded Brass	Pb	Sn	Zn	Fe	Ni	Al	Si	As	Bi	Sb	P	S	Co	Se	Cd	B	Cu								
		1.274	0.0912	35.34	0.299	0.282	0.384	0.022	0.098	0.0257	0.104	0.042	....	0.0053	0.0010	0.0030	0.0014	62.10								
56912	Aluminium Bronze	Sn	Pb	Zn	Fe	Ni	Al	Si	As	Mn	P	Cr	Co	Mg	Ag	C	Cu									
		0.103	0.266	0.247	4.05	4.53	9.60	0.295	0.0066	0.056	0.048	0.0031	....	0.0035	....	....	80.70									
97625	Phosphor Bronze	Sn	Pb	Zn	Fe	Ni	Al	Si	As	Mn	Bi	Sb	P	S	Co	Mg	C	Se	Cu							
		3.39	1.02	1.48	0.555	0.90	(0.001)	0.097	0.199	0.195	0.0033	0.470	0.885	0.023	0.095	0.0035	....	....	90.6							
74808	Gunmetal	Sn	Pb	Zn	Fe	Ni	Al	Si	As	Mn	Bi	Sb	P	S	Cr	Co	Ag	Cu								
		5.22	5.13	4.19	0.136	1.008	(0.007)	(0.001)	0.059	0.0018	0.0225	0.093	0.0112	0.061	....	0.0662	0.0099	83.98								
66955	Copper (Impurities)	Sn	Pb	Zn	Fe	Ni	Al	Si	As	Mn	Bi	Sb	P	S	Co	Cr	Cd	Mg	Ag	Au	Se	Te	In			
		(37)	460	(24)	15	211	2	....	196	23	202	200	(20)	56	2	....	23	....	323	129	120	240	9			
96687	Galvanizing Alloy	Pb	Mg	Al	Cd	Fe	Sn	Cu	Ni	Mn	Bi	Sb	As	Cr	Co	V										
		0.0062	0.0034	0.514	0.0006	0.0028	0.0024	0.0321	0.0441	0.0089	0.0061	0.0287	(0.0003)	0.0007	0.0037	....										
95653	Zinc (Impurities)	Pb	Mg	Al	Cd	Fe	Sn	Cu	Ni	Mn	Bi	Sb	Ti	Tl	In	Si	Hg									
		0.0026	0.0001	0.0008	0.0017	0.0077	0.0016	0.0018	0.0013	0.0005	0.0005	0.0006	....	0.0012	0.0006	....	0.0003									
49892	Zinc-Aluminium-Copper Alloy	Pb	Mg	Al	Cd	Fe	Sn	Cu	Ni	Mn	Bi	Sb	Ti	Cr	Si	Be										
		0.0106	0.0828	3.89	0.0053	0.0081	0.0099	1.004	0.0024	0.0086	0.0022	0.0068	0.0008	0.0063	0.011	....										
43998	Aluminium -Magnesium Alloy	Cu	Mg	Si	Fe	Mn	Ni	Zn	Pb	Sn	Ti	Cr	Co	V	Be	Cd	Ga	Zr	Na	B	Li	As	Mo			
		0.093	3.97	0.21	0.41	0.40	0.090	0.092	0.107	0.096	0.25	0.095	....	....	0.0074	....	....	....	....	....	....	....	....			
91927	Aluminium-Silicon-Copper Alloy	Cu	Mg	Si	Fe	Mn	Ni	Zn	Pb	Sn	Ti	Cr	Co	V	Be	Bi	Sb	Ag	Ca	P	Cd	Zr				
		1.48	0.304	10.19	1.001	0.547	0.234	1.688	0.292	0.368	1.57	0.0106	0.0179	0.0179	0.0025	....	0.0042	....	....	....	0.0032	....				
67226	Tin (Impurities)	As	Bi	Sb	Pb	Cu	Fe	Cd	Zn	Ni	Al	Ag	S	In	Au	Te	Se	Ga	Hg							
		0.0008	(0.0025)	0.0034	0.0113	0.0013	0.0008	0.0015	0.0034	0.0004	0.0197	0.0018	....	0.0047	0.0007	0.0009	0.0005	0.0027	0.0044							
12242	Tin-Base Solder (Lead free)	As	Bi	Sb	Pb	Cu	Fe	Cd	Zn	Ni	Al	Ag	P	Se	Ge											
		0.045	0.175	1.06	0.119	2.97	(0.016)	0.0072	(0.002)	0.0203	(0.008)	0.562	0.018	0.0115	....											
80901	Lead (Impurities)	Sn	Sb	Bi	Cu	As	Ag	Fe	Zn	Cd	In	Ni	Te	Se	Au	Tl	Na	Hg	Pt							
		0.603	0.259	1.186	0.066	0.155	0.505	....	....	0.211	0.224	0.0020	0.0011	0.0064	0.0082	....	0.0073	0.089	....							
30978	Battery Alloy with Calcium	Sn	Sb	Bi	Cu	As	Ag	Zn	Cd	Ni	Al	Te	Ca	Hg												
		0.899	(0.002)	0.0174	0.0009	0.0007	0.0082	0.0048	0.0019	(0.0004)	0.0240	(0.0005)	0.0960	....												
87610	Lead (high Alloy)	Sn	Sb	Bi	Cu	As	Ag	Fe	Zn	Cd	In	Ni	Te	Se	Au	Tl	S	Hg								
		1.45	6.05	0.0194	0.0291	0.217	0.0071	(0.010)	(0.0007)	0.0046	....	0.0062	0.0071	0.0149	....	....	(0.0036)	....								

Table 1 Spectroscopy Products with Certified Values

## Enantiomeric Purities of Pharmaceuticals Using Carbohydrate-based Isothiocyanates

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### Introduction

In the first two papers of this series (Analytix Nr. 1/2010 and 2/2010), the efficient enantiomeric analysis of (a) alkyl oxiranes, and (b) amino acids was described using inexpensive reversed phase columns (as an alternative to high cost so-called “chiral columns”) in combination with monosaccharide based isothiocyanates as derivatization reagents (**Figure 1**). While alkyloxiranes have to be converted first into the corresponding  $\beta$ -amino alcohols by reaction with isopropyl amine, all amino acids (proteinogenic, non-proteinogenic and non-natural) can be converted directly (straight out of the bottle or the body fluid, respectively) into the corresponding diastereomeric thioureas, which can be injected directly into the HPLC. Base-line separations were observed in almost all cases, establishing a highly efficient and generally applicable method for the analysis of these classes of molecules.

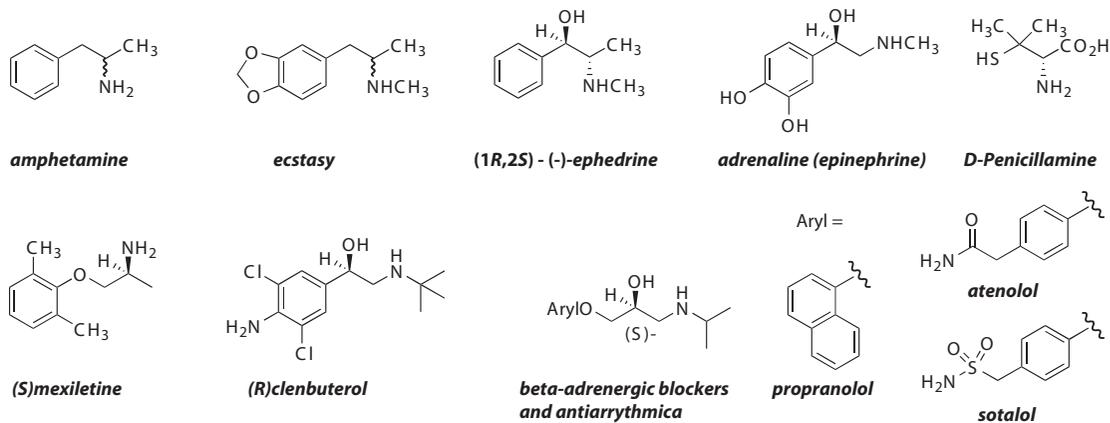
### Pharmaceuticals

The carbohydrate-based isothiocyanates have also been shown to be highly suitable for the enantiomeric analysis of neurotransmitters (e.g. adrenaline and related molecules), numerous pharmaceuticals carrying functional amino groups, such as  $\beta$ -adrenergic blockers, various pharmaceuticals such as penicillamine and mexiletine, and fine chemicals such as 1-phenyl-2-aminoethanol. Representative examples of these classes of molecules are shown in **Figure 1**.

The close relationship between the biological (physiological) activities of these molecules and their absolute configurations is well established. Frequently, only one enantiomer (*Eutomer*) has the desired pharmacological activity, while its antipode is inactive (*Distomer*), shows undesired side-effects, or is even toxic. Inactive enantiomers are also frequently referred to as *Xenobiotics* in the sense of pollutants. Several of these compounds are known for their illicit use in doping,

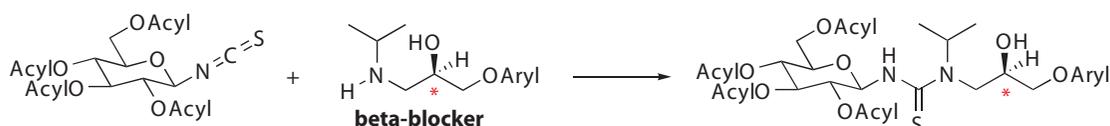
as narcotics or psychotropic agents, and for their illegal use in food and feed. It is well established that in so-called  $\beta$ -adrenergic blockers, the pharmacological activity resides in the (*S*)-enantiomers, while the (*R*)-enantiomer of penicillamine is highly toxic. On the other hand, the neurotransmitter activity of adrenaline resides largely in the (*R*)-enantiomer. Many more similar examples can be found in the literature. Clearly, in view of the fact that novel pharmaceuticals of this kind are increasingly used in the form of single enantiomers, the determination of enantiomeric ratios/purities is of increasing importance. Examples include the monitor of (a) enantioselective syntheses, (b) quality control in manufacturing, (c) stability and metabolic rate in biological systems (e.g. serum), and (d) analysis of illicit drugs and narcotics in body fluids (e.g. doping/body building/athletic sports). Advantageously, all of these compounds can be analyzed without any prior manipulation (“straight out of the bottle” or the reaction medium, e.g. biological fluids). They react under mild conditions and at a rapid rate (at room temperature) with the mono-saccharide isothiocyanates, leading to the corresponding diastereomeric thioureas, as exemplified in **Figure 2**. These, in turn, can be injected – without the need for further purification – directly into the HPLC.

As derivatives of natural mono-saccharides (**Figure 1**), all of the employed reagents are enantiomerically pure by definition, and the ratios of thus produced diastereomers directly reflect the enantiomeric composition of the chiral amino compound in question. This requires, of course, that both enantiomers of a racemic mixture react rapidly and quantitatively, and with the same rate in order to avoid diastereoselectivity during the derivatization process. For new target molecules, this must be ascertained in every case by calibration with the corresponding racemate. The described strategy frequently has distinct advantages over



**Figure 1** Pharmacologically active molecules with functional amino groups

(continued on page 14)



**Figure 2** Pharmaceuticals (here a *beta-blocker*) with functionalized amino groups: formation of diastereomeric thioureas (schematic) [\* denotes center of chirality]

the so-called *direct* method employing chiral stationary phases in that (a) the separation of diastereomers is usually more simple to perform and often provides better resolutions, (b) the choice of chromatographic conditions is much greater and thus can be more easily optimized, and (c) the reagents contain chromophores (fluorophores) for convenient UV- or fluorescence detection. In view of the range of novel derivatization reagents which recently became available (PGITC, PGallTC, NGallTC) [5], the method is an interesting alternative to so-called *chiral columns*.

In principle, all of the above reagents can be employed for the above pharmaceuticals. Thus, Nimura et al. [1] achieved base-line separations in the analysis of adrenaline (epinephrine) and noradrenaline (norepinephrine) using GITC (Aldrich T5783) and AITC (Fluka 90245). Adrenaline is only present in minute quantities in lidocain local anesthetics; nevertheless this method allowed the quantitative determination of the enantiomeric ratio in more than 250 commercially available anesthetics [2]. Using the same reagents, a series of differently substituted amphetamines were analyzed [3]. The method also works well for the analysis of ephedrine, pseudoephedrine and norephedrine (4). Further examples include the enantiomeric analysis of the antiarrhythmic agent mexiletine in human plasma using GITC [5] and the corresponding analysis of a whole series of beta-adrenergic antagonists (*β-blockers*) such as propranolol, atenolol and sotalol (**Figure 2**) [6]. The introduction of benzoyl groups (BGITC Aldrich 335622) [7] and naphthoyl groups (NGallTC Fluka 04669) [8] considerably enhanced the UV- and fluorescence detection of the corresponding thioureas by factors of 6 (BIGTC) to 40 (NGallTC). Furthermore, the introduction of these residues frequently resulted in a considerable improvement in the separation of these diastereomers, as did the incorporation of extremely bulky pivaloyl groups such as in PGITC (Fluka 44891) and PGallTC (Fluka 88102). It should also be noted that the described methodology is not limited to the referenced pharmaceuticals, but can be potentially extended to all chiral compounds carrying functional amino groups such as a wide variety of amino alcohols.

PGallTC	t <sub>1</sub> (min)	t <sub>2</sub> (min)	D t <sub>1/2</sub> (min)
norleucin	9.29	10.81	1.52
sotalol	20.68	22.10	1.42
atenolol	20.49	21.90	1.41
penicillamine	10.93	12.74	1.81
DOPA	20.32	24.41	4.09
2-amino-1-phenylethanol	21.01	22.31	1.30

**Table 1** Mobile phase: acetonitrile: 0.1% TFA/H<sub>2</sub>O = 70:30

NGallTC	t <sub>1</sub> (min)	t <sub>2</sub> (min)	D t <sub>1/2</sub> (min)
atenolol	24.53	27.01	2.48
sotalol	23.20	25.28	2.08
penicillamine	25.50	28.41	2.91
clenbuterol	23.70	27.45	3.75

**Table 2** Mobile phase: acetonitrile : 0.1% TFA/H<sub>2</sub>O = 60:40

BGITC	k <sub>s</sub>	k <sub>R</sub>	a
propranolol	2.65	3.65	1.38
pindolol	16.88	18.95	1.12
atenolol	12.89	16.81	1.30
sotalol	11.94	13.90	1.16
penicillamine	7.37	10.05	1.33

**Table 3** Mobile phase: MeOH: H<sub>2</sub>O = 80:20 to 90:10

While simple RP-18 columns are generally employed, the separation conditions can be varied widely in order to achieve the best separating conditions. Various different mobile phases have been used ranging from MeOH : phosphate buffer (pH 2.8) [1] over MeOH : H<sub>2</sub>O : phosphate buffer (pH 7) to acetonitrile : water : 0.1% trifluoroacetic acid in order to optimize the separation conditions. In certain cases the reagent may interfere with the separation, having the same or similar retention time. The addition of small amounts of ethanolamine or hydrazine is sufficient to destroy excess reagent by formation of the corresponding thioureas, which elute at different retention times.

### Summary

The method described above allows the rapid, efficient and inexpensive determination of enantiomeric purities in a wide variety of structurally varied pharmaceuticals and fine chemicals. By using the suitable derivatization reagent, base-line separations are observed in nearly all cases. The procedure is quite general and applicable to (a) detecting enantiomeric ratios of pharmaceuticals, in addition to biological samples; (b) determining racemizations and differences in metabolic degradation; (c) monitoring asymmetric syntheses; and (d) detecting molecules in illicit drug abuse and doping. The method is clearly adaptable to automation using reaction batteries and auto-samplers. The technique is applicable both on a laboratory scale and in on-line quality control. It is thus highly suitable for monitoring asymmetric syntheses including enzyme-catalyzed transformations.

## Experimental

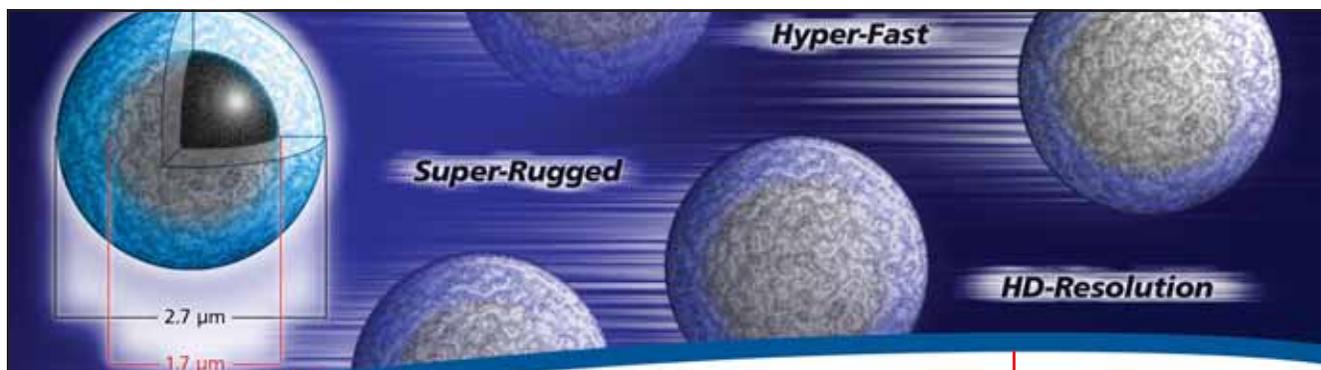
5 mg of the corresponding pharmaceutical is dissolved in 50% (v/v) aqueous acetonitrile (or dimethylformamide) containing 0.55% (v/v) triethyl amine (in this case, hydrochlorides are employed) to give a final volume of 10 mL. To 50  $\mu$ L of this stock solution 50  $\mu$ L of 0.66% (w/v) BGITC in acetonitrile is added. The resulting solution is shaken on a laboratory shaker for 30 min, after which 10  $\mu$ L of 0.26% (v/v) ethanolamine (or hydrazine) in acetonitrile is added and shaking is continued for another 10 min. Ethanolamine (hydrazine) reacts with any excess of BGITC and the resulting thiourea derivative is eluted well behind any of the amino acid derivatives. The mixture is then diluted to a final volume of 1 mL and a 10  $\mu$ L aliquot is injected into the HPLC. (RP-18, mobile phase MeOH : H<sub>2</sub>O [67 mM phosphate buffer (pH 7) = 65:27:8 up to 70:25:5 and 80:15:5], depending on the case, flow rate 0.5 mL/min, compare Tables).

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Cat. No.	Brand	Description	Abbr.	Package Size
90245	Fluka®	2,3,4-Tri-O-acetyl- $\alpha$ -D-arabinopyranosyl-isothiocyanate	AITC	100 mg, 500 mg
T5783	Sigma	2,3,4,6-Tetra-O-acetyl- $\beta$ -D-glucopyranosyl-isothiocyanate	GITC	100 mg, 1 g
335622	Aldrich	2,3,4,6-Tetra-O-benzoyl- $\beta$ -D-glucopyranosyl-isothiocyanate	BGITC	500 mg
44891	Fluka	2,3,4,6-Tetra-O-pivaloyl- $\beta$ -D-glucopyranosyl-isothiocyanate	PGITC	100 mg
88102	Fluka	2,3,4,6-Tetra-O-pivaloyl- $\beta$ -D-galactopyranosyl-isothiocyanate	PGallITC	100 mg, 500 mg
04669	Fluka	2,3,4,6-Tetra-O- (2-naphthoyl)- $\beta$ -D-galactopyranosyl-isothiocyanate	NGallITC	25 mg, 100 mg

Product Table for Carbohydrate-based Isothiocyanates



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Cat. No.	Brand	Product Name	Concentration	Pack sizes
07692	Fluka®	Acetic acid	≥ 99.0%	250 mL/1 L
16748	Fluka	Ammonium hydroxide solution	≥ 25%	250 mL/1 L
23828	Fluka	Hydrobromic acid	≥ 44%	250 mL/1 L
96208	Fluka	Hydrochloric acid	≥ 30%	250 mL/1 L
02658	Fluka	Hydrofluoric acid	≥ 49%	250 mL/1 L
16911	Fluka	Hydrogen peroxide solution	≥ 30%	250 mL/1 L/5 L
02650	Fluka	Nitric acid	~ 65%	250 mL/1 L
64957	Fluka	Phosphoric acid	≥ 85%	250 mL/1 L
77239	Fluka	Sulfuric acid	≥ 95%	250 mL/1 L
14213	Fluka	Tetramethylammonium hydroxide solution (TMAOH)	~ 25% in H <sub>2</sub> O	250 mL
14211	Fluka	Water		1 L



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FCISI004	4 g 40–60 µm	irregular	20
FCISI012	12 g 40–60 µm	irregular	20
FCISI025	25 g 40–60 µm	irregular	15
FCISI040	40 g 40–60 µm	irregular	15
FCISI080	80 g 40–60 µm	irregular	12
FCISI120	120 g 40–60 µm	irregular	10
FCISI240	240 g 40–60 µm	irregular	4
FCISI330	330 g 40–60 µm	irregular	4
97787-U	11 g 20–45 µm	spherical	20
97788-U	23 g 20–45 µm	spherical	20
97789-U	50 g 20–45 µm	spherical	12
97790-U	100 g 20–45 µm	spherical	6

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Cat. No.	Bed Wt.	Tube Volume	Silica Type	Qty/Pk
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97716-U	20 g 70 mL	40–63 µm	irregular	16
97717-U	50 g 150 mL	40–63 µm	irregular	8
97718-U	70 g 150 mL	40–63 µm	irregular	8

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## Analysis of Bioethanol – Detection of Chloride and Sulfate with Dication and Trication Solutions

R. Köhling, N. Reichlin, Sigma-Aldrich Production GmbH (CH), R&D – LC/MS Applications [rudolf.koehling@sial.com](mailto:rudolf.koehling@sial.com)



Biodiesel and bioethanol are increasingly being used as renewable energy sources to replace the use of fossil fuels for common combustion engines. Diesel engines can be converted for use with plant oils or other fats, but significant changes must be made to the vehicle. Modern biodiesel is an alternative to plant oils and used increasingly in diesel vehicles, e.g. trucks. There is also a renewable fuel source for gasoline engines which can run on ethanol-gasoline blends instead of pure gasoline. This sourcing of fuels from biomass is a common technique in countries with a large corn, sugar cane or general biomass emergence (USA, Brazil). The bioethanol can be obtained in large amounts through

fermentation and distillation and is added to gasoline in varying amounts, e.g. 85% ethanol in E85 fuel [1]. Bioethanol and biodiesel contain several impurities despite passing through several cleaning steps. Especially concerning are dissolved salts which can damage modern engines. Thus, the determination of sulfate and chloride in ethanol-based fuels is an important quality criterion. A standard detection method for these analytes is ion chromatography configured with a conductivity detector [2]. It is a very sensitive analytical technique, but it lacks the capability to definitively identify the compounds in addition to retention time. An alternative to IC can be Di- (75128) and tricationic organic compounds (08675), which form positively charged adducts with chloride and sulfate anions, making them detectable for mass spectroscopy in the highly sensitive positive ESI mode [3]. The use of LC/MS also has the advantage of easy sample handling. For a large number of matrices, the samples can be injected without further treatments.

Independent of analytical techniques, the quality of quantitative results strongly depends on the precision and accuracy of the analytical reference standards. A robust calibration method still can lead to results with a high uncertainty, when the content of the reference material is not well defined. In this case, matrix samples are spiked with **TraceCERT®** anion standards for ion chromatography in order to ensure the highest accuracy for the calibration data.

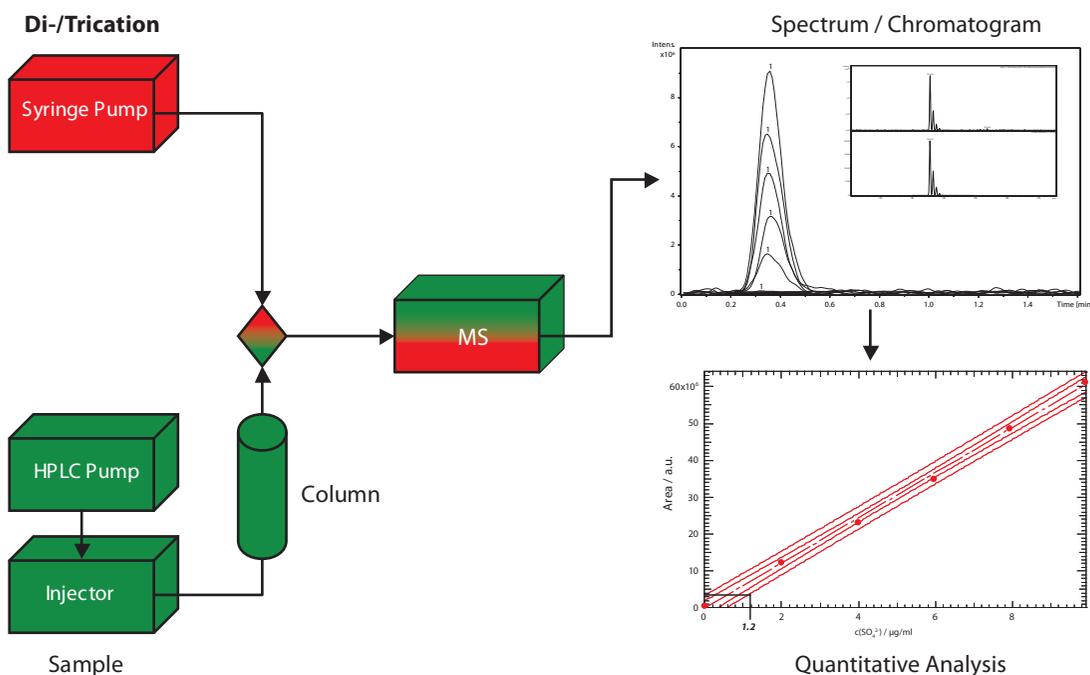


Figure 1 Schematic drawing of the LC/MS set-up and the data processing of the MS data

## Method

There are two favored methods for the application of multiply charged cation solutions with LC/MS: (1) a direct injection of a mixture of cation solution, sample and solvent (e.g. methanol/water) into the MS, or (2) the injection of the sample into the constant flow of a mobile phase, which is mixed with a cation solution post-column via a T-connector (**Figure 1**).

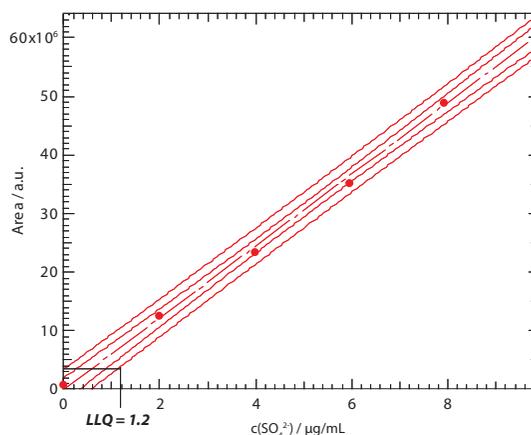
The second method is preferred for the analysis of biofuels since it provides the highest sensitivity and opens the possibility to change all compositions during the method development. Additionally, an analytical column can remove parts of the matrix to prevent suppression effects. However, one should be aware that ionic analytes could interact with metal parts throughout the entire HPLC system, columns or tubing. If this happens and peak broadening occurs, then PEEK tubing, injectors and columns can prevent this negative influence on the peak height and shape.

Starting with the HPLC, the pump delivers a typical mobile phase water and methanol (90/10, v/v) at a low flow rate of 0.2 ml/min. Additives like formic acid, TFA or acetic acid should not be used, as they will form adducts with the multi-cationic reagent and will lower its efficiency. The solvents should have the best available quality to minimize bias and noise. Reducing the flow rate also reduces suppression effects caused by sample matrices [4]. The di- and tricationic fluoride solution is added to the mobile phase with a flow rate of 80  $\mu\text{l/h}$ , but can be increased in case of low signal intensity of the adduct (ion pair of di-/trication and anion).

The calibration samples are prepared according to DIN 38402 part 51 and 32645 with equidistant concentrations. 5 calibration levels and 1 blank sample cover a concentration range from 2 to 10  $\mu\text{g/ml}$  and result in a typical limit of detection (LOD) of 0.5  $\mu\text{g/ml}$  and a lowest limit of quantification (LLQ) of 1.2  $\mu\text{g/ml}$  (**Figure 2**). Ethanol and water serve as matrix for the preparation of the calibration standards. Finally a sample of the lab water supply is analyzed without any sample pre-treatments.

## Discussion

Solutions of di- and tricationic compounds can easily be used for the determination of chloride and sulfate in ethanol or drinking water. The lab water supply was directly injected into the HPLC system and resulted in a typical chloride concentration of 4 ppm. The solutions of the



**Figure 2** Typical calibration curve for sulfate ions in water detected as trication-sulfate adduct. Similar results are obtained for chloride ions in ethanol detected as a dication-chloride adduct. Correlation coefficients of 0.996 to 0.999 are obtained for the linear fit of the calibration data. The calculation of the confidence and prediction bands is based on a confidence level of 0.95.

di-/trications are supplied in a condition capable of being used directly in mixing with the mobile phase post-column. This set-up is installed on most LC/MS systems and does not need additional items except a syringe pump.

Mass spectroscopy with electrospray ionization (ESI) has the advantage of a very sensitive and selective detection, which is responsible for low LODs and LLQs as can be seen in **Figure 2**. Although 2 ppm is the lowest calibration level, it is possible to get lower LLQs even at a confidence level of 0.99 because of the steep regression curve. One important advantage of MS is its capability to definitively identify analytes by their mass (exact mass), isotopic pattern and MS/MS spectra.

In addition to IC, this LC/MS method can also be a useful tool for the analysis of anions in complex matrices like biofuels, especially for those laboratories equipped with an LC/MS system.

## References

- [1] [www.bio-kraftstoffe.info](http://www.bio-kraftstoffe.info)
- [2] Norm ASTM D4806 "Standard Specification for Denatured Fuel Ethanol for Blending with Gasolines for Use as Automotive Spark-Ignition Engine Fuel".
- [3] R. Köhling, N. Reichlin, "Highly Sensitive Detection of Organic and Inorganic Anions with Di- or Tricationic LC/MS Additives", *Analytix*, 2, 2009.
- [4] F. Gosetti, E. Mazzucco, D. Zampieri, M. Gennaro, "Comparison of Matrix Effects in HPLC-MS/MS and UPLC-MS/MS Analysis of Nine Basic Pharmaceuticals in Surface Waters", *J. Am. Soc. Mass. Spectrom.*, 2008, 19, 713–718.

Cat. No.	Brand	Description	Package Size
8675	Fluka®	1,3-Bis[6-(3-benzyl-1-imidazolium)-hexyl]imidazolium trifluoride solution	100 mL
75128	Fluka	1,9-Nonanediy-bis(3-methylimidazolium) difluoride solution	100 mL
79254	Fluka	1,3,5-Tris[(3-butyl-imidazolium)methyl]-2,4,6-trimethylbenzene trifluoride solution	100 mL
78897	Fluka	1,3,5-Tris[(tripropyl)phosphonium)methyl]benzene trifluoride solution	100 mL
56618	Fluka	1,5-Pentanediy-bis(1-butylpyrrolidinium) difluoride solution in water/methanol 1:1	100 mL
76507	Fluka	1,5-Pentanediy-bis(3-benzylimidazolium) difluorid solution in water/methanol 1:1	100 mL

## Increasing Reliability and Value of Analytical Results

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**Helga Hoffmann, Technical Service HYDRANAL Manager** [helga.hoffmann@sial.com](mailto:helga.hoffmann@sial.com)  
**Andrea Felgner, Product Manager Analytical Reagents** [andrea.felgner@sial.com](mailto:andrea.felgner@sial.com)

Sigma-Aldrich's HYDRANAL® service laboratory in Seelze, Germany, proved its expertise for testing and calibration and achieved accreditation as a testing laboratory according to ISO/IEC 17025:2005 "General requirements for the competence of testing and calibration laboratories". It was audited by the German Accreditation Council (Deutscher Akkreditierungsrat DAR) and received accreditation for titration and gravimetry for water standards and reagents for Karl Fischer titration.

From the beginning of our HYDRANAL product line over 30 years ago, the HYDRANAL technical service in Seelze has applied a high standard and continual improvement of testing methods for production and quality control of HYDRANAL water standards and HYDRANAL Karl Fischer reagents. These high-quality testing methods have now been proven to comply with the high standards of the auditors and have led to the accreditation of HYDRANAL Technical Service according to ISO/IEC 17025. This accreditation includes all HYDRANAL products, and the accreditation details, along with the logo of the accreditation body, will now appear on the Certificate of Analysis (see **Figure 1**) and on the label for each produced lot.

### Accreditation requirements and procedure

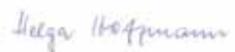
Accreditation means that an authoritative body formally recognizes that an organization or individual is competent to execute a specific service as described in the scope of

accreditation. ISO/IEC 17025 is associated with a well-defined analytical technique and a stated measurement range comprising analyte, matrix and concentration range. This is called the scope of the accreditation. In this case, HYDRANAL Technical Service is accredited to perform titration and gravimetry for water standards and reagents for Karl Fischer titration according to the following methods/norms: ISO 760:1978-12, ASTM E203-08 and HYDRANAL Technical Service's in-house methods 01-04.

To fulfill the requirements of ISO/IEC 17025, a laboratory must demonstrate its general competence and compliance in performing specific tests or calibrations, as well as establishing and documenting its quality management system. Beyond the basic management requirements of ISO 9001, ISO/IEC 17025 requires the following topics:

- Instrument qualification
- Validation of analytical methods
- Traceability statements
- Evaluation of measurement uncertainty
- Ongoing education of personnel
- Periodic participation in proficiency tests to demonstrate technical capability

Following the accreditation, evidence of continued improvement is required, including regular internal audits and the demonstration of efforts towards scientific and technological progress.

<b>Certificate of Analysis</b>		 <b>Fluka</b> Analytical	<a href="http://sigma-aldrich.com">sigma-aldrich.com</a>
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<b>HYDRANAL®-Standard Sodium tartrate dihydrate</b> , reference no. 34696, is intended for the titre determination of volumetric Karl Fischer reagents. The certified water content of this Lot (SZE8347B, Exp.date Nov. 2013) is			
<b>15.63% (expanded uncertainty = 0,02%, k=2.78).</b>			
The water content is analyzed by loss on drying at 150 °C on 5 samples.			
HYDRANAL®-Standard Sodium tartrate dihydrate can be used for standardization of KF reagents according to ISO 760 or ASTM E 203. This standard is traceable to SI-Unit (kg) and tested against <b>NIST SRM 2890</b> .			
Sigma-Aldrich Laborchemikalien GmbH Helga Hoffmann Manager Technical Service HYDRANAL® Wunstorfer Str.40, D-30926 Seelze	 Deutscher Akkreditierungsrat DGA-PL-6670.09		QC release date Seelze, 19.02.2010 page 1 of 1

**Figure 1** Example of HYDRANAL Certificate of Analysis with accreditation details



Analytical results produced in laboratories fulfilling ISO/IEC 17025 can be labeled with a special quality logo that confirms the reliability of the results. Every country has at least one official body that is responsible for accreditation, and each body signifies its accreditation with its own unique logo. Since HYDRANAL® Technical Service was accredited by DAR, the DAR symbol and the individual registration number of the laboratory must appear on the certificate of Karl Fischer reagents and water standards. Beginning in 2010, the authoritative body for accreditations in Germany is the Deutsche Akkreditierungsstelle (DAkkS), which will also administrate accreditations issued by former authorities.

Helga Hoffmann

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**Figure 2** Official accreditation document for HYDRANAL Technical Service

Cat. No.	Product	Description	Pack Size
34849	HYDRANAL-Water Standard 10.0	Standard for volumetric KF titration 1 g (= 1 mL at 20 °C) contains 10.0 mg = 1.00% water Contains 10 glass ampoules of 8 mL. Tested against NIST SRM 2890	80 mL
34828	HYDRANAL-Water Standard 1.00	Standard for coulometric KF titration 1 g (= 1 mL at 20 °C) contains 1.00 mg = 0.10% water Contains 10 glass ampoules of 4 mL. Tested against NIST SRM 2890	40 mL
34847	HYDRANAL-Water Standard 0.10	Standard for coulometric KF titration 1 g contains 0.10 mg = 0.01% water Contains 10 glass ampoules of 4 mL. Tested against NIST SRM 2890	40 mL
34694	HYDRANAL-Water Standard Oil	Mineral oil-based standard with water content in low ppm-range (CoA) Contains 10 glass ampoules of 8 mL	80 mL
34693	HYDRANAL-Water Standard KF-Oven 140–160 °C	Solid standard for control of KF Ovens, Water content ~5%	10 g
34748	HYDRANAL-Water Standard KF-Oven 220–230 °C	Solid standard for control of KF Ovens, Water content: 5.55 ± 0.05%	10 g

**Table 1** HYDRANAL Water Standards (ISO/IEC 17025 accreditation is valid for all Water standards and Karl Fischer reagents)

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38283	Fluka	Hydrochloric acid std., concentrate, pkg of 10 mol (364.61 g HCl)
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38073	Fluka	Potassium hydroxide std., concentrate, pkg of 1.0 mol (56.109 g KOH)
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38215	Fluka	Sodium hydroxide std., concentrate, pkg of 1.0 mol (39.997 g NaOH)
38212	Fluka	Sodium hydroxide std., concentrate, pkg of 2.0 mol (79.994 g NaOH)
38214	Fluka	Sodium hydroxide std., concentrate, pkg of 10 mol (399.97 g NaOH)
38243	Fluka	Sodium thiosulfate std., concentrate, pkg of 0.01 mol (2.482 g Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O)
38200	Fluka	Sodium thiosulfate std., concentrate, pkg of 0.1 mol (24.818 g Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O)
38308	Fluka	Sulfuric acid standard std., concentrate, pkg of 0.005 mol (0.4904 g H <sub>2</sub> SO <sub>4</sub> )
32043	Fluka	Sulfuric acid standard std., concentrate, pkg of 0.05 mol (4.904 g H <sub>2</sub> SO <sub>4</sub> )
38295	Fluka	Sulfuric acid standard std., concentrate, pkg of 0.25 mol (24.519 g H <sub>2</sub> SO <sub>4</sub> )
38294	Fluka	Sulfuric acid standard std., concentrate, pkg of 0.5 mol (49.039 g H <sub>2</sub> SO <sub>4</sub> )
38291	Fluka	Sulfuric acid standard std., concentrate, pkg of 1.0 mol (98.078 g H <sub>2</sub> SO <sub>4</sub> )
32044	Fluka	Sulfuric acid standard std., concentrate, pkg of 5 mol (490.39 g H <sub>2</sub> SO <sub>4</sub> )

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## Analytical Standards of Alkylresorcinols

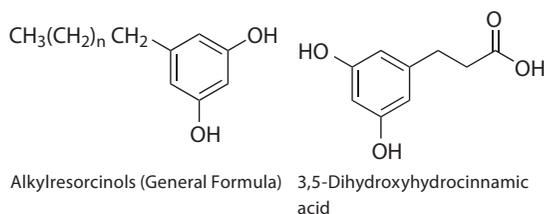
Matthias Nold, Product Manager Analytical Standards [matthias.nold@sial.com](mailto:matthias.nold@sial.com)



Alkylresorcinols are naturally occurring phenolic lipids that are present in substantial amounts in the outer layer of cereal grains. They are especially abundant in the bran layer of wheat and rye.

Alkylresorcinols are known to have antimicrobial activity and are believed to have an antitumor function as well. Since they are not present in the endosperm, they are virtually absent in white bread. Therefore, alkylresorcinols are often used as biomarkers for whole-grain intake in research investigating the health benefits of whole grain consumption.

Under the Fluka® brand, Sigma-Aldrich now launches a series of analytical standards of alkylresorcinols with different chain lengths, as well as the metabolite 3,5-Dihydroxycinnamic acid (3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA)).



Cat. No.	Brand	English desc	Pack Size	n
56453	Fluka	5-Tridecylresorcinol	10 mg	11
91822	Fluka	5-Pentadecylresorcinol	10 mg	13
97001	Fluka	5-Heptadecylresorcinol	10 mg	15
57981	Fluka	5-Nonadecylresorcinol	10 mg	17
49519	Fluka	5-(Nonadecyl-1,1,2,2-d4)resorcinol	10 mg	17
53503	Fluka	5-Eicosylresorcinol	10 mg	18
50851	Fluka	5-Heneicosylresorcinol	10 mg	19
03422	Fluka	5-Tricosylresorcinol	10 mg	21
56452	Fluka	3,5-Dihydroxyhydrocinnamic acid	10 mg	-

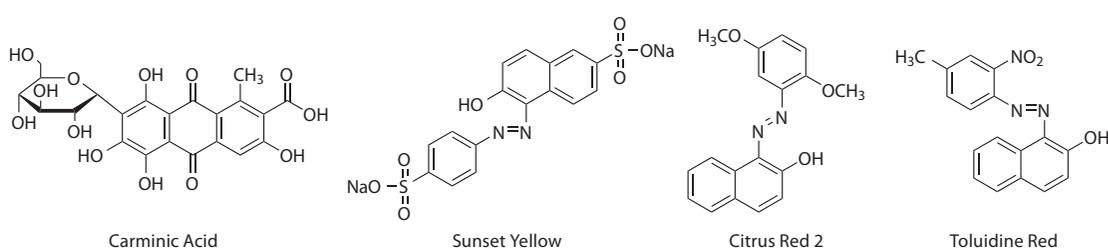
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Matthias Nold, Product Manager Analytical Standards [matthias.nold@sial.com](mailto:matthias.nold@sial.com)



Sigma-Aldrich offers a wide range of reference standards for the accurate detection of regulated dyes. These dyes, although banned worldwide for use as food additives, are still being used illicitly as additives in food products. We recently expanded our portfolio to include reference standards for the detection of additional food dyes. Please visit our webpage at [sigma-aldrich.com/fooddyes](http://sigma-aldrich.com/fooddyes) for a complete product listing.

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68775	Sunset Yellow	Fluka	25 mg
59659	Toluidine Red	Fluka	25 mg



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