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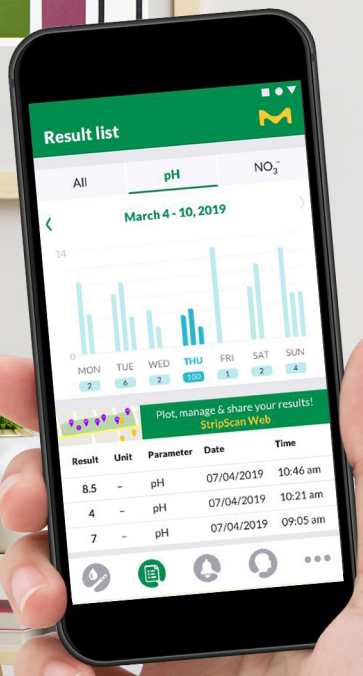

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Dear Reader,

The SARS-CoV-2 pandemic has forced us to transform the way we work and confronted us with additional challenges, such as fewer people on-site, social distancing, the need to work remotely, and reduced direct employee contacts. All this has amplified the need for more efficient work and reduced costs - now more than ever.

It is with these restraints and challenges that we seek to develop and apply new ways of conducting analytical chemistry. By adopting solutions for the digital transformation of the laboratory, we can create more efficient and reliable methods. And the good news is, this transformation is going to be beneficial for long after the pandemic is gone.

So how can that be done without painful investments? One way is to use the now ubiquitiously available smartphones and tablets as portable and affordable chemical analytical devices. They allow a paper-free and seamlessly connected documentation workflow. An example for smartphone-based chemical analysis is the MQuant® StripScan app for the readout of test strips. It allows a reliable mobile on-site result acquisition and documentation of in-process chemical analyses (see more on page 3). The potential of this app was recently recognized by the University of St. Gallen in Switzerland, who awarded the Mobile Business Award 2020 to us and our software development partner, Incloud Engineering GmbH. The prestigious award praises the app's data-based added value, its many possible uses, e.g. in the environmental sector and food and water analysis, as well as the app's potential for new user benefits and services.

Mobile Business Award Winner 2020

But with that being said, we are still at the beginning of leveraging the power of smartphones for our laboratory analyses. In the meantime, other data transfer solutions like Spectroquant® PROVE Connect for the transfer of result data from benchtop photometers, or Spectroquant® MOVE Data Transfer for the data transfer from portable colorimeters make life easier, with fewer errors and reduced pen-and-paper documentation. These are only a few examples from the Supelco® digital solutions portfolio, to help us access and keep track of our data in a seamlessly connected manner. The whole portfolio of solutions contributing to lab efficiency and reliability can be found at **SigmaAldrich.com/Connected.**

Yours sincerely,

Dr. Saskia Neubacher
Product Manager for
Mobile Analytical Workflows

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FOOD & BEVERAGE

Beverage Testing with the MQuant® StripScan Mobile App

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Abstract

pH and nitrate content are two of the key parameters used to measure the quality and safety of food and beverages (F&B). Generally, pH measurements are carried out using pH strips, pH meters, or electrodes, whereas spectrophotometers or electrodes are used for measuring nitrate content. This article examines the use and efficiency of the MQuant® StripScan mobile app to measure the pH of orange juice samples and nitrate concentration of mineral water samples. The results indicate towards the possible use of the MQuant® StripScan mobile app as a viable alternative to more sophisticated and instrument-based methods for pH and nitrate measurements in food and beverage analysis.

Introduction

Ensuring delivery of quality and safe food and beverage products is critical to the global F&B industry. pH plays an important role in preserving the color, flavor, texture, taste, and nutritional overall value of the product and significantly impacts the quality of final food and beverage product delivered. Moreover, maintaining the proper pH is a food safety issue.[1] For instance, keeping the pH of canned or preserved food at 4.6 helps prevent the growth of toxic bacteria, such as *Clostridium botulinum*.[2] Additionally, most microorganisms including bacteria, yeasts, and molds cannot survive or grow under very low or high pH values.

In the food industry, monitoring the pH of raw materials is essential to prevent their deterioration, which in turn, can affect the shelf-life of the final product. For example, in juices and brews, measuring the pH of water before addition in food processing guarantees a good quality and safe end-product.[3]

This is particularly important when the water source is a municipal water system, where quality can vary considerably over time.

The traditional method for pH measurement involves the use of a pH meter, which uses the difference in electrical potential between a pH electrode and a reference electrode to generate a reading. The logarithmic nature of pH scale makes even a small change very significant, with even a change of just 0.3 units denoting doubling of acid concentration.[4] Additionally, pH measurements by electrodes are greatly influenced by factors like temperature, electrode stability (drift and hysteresis), the quality of the response slope/calibration curve, as well as the accuracy of the instrument.[5]

A novel method for measuring pH involves the use of the MQuant® StripScan mobile app in combination with test strips. This method provides pH measurements in a few seconds, and also offers additional benefits of automatic data storage, generation of graphs for further documentation, and an automatic data transfer to desktop devices.[6]

Nitrate (NO_3^-) is another compound that has a significant influence on the quality of F&B products. Although it occurs naturally as part of earth's nitrogen cycle, various human activities contribute to its presence through agricultural operations (via excessive use of inorganic fertilizer), sanitation, diffusion from industrial processes, and disposal of solid waste.[7]

Consumption of food or beverages with high levels of nitrates can have adverse health effects. For example, it can generate carcinogenic nitrosamines upon reaction with amines or amides. Under some conditions, nitrates can also produce nitrite (NO_2^-) through bacterial reduction in the stomach, causing a rare blood disorder called methaemoglobinaemia, a serious condition resulting from impaired oxygen transport by red blood cells.[8] For this reason, regulatory agencies have set safety limits for nitrate in food and beverages, as well as in water supplies. For example, the current acceptable daily intake (ADI) for nitrate set forth by the European Food Safety Authority (EFSA) is 3.7 milligrams per kilogram of body weight per day (mg/kg bw/day). The U.S. Environmental Protection Agency (EPA) has implemented a maximum contaminant level (MCL) for nitrate in water at 10 mg/L (as nitrogen; equivalent to 45 mg/L of nitrate).[9] The Food and Drug Administration's (FDA) allowable nitrate

level in bottled water is the same as EPA, while in food, the level should not exceed 500 part per million (ppm) in the finished product.[10]

Different methods available for measuring nitrate content include spectrophotometric, chemiluminescence, electrochemical detection, chromatographic, capillary electrophoretic, and spectrofluorimetric methods.[11] Although these techniques offer high sensitivity and selectivity, they also involve laborious chemical work, and require specialized and expensive instrumentation. To address these issues, rapid detection techniques based on test strips are gaining popularity. Combined with the MQuant® StripScan smartphone app, test strips are fast, affordable, non-hazardous for most measurements, and do not require the handling of liquid chemical waste.

In this article, we describe the use of MQuant® StripScan mobile app and test strips for measuring pH and nitrate content of food and beverage products.

Methods and Materials

To measure pH and nitrate in F&B samples, MQuant® test strips (Merck KGaA, Darmstadt, Germany) were used; specifically pH test strips with the range 0-14 and nitrate test strips with the range 0-500 mg/L. The test strips were used according to manufacturer instructions, and readout was performed using the MQuant® StripScan app in conjunction with the appropriate reference cards (Merck KGaA, Darmstadt, Germany):

1. Dip the test strip in the sample and select the parameter to measure (pH or nitrate) in the app.
2. As the app displays a countdown, remove excess sample from the strip and place the strip on the reference card.
3. Position the reference card within the mobile phone's camera view. Align the marks on the screen with the reference card. An image is acquired automatically, and the result is displayed immediately.

A. pH of orange juice

Four orange juice samples were analyzed using the MQuant® pH-indicator strips (pH 0-14) and the corresponding pH reference card. Five individual measurements were made for each sample.

The pH measurements using the test strips were compared with the measurements made using a pH meter.

B. Nitrate in mineral water

Three samples of mineral water were tested using MQuant® nitrate test strips (0-500 mg/L) and the corresponding reference card. Five measurements were made for each sample.

The nitrate measurements were compared with two different reference methods:

1. photometric determination using a Spectroquant® photometer
2. reflectometric determination using the Reflectoquant® system (reflectometer)

Results and Discussion

A variety of modern analytical techniques are being used to support the quality control of food and beverages. These include mobile photometry (e.g. Spectroquant® Move 100) or reflectometry (Reflectoquant® RQflex 20), bench top spectrophotometry (e.g. Spectroquant® Prove series), chromatography, mass spectrometry, NMR, X-ray analysis, and atomic spectroscopy.[5, 6] Although these techniques offer high sensitivity and selectivity, most of them also involve laborious chemical work and expensive investment in instruments.

Rapid detection techniques based on test strips are gaining popularity. A visual observation of color or fluorescence forms the basis of the test strip detection method. Typically, the test substance reacts with chemicals on the reaction pad(s) of the test strip and results in a color change, which is then compared

with a color reference for validation.[12] One drawback of this technique, however, is that a semiquantitative readout is not very accurate, and is prone to individual variations and documentation errors. These aspects are addressed by a new app reader for test strips, the MQuant® StripScan app.[6]

A. pH measurement of orange juice samples

Amongst others, pH measurement is relevant in the food and beverage industry, for instance in quality monitoring of fruit juices. Juices are prone to spoilage due to their possible contact with air and microorganisms in the environment during handling. This is a concern because spoiled fruit juice products can lead to various foodborne illnesses. Despite this risk, microorganisms are not usually present in significant amounts because the low pH of these products is not conducive to their growth. Therefore, monitoring the pH of juice products is critical for both their shelf life and safety.

Four orange juice samples were analyzed using the MQuant® pH-indicator strips (pH 0-14), the MQuant® StripScan app, and the corresponding reference card. The pH measurements were compared with the measurements using a pH meter. The results are shown in **Table 1**.

Table 1. Comparison of pH values obtained by MQuant® StripScan and a pH meter

Orange juice sample number	pH	
	MQuant® StripScan	pH meter
1	4	3.95
2	4	3.85
3	4	3.83
4	4	3.87

The results in **Table 1** are averages of five measurements. The MQuant® StripScan app yields results in increments of 0.5 pH units, while the pH meter provides an accuracy of two decimal places. The experimental data indicates that results obtained by the app correspond with the values measured with the pH meter, showing that MQuant® test strips along with the StripScan app are an adequate alternative to measure pH, if the accuracy provided by the app is sufficient for the use case.

B. Nitrate in mineral water

Monitoring of nitrate is important because of its potentially adverse health effects when consumed in excess. An example is the preparation of infant formula, prepared usually with mineral water. In a hypothetical scenario, where a formula is made from water containing 50 mg of nitrate per liter (50 mg/L), that would average about 8.3–8.5 mg of nitrate per kilogram of body weight per day, which is more than double the current acceptable daily intake (ADI) of 3.7 mg/kg bw/day set forth by the EFSA. Water with high nitrate levels used in making infant formula has a serious impact on the daily exposure levels among the formula-fed infants.

Two mineral water samples were measured for their nitrate content. The rapid MQuant® StripScan method was compared to measurements using reflectometry with the Reflectoquant® system, and photometric measurements with the Spectroquant® system. The results are shown in **Table 2**.

Table 2: Nitrate concentration of mineral water samples measured using MQuant® StripScan app, Reflectoquant® system and Spectroquant® measurements

Sample	NO_3^- concentration [mg/L]		
	*MQuant® StripScan	*Reflectoquant®	Spectroquant®
Water 1	0	<3	2.7
Water 2	10	12	12.0
Citrus flavored water	10	10	>25.0

*MQuant® StripScan app and Reflectoquant® instrument results are based on the average of 5 measurements

As expected, the photometric approach provided the most accurate results out of the three methods that were compared in this experiment.

As the nitrate content in water sample 1 was very low, it was below the detection limit of the reflectometric and app readers. Accordingly, the Reflectoquant® system correctly determined NO_3^- content to be below the detection range of 3 mg/L. The MQuant® StripScan method also correctly determined the concentration to be below the detection limit of 5 mg/L.

The nitrate content of water sample 2 was concurrently determined by reflectometry and photometry to be 12 mg/L. The MQuant® StripScan app determination of 10 mg/L was also in accordance with these reference values, as the incremental values determined by the app are 0 - 5 - 10 - 15 - 20 - 25 - 35 - 50 - 75 - 100 - 250 - 500 mg/L. Thus, the increment value determined by the app is the closest match with the results of the other two methods.

The nitrate concentration of citrus flavored water was also measured. In this case, the Spectroquant® photometric measurements did not give an accurate result because the high sugar content of the sample interfered with the measurement. The results obtained by reflectometry and the MQuant® StripScan app correspond with each other, suggesting that similar method precision is observed here.

To summarize, the measurements obtained with the MQuant® test strips and StripScan app were in range with the reference methods. The accuracy of measurements made was lower due to the system's semiquantitative nature. For accurate values at very low concentrations, the photometric method is best suited. At the same time, the MQuant® test strips and StripScan app are well-suited as a rapid alternative if only binary answers are required (i.e. whether a concentration is above or below a threshold) and to determine the general concentration range of nitrate content in a sample.

Test strip-based methods show advantages with samples containing additives that may interfere with photometric measurements. Here, measurements with test strips yielded adequate results in direct measurements of the sample without the necessity of sample preparation.

Conclusion

The pH of orange juice samples and nitrate concentration of mineral water samples were measured using the MQuant® StripScan mobile app and MQuant® test strips. In all samples, the data obtained with the MQuant® StripScan mobile app was in range in comparison with the results obtained by the reference methods. This allows for the conclusion that this smartphone-based analytical tool presents a viable alternative to more sophisticated, instrument-based methods, such as pH meters for measuring pH, and spectrophotometers for nitrate measurement.

A general advantage of using test strips over pH electrodes or wet chemical methods is their ease of use, speed, low cost as well as the fact that the strips can be discarded with regular waste after use. This can streamline measurement processes drastically, as no cleaning of equipment and disposal of hazardous liquid waste is necessary, saving time and money.

By combining test strips with the readout by the MQuant® StripScan mobile app, accuracy and reproducibility of the test strip readout are improved without the need to purchase a dedicated readout instrument. As an added value, digital data acquisition and traceability are provided by the app solution, together with an easy way to graph, share, and export data for better documentation. This makes this method suitable for on-site and in-process testing which does not require highly accurate results, and for routine use in laboratories or production sites where pH and chemical screenings are routinely executed.

Acknowledgements

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Featured Products

Description	Cat. No.
MQuant® pH-indicator strips pH 0-14 Universal indicator, Pk.100	**1.09535**
MQuant® StripScan Reference Card for analyzing MQuant® pH indicator strips pH 0-14 (Cat. No. **1.09535**)	**1.03736**
MQuant® Nitrate Test, colorimetric with test strips, Pk. 25 or 100	**1.10020**
MQuant® StripScan Reference Card for analyzing MQuant® Nitrate Test (Cat. No. **1.10020**)	**1.03733**

MQuant® StripScan App can be downloaded via the Apple Appstore and Google Play.

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Description	Cat. No.
Reflectoquant® Reflectometer RQflex® 20	**1.17246**
Nitrate Test 5-225 mg/L (NO_3^-), for use with RQflex®20, 50 Tests	**1.16971**
Spectroquant® Prove 100 Photometer, suitable for UV/Vis spectroscopy	**1.73016**
Spectroquant® Prove 300 Photometer, suitable for UV/Vis spectroscopy	**1.73017**
Spectroquant® Prove 600 Photometer, suitable for UV/Vis spectroscopy	**1.73018**
Nitrate Test photometric, 0.3-30.0 mg/L (NO_3-N), Spectroquant®, 100 Tests	**1.01842**

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FOOD & BEVERAGE

Ensure Reliable Results of Your Microbiological Tests

New Vitroids™ Certified Reference Materials (CRMs) compliant to ISO 11133:2014

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Foodborne illnesses caused by contaminated food products are a major global concern. According to the World Health Organisation (WHO), every year about 10% of the global population falls ill after eating contaminated food, causing almost half a million deaths per year.[1] While chemical contamination e.g. biotoxin poisoning is also a concern, the consumption of food contaminated by pathogens like viruses or bacteria causes most of the fatalities.

The international standard, ISO 11133:2014 (plus amendment 1:2018 and amendment 2:2020) outlines the regulatory requirements to be met in the preparation of culture media meant for use in the microbiological analysis of food, animal feed, and water meant for consumption or use in food production.[2]

Reliable and reproducible test results in microbiological analysis, necessitate a thorough quality check of the containing culture media. With Vitroids™ discs and LENTICULE® discs Certified Reference Microorganisms (CRMs) from the Supelco® portfolio of analytical products, we offer a comprehensive range of microbiological reference materials. These reference materials have a confirmed identity of the strain along with traceability to the strains from internationally recognized institutions like NCTC/NCPF or CECT. The products are certified reference materials produced in our ISO/IEC 17025 and ISO 17034 accredited lab in Buchs, Switzerland.

These CRMs are developed with an intention to provide laboratories with suitable ready-to-use microbiological controls for every media quality control test. The concentrations of CFUs are designed in ranges ensuring that no or only minimal dilution steps are required, thereby saving time and reducing the chances of cross-contamination

Portfolio expansions

The product portfolio undergoes steady expansion and adaptation based on parameters such as, ISO standard requirements, customer suggestions for new organisms, different concentration levels, or certification methods.

The table below shows the most recent additions to the Vitroids™ CRM range focusing on ISO 11133 relevant reference strains.

Description	WDCM number	CFU Level	Cat. No.
Citrobacter freundii	00006	15-80	**VT000062**
Citrobacter freundii	00006	80-130	**VT000063**
Citrobacter freundii	00006	1,000-10,000	**VT000066**
Citrobacter freundii	00006	50,000-150,000	**VT000067**
Enterococcus faecalis	00009	50,000-150,000	**VT000097**
Listeria monocytogenes	00021	15-80	**VT000212**
Listeria monocytogenes	00021	1,000-10,000	**VT000216**
Staphylococcus aureus	00034	15-80	**VT000342**
Staphylococcus aureus	00034	1,000-10,000	**VT000346**
Staphylococcus aureus	00034	50,000-150,000	**VT000347**
Staphylococcus epidermidis	00036	1,000-10,000	**VT000366**
Aerococcus viridans	00061	10,000-100,000	**VT000617**
Enterococcus faecalis	00087	15-50	**VT000872**
Enterococcus faecalis	00087	80-130	**VT000873**
Enterococcus faecalis	00087	1,000-10,000	**VT000876**
Escherichia coli	00090	600-1,400	**VT000905**
Staphylococcus saprophyticus	00159	1,000-10,000	**VT001596**
Heterotrophic organism replacement	-	1,000-10,000	**VT025046**

Find the complete product range at **SigmaAldrich.com/mibi-crm**

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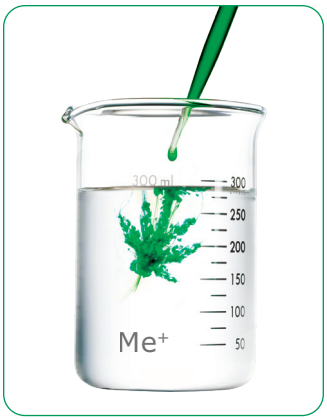
1. https://www.who.int/news-room/fact-sheets/detail/food-safety
2. ISO 11133:2014

CANNABIS

Heavy Metal Analysis of *Cannabis sativa* by ICP-MS and the Need for Proper Sample Homogenization

Stephan Altmaier, Principal Scientist Advanced Analytical R&D, Analytix@milliporesigma.com

Abstract



A step-by-step protocol for the analysis of various heavy metals in *Cannabis sativa* plant material by ICP-MS was developed. Cannabis is known to accumulate metals in various parts of the plant, such as seeds, leaves or stems, to a different extent. As a consequence, several protocols for the homogenization of cannabis buds were developed in order to prepare reproducible samples and analysis results. For comparison, cannabis buds were separated into seeds, stems, and leaves and the plant parts were subjected to ICP-MS.

Introduction

Cannabis is a plant genus that consists of three different species (*Cannabis sativa, indica* and *ruderalis*) and all of these are known to accumulate heavy metals in different parts of the plant (roots, leaves, seeds etc.). Due to this ability cannabis has been used for the remediation of contaminated soil (phytoremediation and phytoextraction).[1,2,3,4] On the other hand this inclination can hinder the use of cannabis in the food or medical industry. Therefore, all plant materials used in either food or pharma products, should be tested for their heavy metal content.

As of July 2020, 24 US states and Canada issued regulations for the testing of heavy metal content in cannabis, and all of them provided limits for arsenic, cadmium, lead and mercury metals (referred to as the "big four"). In addition, several states have set limits for one or more of these metals: chromium, barium, silver, selenium, antimony, copper, nickel and zinc.

Dried cannabis plant material is a very inhomogeneous matter and consists of leaves, buds including resin, stems of various thickness and seeds. All these plant parts accumulate heavy metals to a different extent. As it was shown in numerous studies, the heavy metal uptake depends on both the plant part and the element[1,5,6] In addition, uptake is influenced by external factors such as fertilization and liming,[1] ultimately causing an uneven distribution of metals throughout the plant. Hence, if the focus of studies is on the overall heavy metal content of cannabis, the material needs to be thoroughly homogenized before sample analysis. The recommended process for this sample breakdown is grinding. Various milling techniques exist, each differing in their technical complexity, such as mortar and pestle, rolling pin, knife mill, cutting mill, rotor mill, and ball mill.

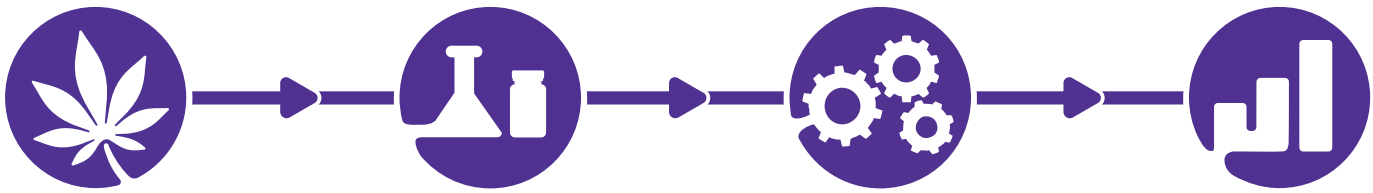
Experimental

In this paper, a four-step workflow was applied for the process of sample homogenization, standardization, sample digestion, and analysis.

The cannabis resins which have a sticky, smearing appearance make it necessary to always freeze samples prior to milling.

Freezing can either be accomplished at -20 °C in a freezer, by making use of dry ice (-78 °C) or by utilizing liquid nitrogen (-196 °C) as a cooling agent.

The setup of any grinder must be performed according to the target analytes. For the analysis of the big



four heavy metals, irrespective of the type of grinder chosen, stainless steel tools can be used. In contrast, if the abundance of additional metals such as Cr or Ni in a sample is of interest, knife mills with titanium blades or mills with grinding tools made of ZrO_2 or PTFE need to be selected. As these tools are normally smaller in size, the milling process is more time consuming.

The seeds of the cannabis species are the plant part that is most potent and is investigated in this homogenization study.

Four different grinding methods were applied for the milling of three *Cannabis sativa* hemp varieties. ICP-MS was used to determine the heavy metal content of the samples and based on the results the homogenization efficiency of all approaches was compared. In a second set of experiments, one hemp variety was separated into seeds, leaves, and stems, followed by ICP-MS analysis to identify possible variations of heavy metal concentrations in the different plant parts.

Sample Homogenization / Milling

Three different *Cannabis sativa* varieties: "Finola", "Felina" and "Santhica", were purchased from a drug store. All of them were qualified as industrial hemp per German regulation and were sold as "hemp flowers". The samples were obtained as 25 g batches of dried buds.

The experiments for the analysis of homogenization efficiencies of milling and grinding techniques were performed by applying four different methods, described below:

- Rolling pin (RP)
- Mortar and pestle (MP)
- Knife mill (KM)
- (Cryo) Ball mill (CM)

All four procedures are very different in terms of their speed, throughput, and ability to provide homogeneous samples in a reproducible way. In addition, using the knife mill can lead to an increase in sample temperature and hence in a change of sample composition. This needs to be considered, if samples are meant to be subjected not only to ICP-MS, but also to HPLC or GC analysis (e.g., for the determination of cannabinoid or terpene content). The detailed proceedings for each of the milling techniques applied are described in the following sections.

Rolling Pin

Approximately 10 g of dried buds were weighed into an airtight zip bag and cooled in a freezer at -20 °C for one hour. Subsequently the bag was placed on a hard support and the sample was ground using a wooden household rolling pin. After three to five minutes no further sample breakdown was observed and the process was stopped. Stems in the sample needed to be broken into shorter pieces of approximately 10 mm length by hand. An image of the final sample revealed rather large pieces of stem segments and undamaged seeds in an overall inhomogeneous material (**Figure 1**).

Figure 1. Hemp Buds "Finola" After Three Minutes Grinding Using a Wooden Rolling Pin.

Mortar and pestle

Approximately 10 g of aliquoted, dried buds were weighed into an airtight zip bag and cooled in a freezer at -20 °C for one hour. Then one or two buds were withdrawn from the bag, placed in a china mortar and ground for five minutes utilizing a porcelain pestle. The resulting coarse powder still contained large pieces of rigid stems, which needed to be broken by hand in order to obtain shorter pieces of approximately 10 mm length. The entire process was repeated, until a 10 g sample had been prepared. Rather large pieces of stem material were visible in the otherwise homogeneous, final sample.

Knife mill

For knife milling 18 g of hemp buds were placed in a mill equipped with titanium blades and ground for 30 seconds at 4,000 rpm (one cycle) or 10,000 rpm (two cycles), respectively. The 30 sec/4000 rpm method resulted in a sample that looked similar to that obtained with the rolling pin. In contrast, the 30 sec/10,000 rpm milling process generated a coarse powder comparable to ground coffee beans, with no stem segments or seeds visible.

(Cryo) Ball mill

One hemp bud (approx. 2.5 g) was manually broken into pieces and placed into a 50 mL stainless steel milling beaker. A 25 mm stainless steel milling ball was added and the sealed beaker was mounted to a cryo ball mill equipped with a liquid nitrogen filling system. The grinding parameters were as follows: Pre-cooling at 5 Hz, two cycles of 90 s at 30 Hz and 30 s at 5 Hz (for intermediate cooling). This process resulted in a very fine powder with a particle size of <100 µm (**Figure 2**).

Figure 2. Hemp Buds "Finola" after Grinding in a Cryo Ball Mill. Resulting Particle Size <100 µm.

For a second set of experiments, the hemp variety "Santhica" was manually separated into seeds, leaves, and stems to identify possible variations of heavy metal concentrations in the different plant parts (**Figure 3**).

These samples were directly subjected to digestion (without a preceding grinding step) and then analyzed by ICP-MS.

Figure 3. *Cannabis sativa* "Santhica" Separated into Leaves (top), Stems (middle) and Seeds (bottom).

Digestion of samples

All hemp samples were digested as follows: 50 (±1) mg of ground sample was weighed into a microwave vial and after addition of 3 mL nitric acid (65%), 1 mL hydrogen peroxide (30%) and a metal spiking solution, the sample was digested. After completion of digestion, the solution was quantitatively transferred into a 50 mL polypropylene tube, and 50 µL of indium internal standard solution was added. The final solution was then brought up to 50 mL final volume with ultrapure water. For detailed microwave digestion conditions please contact the author.

Preparation of standard solutions

In order to compensate for sample matrix effects, a standard addition approach utilizing various Certified Reference Material Heavy Metal Mix *Trace*CERT® standard solutions was applied for the preparation of all calibration curves. The final calibration curve covered a range from 1-20 µg/g total of heavy metals. The curve comprised of four data points; three standard addition solutions plus one unspiked sample solution. Alternatively, standard solutions were also prepared by using ICP Certipur® single element standards and ICP *Trace*CERT® single element standards (data not shown). For accuracy reasons, the composition of the addition solutions was adjusted to the heavy metal concentration in each of the three samples.

ICP-MS analysis

Analysis of samples, standards, and spikes was done by ICP-MS. For detailed conditions kindly contact the author.

The analysis was performed in the sequence: Blank, sample 1 – x, additions.

The resulting calibration curves for As, Cd, Hg, and Pb revealed excellent linearity over the entire calibration range, with r^2 values of > 0.9995 for all.

Addition solution for the determination of recovery rates

The recovery rates for the big four heavy metals are listed in **Table 1**. All recoveries were in the range of ±10%.

Table 1. Recovery rates for three hemp varieties (RP and MP grinding were applied to each sample) using a CRM Heavy Metal Mix *Trace*CERT® standard solution I, II or III (see details in products section).

	Cannabis Finola		Cannabis Santhica		Cannabis Felina	
Element	RP Mix I [%]	MP Mix I [%]	RP Mix II [%]	MP Mix II [%]	RP Mix III [%]	MP Mix III [%]
As	104	102	98	99	104	98
Cd	98	97	99	94	102	100
Hg	90	91	108	110	100	97
Pb	94	95	96	102	98	99

Results & discussion

Seed milling method investigation

The heavy metal content of the three hemp varieties that were subjected to the different milling processes are listed in **Table 2**.

The mercury content of all samples was below the limit of detection, and only one sample, out of the 14 samples displayed a cadmium level above the detection limit (0.1 µg/g). For arsenic the results were similar, with five samples containing As close to the LOD (0.1 µg/g). The findings for lead were a bit different, and the detected concentrations ranged from 0.3 to 1.0 µg/g. It is noteworthy, that these values do in part exceed the limits of various US states and Canada by a factor of 2-3 (depending on the intended use). The analysis of the "big four" elements was reproducible, and except for one deviation (Pb content of hemp variety "Felina" ground with MP) the milling technique did not affect the detected heavy metal concentrations.

Cryo milling was performed utilizing stainless steel equipment and resulted in the detection of elevated levels of chromium in all cryo-ground samples. Though nickel is also a content of stainless-steel alloys, increased amounts (compared to RP and MP milling) were only found in the "Finola" sample. This result can be attributed to the difference in grinding time. Some US states (as of now MI, MD, MO, NY) issued regulations, which make the analysis of chromium in cannabis necessary. In this case, it is essential to utilize a cryo milling approach and zirconia or PTFE grinding equipment in order to avoid sample contamination. In contrast to cryo milling, knife milling was performed (highlighted in **Table 2**) using titanium blades and therefore did not affect the Cr (and Ni) content of cannabis samples.

Plant part analysis

The heavy metal content of stems, seeds, and leaves of the hemp variety "Santhica" and the respective recovery rates are listed in **Table 3**. All results but the lead content of seeds are in line with the data shown in the previous section. This finding corresponds to results published in various publications, that also reported the Pb concentration in seeds being lower than in other plant parts such as leaves, stems, flowers, or roots.[5,6]

Table 2. Heavy Metal Content of three Hemp Varieties Determined by ICP-MS. Four different Grinding Procedures were Applied. Roman Figures Indicate the Use of a Specific CRM Heavy Metal Mix *Trace*CERT® Standard Solution (III to VIII) for the Preparation of Respective Addition Solutions.

	Cannabis Finola				Cannabis Santhica					Cannabis Felina				
Element	RP [µg/g]	MP [µg/g]	CM-VII [µg/g]	CM-VIII [µg/g]	RP [µg/g]	MP [µg/g]	KM [µg/g]	CM-V [µg/g]	CM-VI [µg/g]	RP [µg/g]	MP [µg/g]	KM [µg/g]	CM-III [µg/g]	CM-IV [µg/g]
As	0.1	0.1	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cd	< 0.1	< 0.1	< 0.1	<0.1	< 0.1	0.1	<0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cr	0.3	0.4	**12.0**	**12.0**	0.2	0.3	0.3	3.6	2.5	0.3	0.5	0.6	3.7	4.3
Hg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Ni	0.8	1.1	**2.0**	**2.1**	0.4	1.7	0.4	0.7	0.6	0.5	0.6	0.6	0.7	0.7
Pb	0.6	0.7	0.7	0.7	0.3	0.5	0.5	0.4	0.4	0.5	1.0	0.5	0.5	0.5

Table 3. Heavy Metal Content of "Santhica" Stems, Seeds, and Leaves as Determined by ICP-MS (Duplicates) and Respective Recovery Rates (RR) using CRM Heavy Metal Mix *Trace*CERT® Standard Solution II. No Grinding was Performed prior to Digestion.

	Stems			Seeds			Leaves		
Element	#1 [µg/g]	#2 [µg/g]	RR [%]	#1 [µg/g]	#2 [µg/g]	RR [%]	#1 [µg/g]	#2 [µg/g]	RR [%]
As	< 0.1	< 0.1	99	< 0.1	< 0.1	96	< 0.1	< 0.1	99
Cd	< 0.1	<0.1	100	< 0.1	< 0.1	98	< 0.1	<0.1	102
Hg	< 0.1	< 0.1	96	< 0.1	< 0.1	103	< 0.1	< 0.1	100
Pb	0.4	0.4	97	< 0.1	< 0.1	101	0.4	0.4	99

Conclusion

This work demonstrates a comprehensive ICP-MS workflow, using the standard addition calibration method, for the determination of heavy metals in *Cannabis sativa hemp* variety plant materials. Critical elements in the process include homogenization of samples and use of accurate traceable Certified Reference Material mixes, that are tailored to state specific regulations for heavy metals in cannabis. Reproducible samples were prepared by grinding cannabis with different mill types and techniques. Samples were then digested utilizing a specific digestion protocol, optimized to provide clear digestion solutions. The resulting solutions were subjected to ICP-MS analysis. Calibration data was obtained by the preparation and analysis of standard addition solutions obtained by diluting various different heavy metal CRM mixes containing arsenic, cadmium, lead and mercury. The final results were consistent for all samples and revealed an As, Cd, and Hg concentration of <0.1 to 0.1 µg/g. The detected lead content of the three cannabis varieties ranged from 0.3 to 1.0 µg/g.

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Featured products

Description	Cat. No.
Certified Reference Materials (CRMs)	
Single element standards for ICP Certipur® 1000 mg/L	
Arsenic	**1.70303**
Cadmium	**1.70309**
Indium	**1.70324**
Lead	**1.70328**
Mercury	**1.70333**
Single element standards for ICP TraceCERT® 1000 mg/L	
Arsenic	**01969**
Cadmium	**36379**
Indium	**00734**
Lead	**41318**
Mercury	**28941**
State specific heavy metals CRM mixes TraceCERT®, 100 mL each	
Heavy metal mix I Arkansas, (As 20, Cd 20, Hg 10, Pb 50) mg/L	**94846**
Heavy metal mix II California, (As 15, Cd 5, Hg 30, Pb 5) mg/L	**94794**
Heavy metal mix III Colorado, (As 40, Cd 40, Hg 20, Pb 100) mg/L	**94772**
Heavy metal mix IV Nevada and Washington, (As 200, Cd 82, Hg 40, Pb 120) mg/L	**95094**
Heavy metal mix V Connecticut and New Mexico, (As 14, Cd 9, Hg 29, Pb 29) mg/L	**95117**
Heavy metal mix VI Vermont, (As 100, Cd 41, Hg 20, Pb 100) mg/L	**04295**
Heavy metal mix VII Pennsylvania, (As 15, Cd 3, Hg 5, Pb 10) mg/L	**03056**
Heavy metal mix VIII New Hampshire, (As 5, Cd 3, Hg 9, Pb 9) mg/L	**95562**
Heavy metal mix IX (As, Cd, Hg, Pb all 100 mg/L each)	**89471**
Heavy metal mix according to USP <561> articles of botanical origin, (As 20, Cd 5, Hg 10, Pb 50) mg/L	**18208**
Metal mix I for cannabis testing (As, Hg, Cd, Pb, Cr, Ba, Ag, Se, Sb, Cu, Ni, Zn, all 100 mg/L each)	**91539**
Solvents, reagents and acids	
Ultrapure water from Milli-Q® system e.g. IQ 7003/05/ 10/15 or bottles	
Nitric acid 60% Ultrapur	**1.01518**
Nitric acid 65% Suprapur®	**1.00441**
Hydrogen peroxide 31% Ultrapur	**1.06097**
Hydrogen peroxide 30% Suprapur®	**1.07298**

Related products

Description	Cat. No.
Milling Equipment	
IKA® MF10 basic microfine grinder drive, 115 V	**Z645168**
IKA® MF10 basic microfine grinder drive, 230 V	**Z645176**
IKA® MF 10.1 cutting-grinding head	**Z645249**
IKA® MF 10.2 impact grinding head	**Z645257**
IIKA® M 20 universal mill, 115 V	**Z645133**
IKA® M 20 universal mill, 230 V	**Z645141**
IKA® A11 basic analytical mill	**Z341789**
Agate mortar and pestle	**Z409111**
Porcelain mortar and pestle	**Z247499**
Vials and bottles	
Centrifuge tubes polypropylene 50 mL	**T2193**
Laboratory glass bottles 100 mL	**Z232173/ DWK218062454**

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Analyzing Terpenes in Cannabis

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Abstract

An efficient and easy workflow was developed for the extraction and GC-MS analysis of 31 terpenes in cannabis. The method utilized a simple solvent extraction followed by a fast and efficient GC-MS analysis on an SLB®-5ms capillary column.

Introduction

Terpenes are a class of compounds responsible for the aroma and fragrance of the cannabis flower. Labeling of terpene content on cannabis products is important to many consumers in that different varieties exhibit very different and characteristic profiles. At the time of this article, no consensus test method exists for terpene testing. Currently there are two popular approaches – headspace or solvent extraction followed by GC analysis. Headspace analysis is a "cleaner" technique than solvent extraction in that nonvolatile matrix components will not be co-extracted with the terpenes. However, traditional headspace analysis can require special instrumentation in the form of a headspace analyzer. Headspace analysis by solid phase microextraction (SPME) offers similar advantages as traditional headspace analysis, often even more sensitivity, and it can be performed manually or with an appropriate autosampler.[1,2]

Solvent extraction also does not require special instrumentation and has been used effectively to determine terpene profiles.[3] In this work, we demonstrate a solvent extraction method in combination with certified reference materials and GC-MS analysis for the identification and quantitation of terpenes in hemp flower. The method used included a simple and quick solvent extraction followed by analysis on a highly efficient 20 m x 0.18 mm x 0.18 µm SLB®-5ms column. The short length of this column in combination with the small ID allowed for separation and elution of 31 targeted terpenes in under 17 minutes. GC-MS in full scan mode allowed for spectral identification via library match to be used in combination with retention time to verify the identity of the targeted terpenes in the hemp sample. With additional reference materials, the method could potentially be expanded to include more terpenes.

Sample preparation and analytical methods

An extract of coarse ground hemp was prepared following the scheme shown in **Figure 1**. After the centrifugation step, the supernatant was removed and placed into a 2 mL amber autosampler vial for GC analysis. GC-MS analysis proceeded following the conditions listed in **Table 1**. A calibration curve ranging from 0.75 to 200 ppm (compound dependent) was prepared from two separate cannabis terpene CRM mixes. Tridecane (100 ppm) was added as an internal standard (IS) to both samples and standards upon injection using a sandwich injection technique. To aid in identification as part of the MS method, the CRMs were used to define specific retention times for each terpene and to generate reference spectra along with corresponding ratios for quantitation and qualification ions.

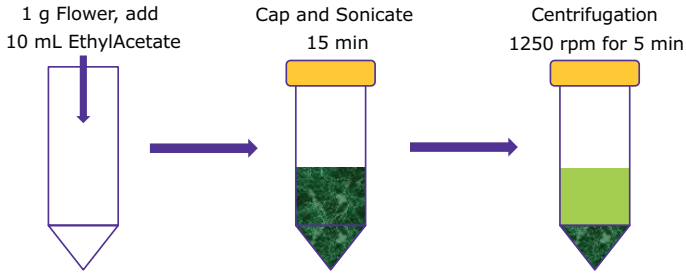


Figure 1. Sample Preparation Scheme

Table 1. Instrument Conditions

Gas Chromatograph Conditions	
column:	SLB®-5ms 20 m x 0.18 mm ID; 0.18 µm (**28564-U**)
oven:	45 °C (2 min), 10°C/min to 140°C (0.5 min), 30°C/min to 300°C (2 min)
inj. temp.:	300° C
carrier gas:	helium, 0.75 mL/min constant Flow
detector:	MSD
injection:	2.0 µL – pulsed split 50:1
liner:	Single Taper FocusLiner™ with wool (**2879905-U**)
sample:	Hemp extract (1 g flower in 10 mL ethyl acetate)

MS Conditions	
tuning:	Auto-tune
acquisition:	Full Scan Mode (EI); 40-400 amu
solvent delay:	4 min
MS source temperature:	300°C
MS quad temp.:	150°C
MS transfer line temp.:	300°C

Results

The GC method eluted the 31 targeted terpenes in under 17 minutes, with excellent peak shape and resolution, as shown in **Figure 2**.

The GC-MS method showed excellent linearity for all analytes (**Table 2**). In addition, retention time stability was evaluated. With the presence of terpenes with

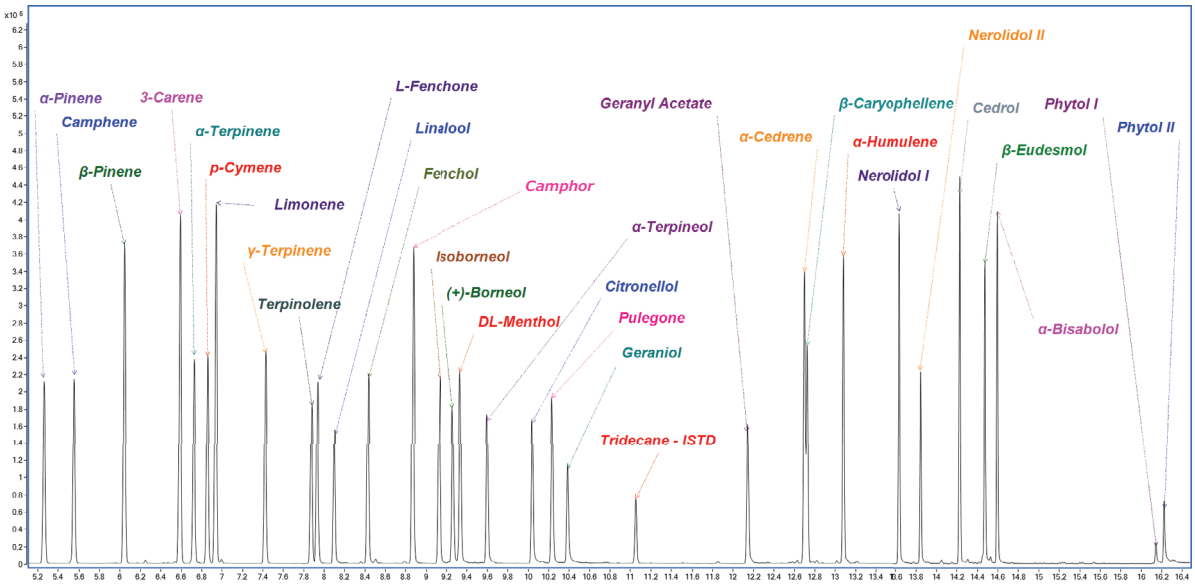


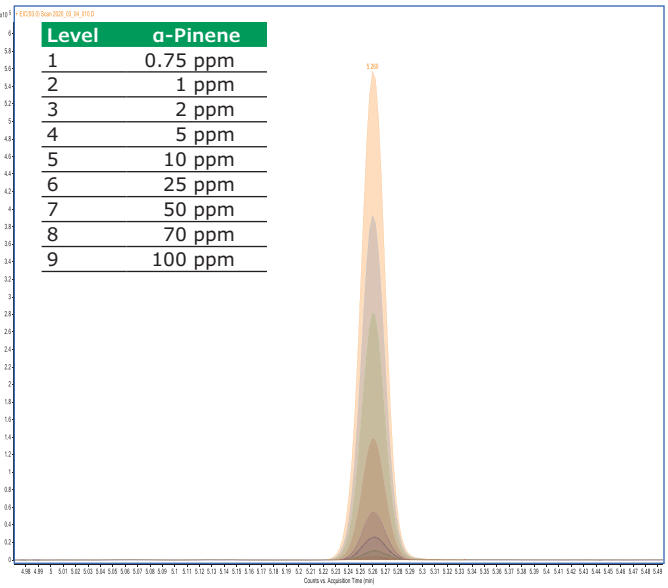
Figure 2. Standard Injection of Cannabis Terpene Standard Analyzed by GC-MS (Retention Time Range 5 to 16.5 min)

Table 2. Tabulated Results for 31 Cannabis Terpenes and IS

Peaks	Compound	CAS #	Ret. Time [min]	Lib Match Factor	r^2	Range [µg/mL]	Detected in hemp sample?
1	α-Pinene	80-56-8	5.257	98.99	0.99991	0.75-100	Y
2	Camphene	79-92-5	5.553	99.00	0.99990	0.75-100	Y
3	β-Pinene	127-91-3	6.049	99.17	0.99995	1.50-200	Y
4	3-Carene	13466-78-9	6.589	99.15	0.99992	1.50-200	Y
5	α-Terpinene	99-86-5	6.726	99.62	0.99987	0.75-100	Y
6	p-Cymene	99-87-6	6.856	99.58	0.99987	0.75-100	Y
7	Limonene	138-86-3	6.938	99.21	0.99997	1.50-200	Y
8	γ-Terpinene	99-85-4	7.422	99.35	0.99994	0.75-100	Y
9	Terpinolene	586-62-9	7.872	99.21	0.99990	0.75-100	N
10	L-Fenchone	7787-20-4	7.930	99.47	0.99989	0.75-100	Y
11	Linalool	78-70-6	8.101	99.37	0.99915	0.75-100	Y
12	Fenchol	2217-02-9	8.426	99.43	0.99996	0.75-100	Y
13	Camphor	76-22-2	8.866	99.57	0.99997	1.50-200	Y
14	Isoborneol	124-76-5	9.126	99.45	0.99983	0.75-100	Y
15	(+)-Borneol	464-43-7	9.259	99.36	0.99968	0.75-100	Y
16	DL-Menthol	89-78-1	9.331	99.52	0.99988	0.75-100	Y
17	α-Terpineol	10482-56-1	9.596	99.34	0.99942	0.75-100	Y
18	Citronellol	106-22-9	10.036	99.05	0.99961	0.75-100	Y
19	Pulegone	89-82-7	10.234	99.55	0.99956	0.75-100	Y
20	Geraniol	106-24-1	10.386	98.21	0.99946	0.75-100	Y
21	Geranyl Acetate	105-87-3	12.145	98.64	0.99980	0.75-100	Y
22	α-Cedrene	469-61-4	12.699	99.62	0.99997	0.75-100	N
23	β-Caryophellene	87-44-5	12.728	99.68	0.99953	0.75-100	Y
24	α-Humulene	6753-98-6	13.082	98.90	0.99975	0.75-100	Y
25	Nerolidol I	7212-44-4	13.636	98.73	0.99913	1.50-200	Y
26	Nerolidol II	7212-44-4	13.845	99.19	0.99905		Y
27	Cedrol	77-53-2	14.226	99.46	0.99978	0.75-100	Y
28	β-Eudesmol	473-15-4	14.471	99.60	0.99945	0.75-100	Y
29	α-Bisabolol	23089-26-1	14.594	99.62	0.99965	0.75-100	Y
30	Phytol I	7541-49-3	16.145	92.91	0.99975	0.75-100	Y
31	Phytol II	7541-49-3	16.225	92.91	0.99950		Y
ISTD	**Tridecane**	**629-50-5**	**11.050**	**96.17**	******	**100 ppm**	

similar MS spectra, it is important that retention times do not vary with concentration, or in the presence of matrix components. An example of the retention time stability provided by the SLB®-5ms column is demonstrated in **Figures 3** and **4** for α-pinene. **Figure 3** is an overlay of the extracted ion chromatogram (EIC) of the quantitation ion from the 9 calibration standards used. No retention time shift is discernable with the change in concentration. Similarly, in **Figure 4**, an overlay of the α-pinene peak from 3 injections of a 50 ppm standard show no difference in retention or response. Retention time stability in matrix was evaluated also and will be discussed later in this article.

Analysis of extracted hemp samples identified the presence of 29 of the 31 terpenes targeted for this analysis (**Table 2** & **Figure 5**). The concentrations of the different terpenes present in the hemp varied,



Level	α-Pinene
1	0.75 ppm
2	1 ppm
3	2 ppm
4	5 ppm
5	10 ppm
6	25 ppm
7	50 ppm
8	70 ppm
9	100 ppm

Figure 3. Overlaid EIC of Nine Alpha-Pinene Standards.

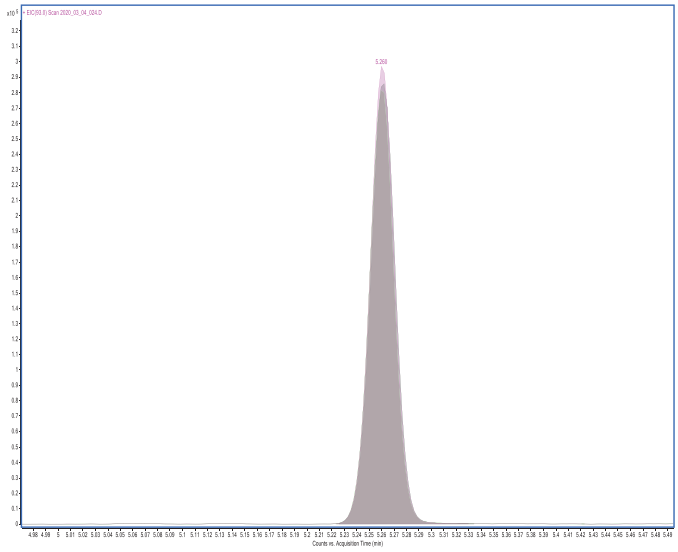


Figure 4. Three Injections of 50 µg/mL Alpha-Pinene Standard.

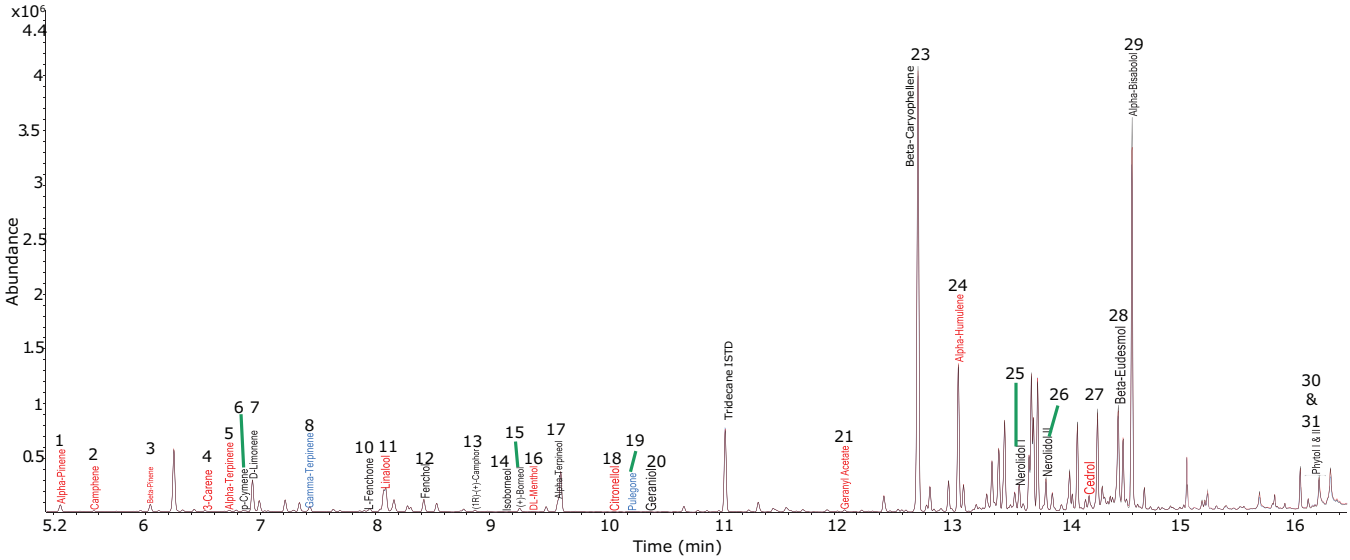


Figure 5. Chromatogram of GC-MS Analysis of Hemp Extract.

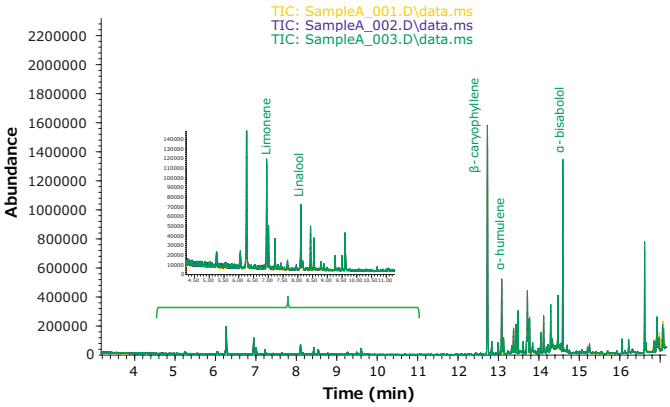


Figure 6. Overlay of TICs from 3 injections of hemp extract. Several prominent terpenes are noted.

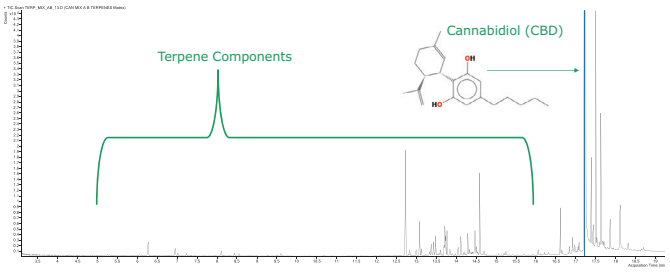


Figure 7. TIC of hemp extract showing elution of co-extracted cannabinoids relative to terpenes.

with several terpenes such as β-caryophyllene and α-bisabolol being at much higher levels than others. The approx. 100-fold calibration range of the method made it possible to quantitate the varied concentrations of terpenes present in the hemp. In addition, retention time stability from matrix, in combination with spectral ID, helped with peak identification. **Figure 6** illustrates this with overlaid total ion chromatograms (TICs) of multiple injections of a hemp extract. As with the calibration standards, no retention shifts were observed. It was noted that the solvent extraction method did result in co-extracted cannabinoids (**Figure 7**), however these eluted late enough in the run so as not to interfere with the terpenes.

Conclusion

The utility of a simple solvent extraction method in combination with GC-MS was demonstrated for the analysis of targeted terpenes in hemp flower, with identification of 29 terpenes. The use of certified reference materials in combination with MS spectra provided for proper identification in matrix, and the 20 m x 0.18 mm I.D. x 0.18 µm SLB®-5ms column provided a combination of both speed and efficiency for the analysis. While this method targeted 31 specific terpenes, it could be expanded to more by using additional CRMs.

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Related products

Description	Cat.No
Reference Materials & Solvents	
Cannabis Terpene Mix A, TraceCERT® certified reference material, 2000 µg/mL each component in methanol, 1 mL	**CRM40755**
Cannabis Terpene Mix B, TraceCERT® certified reference material, 2000 µg/mL each component in methanol, 1 mL	**CRM40937**
Tridecane analytical standard	**91490**
Ethyl acetate for gas chromatography MS SupraSolv®	**1.00789**
GC	
SLB®-5ms Capillary GC Column L × I.D. 20 m × 0.18 mm, df 0.18 µm	**28564-U**
Inlet Liner, Split/Splitless Type, Single Taper FocusLiner™ Design (wool packed), Pk.5	**2879905-U**
Molded Thermogreen® LB-2 Septa, solid discs diam. 11 mm, Pk.50	**28676-U**
Hamilton® Microliter™ syringe, cemented needle 701 ASN, volume 10 µL, needle size 23s ga (cone tip), needle L 43 mm (1.71 in.), Pk.6	**21317**
Accessories	
BenchMixer™ XLQ QuEChERS Shaker/Vortexer AC/DC input 115 V AC, US 2-pin plug	**Z742705**
BenchMixer™ XLQ QuEChERs Shaker/Vortexer AC/DC input 230 V AC, Schuko plug	**Z742706**
BenchMixer™ XLQ QuEChERs Shaker/Vortexer AC/DC input 230 V AC, UK plug	**Z742707**
Certified Vial Kit, Low Adsorption (LA), 2 mL, pk of 100 volume 2 mL, amber glass vial (with marking spot), natural PTFE/silicone septa, thread for 9 mm	**29653-U**
Corning® 50 mL centrifuge tubes 50 mL centrifuge tubes, polypropylene, conical bottom w/ CentriStar cap, rack packed, sterile, natural, 25/rack, 500/cs	**CLS430828**
Eppendorf® epT.I.P.S. box volume range 2-200 µL	**Z640220**
Eppendorf® epT.I.P.S. box volume range 50-1000 µL	**Z640247**
Eppendorf® epT.I.P.S. box volume range 100-5000 µL	**Z640271**

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The analysis of only a few markers might not always be sufficient to ensure the safety and quality of botanical products. Therefore, the availability of well characterized reference materials of the most relevant plant constituents is crucial. Our offering includes a wide selection of close to 2000 reference materials of phytochemicals. A big part of this portfolio consists of phyproof® reference materials, which are manufactured and qualified by the company PhytoLab based in Germany. This range has recently been considerably expanded.

New phytochemical reference materials from PhytoLab

PhytoLab is a global leader in the testing of phytochemicals and the manufacturing of phytochemical reference materials. Last year we announced our collaboration with PhytoLab for the global distribution of their phyproof® reference materials. In the due course of last year, we have added more than 1300 products to our system, making these high-quality reference materials of purified plant constituents available through our ordering systems.

After adding an additional 239 new products, we can now proudly claim the availability of the complete range of phyproof® reference materials with us.

The list below (**Table 1**) only shows a few examples of the new additions, demonstrating the broad range of different product classes including flavonoids, isoprenoids, alkaloids, and glucosinolates.

For the complete list, please visit **SigmaAldrich.com/phytolab**

Table 1. Selected new Product Additions of Phytopharma Standards manufactured by PhytoLab

Description	Product Class	Pack Size	Cat. No.
Integerrimine	Alkaloids	5 mg	**PHL83968**
Littorine hydrochloride	Alkaloids	10 mg	**PHL84100**
Riddelliine	Alkaloids	5 mg	**PHL84102**
Cirsimarin	Flavonoids	10 mg	**PHL85726**
Petunidin chloride	Flavonoids	5 mg	**PHL80225**
Tamarixetin	Flavonoids	10 mg	**PHL85778**
Glucoalyssin potassium salt	Glucosinolates	5 mg	**PHL85742**
Glucoraphasatin potassium salt	Glucosinolates	5 mg	**PHL84216**
Absinthin	Isoprenoids	10 mg	**PHL84170**
Terminoloside	Isoprenoids	10 mg	**PHL84263**
Fukinolic acid	Organic Acids	10 mg	**PHL84767**
Fumarprotocetraric acid	Organic Acids	5 mg	**PHL82266**

New phytochemical certified reference mix for ginger components

For the quality control of herbal medicinal products usually various key components are tested rather than just a single marker compound. To save time and expenses spent on purchasing the components and produce a working standard multi-component solution mix, we offer a range of convenient ready to use mixes for key components of some of the most common herbal medicinal products.

These Supelco® phytochemical certified reference material (CRM) mixes are produced at our Round Rock site in Texas, USA under ISO/IEC 17025 and ISO 17034 accreditation.

In issue 8 of Analytix Reporter, we presented a new certified reference mix for kava including an HPLC application. Now we bring you a CRM mix for Ginger containing certified levels of gingerols and shogaols, the major pungent compounds present in the rhizomes of fresh and dried ginger (*Zingiber officinale*):

Description:	Ginger Gingerols and Shogaols Mix
Components:	6-Gingerol, 8-Gingerol, 10-Gingerol, 6-Shogaol, 8-Shogaol, 10-Shogaol
Concentration:	500 µg/mL each component in acetonitrile
Package Size:	1 mL
Cat. No.:	**G-027**

The complete range of our phytochemical CRM mixes can be found at **SigmaAldrich.com/phytochemicalmixes**

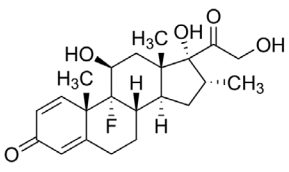
Our entire offering of phytochemical reference materials, including standards and certified reference materials in neat and solution form, and the reference materials of plant extracts and essential oils, can be found by compound class or by plant genus on our website **SigmaAldrich.com/Medicinalplants**

COVID-19 Related

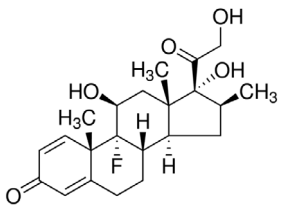
Dexamethasone: An HPLC Assay and Impurity Profiling Following the USP Monograph

Sophia Kwende, Quality Control Scientist, Analytix@milliporesigma.com

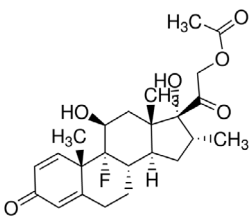
A simple, precise, and sensitive reversed-phase high performance liquid chromatography (RP-HPLC) gradient method was adapted for establishing traceability and total chromatographic analysis of dexamethasone. The given experimental conditions follow the USP43-NF38 monograph method for dexamethasone assay and organic impurity profiling. Baseline-resolved peaks were obtained for dexamethasone, betamethasone, dexamethasone acetate, and desoximetasone within 20 minutes with a Titan™ C_{18} UHPLC column (10 cm x 2.1 mm, 1.9 µm particles). This column has the same phase, length, and ID like in the monograph, but the packing is based on monodisperse particles with a slightly larger average particle size (1.9µm), than referenced in the monograph (1.7 µm). The method was validated following the guidelines in USP General Chapters <621>, <1225>, and <1226>. The use of lower sample concentrations was compensated by a larger injection volume (to maintain mass on column) to improve reproducibility. The chromatographic separation was achieved using a mixture of 3.4 g/L monobasic potassium phosphate solution (pH 3.0) and acetonitrile as the mobile phase with gradient elution and UV detection at 240 nm. Although, comparatively a shorter relative retention time (RRT) was observed for dexamethasone acetate and desoximetasone, both the compounds showed an excellent chromatographic resolution (Rs > 10). Under the applied conditions, system suitability requirements are met, and the method demonstrates good selectivity, reproducibility, sensitivity, and accuracy.



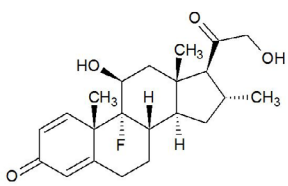
Dexamethasone



Betamethasone



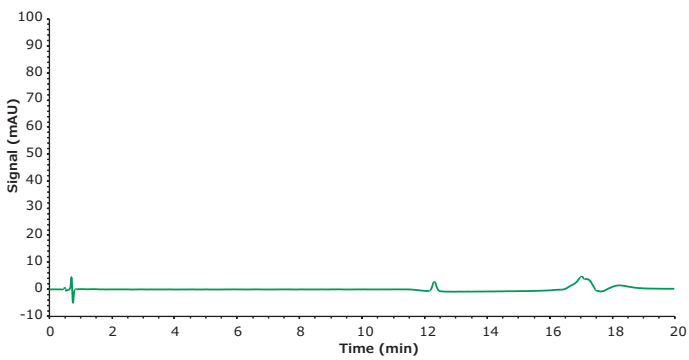
Dexamethasone Acetate



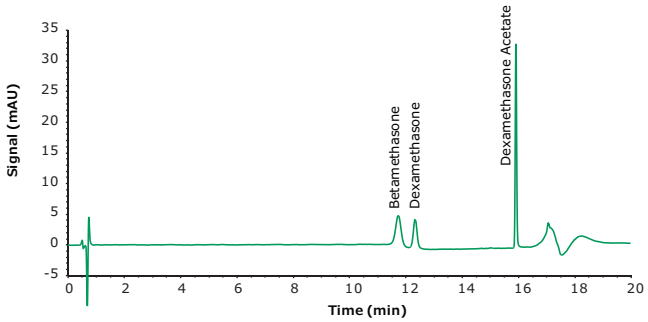
Desoximetasone

Experimental Conditions			
column:	Titan™ C_{18} UHPLC Column 10 cm x 2.1 mm, 1.9 µm		
mobile phase:	[A] 3.40 g of potassium dihydrogen phosphate in 1000 mL water and adjust to pH 3.0 with phosphoric acid [B] acetonitrile		
gradient:	**time (min)**	**% A**	**% B**
	0 – 10	76	24
	10 – 15	76 – 45	24 – 55
	15 – 16	45 – 10	55 – 90
	16 – 16.1	10 – 76	90 – 24
	16.1 – 20	76	24
flow rate:	0.4 mL/min		
pressure drop:	320 - 470 bar		
column Temp.:	35 °C		
detector:	UV @ 240 nm (analytical flow cell; 13 µL)		
injection:	5 µL		
Samples			
diluent:	acetonitrile and water (56:44) v/v.		
test solution:	dissolve 0.04 g of Dexamethasone CRS in 25 mL diluent (1.6 mg/mL).		
system suitability solution:	dissolve 12 mg of Dexamethasone and 8 mg of Betamethasone in 100 mL diluent		
standard solution:	dissolve 1.6 mg of Dexamethasone, 2.4 mg of Betamethasone, 4.8 mg of Dexamethasone acetate using 10 mL diluent further take 1.0 mL of this solution and dilute to 100 mL using the diluent.		
Reference solution	dilute 5 mg of EP dexamethasone for system suitability in 5 mL diluent		

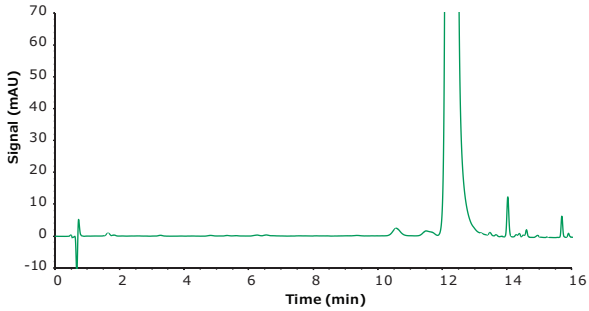
Blank



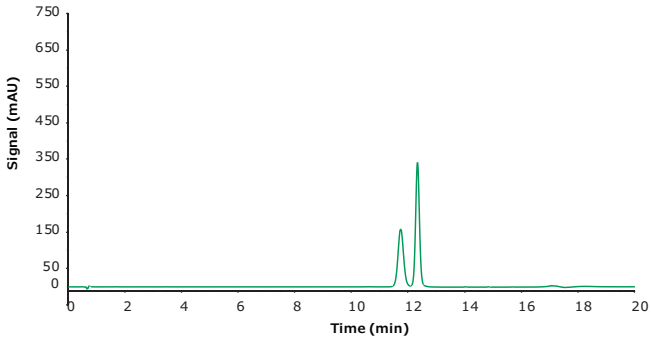
Standard Solution



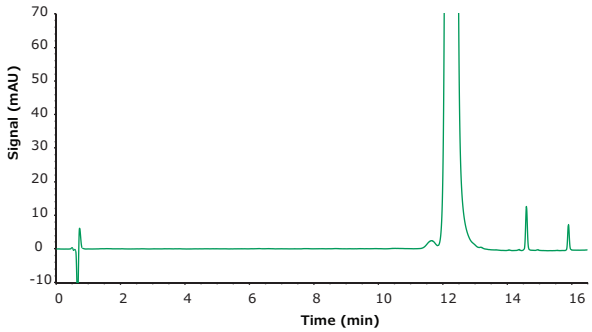
Dexamethasone (Test Solution)



System Suitability Solution



Reference Solution



Chromatographic Data (System Suitability Solution)

Peaks	Compound	Retention Time (min)	RRT	Resolution	Resolution requirement per USP43-NF38	Tailing factor	Tailing requirement per USP43-NF38	Theoretical Plates
1	Betamethasone	11.7	0.95	-		1.03	NMT 2.0	17101
2	Dexamethasone	12.3	1.00	1.99	NLT 1.5	1.0	NMT 2.0	38792

Chromatographic Data (Reference Solution)

Peaks	Compound	Retention Time (min)	RRT	RRT Reference per USP43-NF38	Resolution	Tailing factor	Theoretical Plates
1	Betamethasone	11.7	0.95	0.94	-	1.15	10,612
2	Dexamethasone	12.3	1.00	1.00	1.5	1.03	20,692
3	Desoximetasone	14.6	1.19	1.58	10.2	1.06	340,463
4	Dexamethasone Acetate	15.9	1.29	1.74	13.4	1.02	449,570

Repeatability (Reference Solution)

Peaks	Compound	Area Response (n=3)	Standard deviation	RSD (%)	RSD (%) Reference per USP43-NF-38
1	Betamethasone	39.70	0.48	1.2	5.0
2	Dexamethasone	25,221.73	263.78	1.0	5.0
3	Desoximetasone	50.18	0.50	1.0	5.0
4	Dexamethasone Acetate	27.44	0.34	1.2	5.0

Featured Products

Product list	Cat. No
Titan™ C_{18} UHPLC Column (100 x 2.1 mm, 1.9 µm)	**577124-U**
Water for chromatography (LC-MS grade) LiChrosolv® or tap fresh from a Milli-Q® ultrapure water system	**1.15333**
Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	**1.00030**
Potassium phosphate monobasic, , anhydrous, puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., 99.5-100.5%	**60220-M**
Ortho-phosphoric acid EMSURE®	**1.00573**
Millex® syringe filter units, disposable, Durapore® PVDF, pore size 0.22 µm, non-sterile	**SLHVX13NK**

Product list	Cat. No
Pharmacopeia Reference Materials	
Dexamethasone United States Pharmacopeia (USP) Reference Standard	**1176007**
Betamethasone United States Pharmacopeia (USP) Reference Standard	**1066009**
Dexamethasone acetate United States Pharmacopeia (USP) Reference Standard	**1176506**
Desoximetasone United States Pharmacopeia (USP) Reference Standard	**1173508**
Dexamethasone for system suitability European Pharmacopoeia (EP) Reference Standard	**Y0001177**

To read more about Titan HPLC columns visit us at **SigmaAldrich.com/Titan**

COVID-19 related topics can be found at **SigmaAldrich.com/COVID-19**

Released *N*-Glycan Analysis of a Therapeutic Antibody Using BIOshell™ Glycan Column

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Introduction

Therapeutic monoclonal antibodies (mAbs) have seen an explosive growth since the first mAb was approved by the US FDA over thirty years ago. In fact, over the past five years, therapeutic antibodies have become the best-selling drugs,[1] and they continue to grow in terms of new approvals and targets.[2]

Monoclonal antibodies are target specific, which means that they have high-efficacy and few side effects. However, compared to chemically synthesized small molecule therapies, mAbs are considerably more complex owing to their size and the nature of their development and production. These mAbs are expressed using recombinant technologies in mammalian cell lines or other expression systems, giving rise to heterogeneity mainly through post-translational modifications (PTMs).[3] These PTMs need to be characterized as they affect the efficacy, stability, half-life, and safety of mAbs.

Glycosylation is one of the most common and important PTMs for mAbs. Glycosylation involves the attachment of glycans at specific sites on a protein, most commonly at asparagine (Asn) (*N*-linked) or serine/threonine (Ser/Thr) (*O*-linked) amino acid residues.[4] There are four levels of analytical approaches to *N*-glycan analysis: intact glycoproteins, glycopeptides, released glycans, and monosaccharide analyses.[5] This article focuses on the analysis of released *N*-glycans by high-pressure liquid chromatography (HPLC).

The steps for a released *N*-glycan analysis are outlined in **Figure 1**. The *N*-linked glycans are released by an amidase such as peptide-N-glycosidase F (PNGase F). The released glycans are then labeled with a fluorescent tag, like aminobenzamide (2-AB) or procainamide (4-amino-N-[2-(diethylamino)ethyl] benzamide). Prior to the HPLC analysis, a clean-up step is needed to remove excess tags and salts. Hydrophilic interaction liquid chromatography (HILIC) is a proven technique for the separation and quantitation of glycans over other HPLC methods (*e.g.* reverse phase, anion exchange).[4]

In this article, a BIOshell™ Glycan HPLC column is used to analyze Cetuximab (Erbitux®) *N*-glycans labeled with procainamide. BIOshell™ Glycan HPLC columns are specifically engineered to deliver a fast, high-resolution, and reproducible glycan identification using HILIC.

Experimental

Glycan Release and Labeling - PNGase Fast Kit was used for glycan release with FASP (filter aided sample prep). The released glycans were labeled using procainamide with reductive amination.

Sample Cleanup - The labeled samples were diluted with 99% acetonitrile and loaded to the conditioned Discovery Glycan SPE cartridges. They were allowed to flow through the cartridges slowly, using gravity (slight pressure or vacuum would also be suitable), making sure that the sample was entirely in the resin bed. The cartridges were washed five times with 99% acetonitrile using vacuum. After the washing step, 20% acetonitrile was added to each cartridge, and allowed to elute slowly, using gravity (applying slight pressure or vacuum would also be suitable). After all the eluent had passed into the resin bed, vacuum was used to evacuate all liquid from the SPE into the collection tube. The eluted labeled glycans were then dried by vacuum centrifugation. **Table 1** shows the SPE conditions.

Figure 1. Workflow for Released *N*-Glycan Analysis by HPLC.

Table 1. SPE Conditions

sample matrix:	Labeled sample diluted with 1 mL 99% acetonitrile/1% water
SPE tube:	Discovery® Glycan SPE Tube (**55465-U**)
conditioning:	1 mL 99% acetonitrile/1% water
sample loading:	diluted labeled sample slowly sink into bed
washing:	5 x 1mL 99% acetonitrile/1% water
eluent:	400 µL 20% acetonitrile/80% water, slowly sink into bed
eluate post-treatment:	drying by vacuum centrifugation

HPLC Analysis - The dried and labeled glycans were solubilized by dissolving in 50 µL of 75% ACN / 25% 75 mM ammonium formate pH 4.4, vortexing for 2 mins, followed by centrifugation at 16,000 x g for 2 mins. **Table 2** shows the chromatographic conditions.

Table 2. HPLC Condition for the Analysis of Procainamide Labeled Cetuximab N-Glycans

column:	BIOshell™ Glycan; 15 cm x 2.1 mm I.D., 2.7 µm
mobile phase:	[A]: 75 mM ammonium formate pH 4.4 (50 mM ammonium hydroxide, adjusted to pH 4.4 with formic acid) [B]: Acetonitrile
gradient:	75% B to 59% B in 75 min
flow rate:	0.37 mL/min
column temp.:	58 °C
detector:	Fluorescence, 308 nm excitation,359 nm emission
injection:	10 µL
sample:	dried procainamide labeled Cetuximab reconstituted in 50 µL 25% 75 mM ammonium formate pH 4.4 and 75% acetonitrile

Results

In this study, cetuximab was used as a model therapeutic mAb to analyze released N-glycans. This mAb is a chimeric mouse-human IgG_1 monoclonal antibody, against the epidermal growth factor receptor (EGFR). Cetuximab is used to treat head, neck, as well as colorectal cancers. The antibody is N-glycosylated both in the fragment crystallizable (Fc) and fragment antigen binding (Fab) regions. There are numerous studies and reports showing the attachment of N-glycans to mAbs and the subsequent effect of the attachment on various biological and physicochemical processes, leading to safety and quality issues.[6,7] Some of the processes affected by the glycosylation include enhancement of the structural integrity of the mAb, serum half-life, antibody-dependent cellular toxicity (ADCC), anti-inflammatory activities, immunity, and antigen recognition. This clearly indicates towards the important need for understanding glycosylation patterns.

BIOshell™ HPLC columns are based on Fused-Core® particles (also called core-shell or superficially porous particles (SPPs)), characterized by a thin, porous shell of high-purity silica surrounding a solid, silica core. This design allows for a shorter diffusion path compared to traditional fully porous particles, as illustrated in

Figure 2. The short diffusion path accelerates mass transfer of solutes ("C" term in the van Deemter equation), concomitantly resulting in high column efficiency.

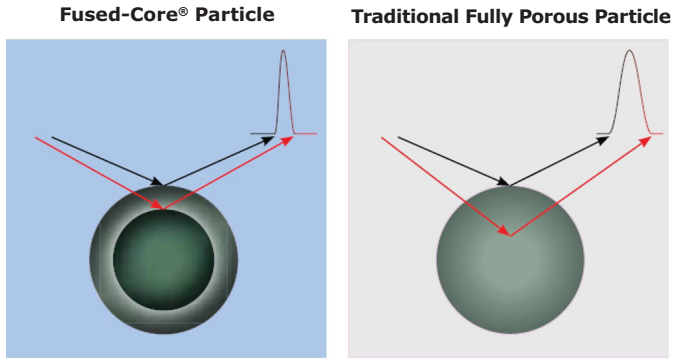

Figure 2. Fused-Core® Particles have Shorter Diffusion Paths Compared to Traditional Fully Porous Particles.

The stationary phase in the BIOshell™ Glycan column is a highly polar ligand that has five hydroxyl (-OH) groups tethered to the silica via a novel and proprietary chemical linkage. This unique column chemistry is suitable for analysis of oligosaccharides, particularly for protein-linked glycans using the typical mobile phases for HILIC of oligosaccharides.

A fluorescence chromatogram of procainamide-labeled cetuximab glycans is shown in **Figure 3**. The BIOshell™ Glycan column was able to elucidate the complex glycosylation of this mAb. The corresponding glycans were identified by MS analysis (mass spectrometry data not shown).

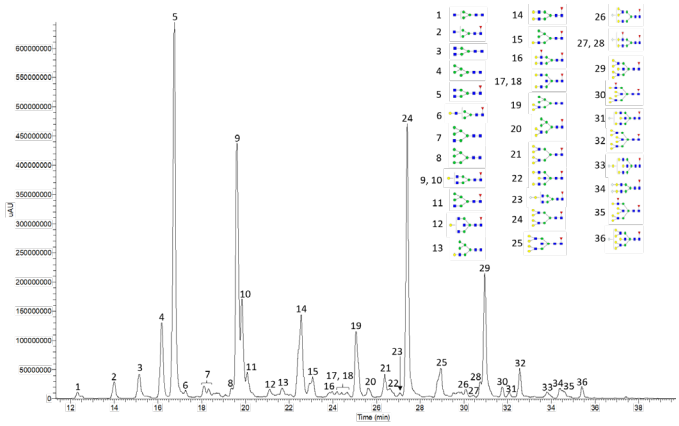

Figure 3. Fluorescence Chromatogram of Procainamide-Labeled Cetuximab Glycans on BIOshell™ Glycan column. LC-MS was Used to Characterize each Peak (Chromatogram not shown). A rapid Release Glycan Protocol was used.

Successful analysis of N-linked glycans by HPLC requires an efficient and reproducible glycan release step. The traditional protocol involves multiple wash steps and an overnight digestion of the native or denatured mAb. The protocol described in the experimental section of this article is a fast protocol

that uses a proprietary detergent-based buffer for rapid deglycosylation of N-linked glycans using PNGase F. In this fast protocol, complete release of N-glycans is achieved in a 15-minute incubation, compared to the traditional overnight digestion.

In another experiment, the BIOshell™ Glycan column was used to compare released glycans from cetuximab using three glycan release protocols:

- Traditional overnight protocol, denatured using guanidine hydrochloride
- Fast protocol, non-reduced (rapid deglycosylation)
- Fast protocol, reduced (rapid deglycosylation under reducing conditions using 2-mercaptoethanol)

The results are shown in **Figure 4**.

With this particular analyte (Cetuximab), all three protocols were found to be equally efficient for some glycan species, such as G0F-N, Man5, G0F, G_1(F1,6), Man5G0F Hybrid, and G1F (1,3). But there were some glycans that were not efficiently released

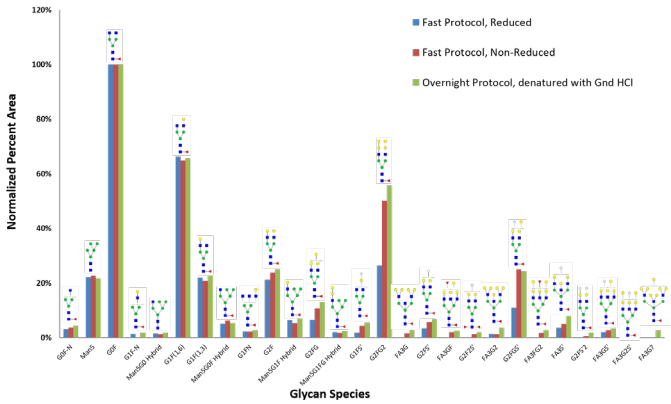

Figure 4. Comparison of Cetuximab Glycan Distribution Using three Glycan Release Protocols: ■ Fast (reduced), ■ Fast (non-reduced), and ■Traditional overnight protocol. A BIOshell™ Glycan Column was Used in all three Samples.

with the fast protocol when a reducing step using 2-mercaptoethanol was included, for example G1F5', $G2FG_2$, FA3G, FA3GF, G_2F2S', and G2FGS'. Indeed, the use of 2-mercaptoethanol in the denaturing step is not required for most proteins while using the fast protocol. But, there are proteins, like RNAse B, that seem to require it. It is recommended that as part of optimizing a method for released glycan analysis, the fast protocol must be tested with and without 2-mercaptoethanol to check for best results.

It is also worth noting that some proteins are not amenable to fast deglycosylation techniques. When working with mAbs without established protocols for glycan analysis, it is best to compare the results of the traditional overnight digest to the fast/rapid digestion protocol.[8]

Conclusion

Characterizing and monitoring the glycosylation pattern of a therapeutic mAb is required by regulatory authorities to ensure efficacy and safety of the drug. While analysis and identification of glycans can be challenging because of their structural complexity, this article has shown that a BIOshell™ Glycan HPLC column was able to elucidate the complex glycosylation of cetuximab after an appropriate glycan release and labeling protocol. Another key consideration in glycan analysis is the deglycosylation protocol. While there is a fast method that significantly saves time, it is recommended to compare the results with the traditional overnight digestion and choose the one that gives more efficient deglycosylation.

Featured Products

Description	Cat. No.
Glycan Release	
PNGase Fast Kit	**EMS0001-1KT**
30 kDa MWCO Centrifugal Filtration Units, 0.5 mL	**MRCF0R030**
Labeling	
Procainamide HCl	**SML2088 or PHR1252**
Cleanup	
Discovery® Glycan SPE Tube 50mg / 1mL, Pk.108	**55465-U**
Visiprep™ SPE Vacuum Manifold DL (Disposable Liner), 12-port	**57044**
Acetonitrile, HPLC gradient grade or hypergrade for LC-MS LiChrosolv®	**34851 or 1.00029**
HPLC	
BIOshell™Glycan, 15 cm x 2.1 mm I.D., 2.7 µm	**50994-U**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Ammonium formate eluent additive for LC-MS, LiChropur™, ≥99.0%	**70221**
Formic acid 98% - 100% for LC-MS LiChropur™	**5.33002**

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Ion Chromatography & Cation Suppression

Straightforward cation analysis at trace levels

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Daniel Weibel, Product Manager Trace Organic Analysis & Sensorics, MilliporeSigma, Analytix@milliporesigma.com

Trace analysis of cations, amines, and transition metals by ion chromatography can be carried out with or without ion suppression. However, some applications require particularly a highly sensitive analysis. This can only be achieved by sequential suppression, as suppression considerably lowers the detection limits of the analytes.[1] Such analyses are common, for instance, in power plant or pharmaceutical applications. Moreover, there are several norms and standards that request for a suppressed cation analysis (e.g. the ASTM D6919 - 17 Standard Test Method for Determination of Dissolved Alkali and Alkaline Earth Cations and Ammonium in Water and Wastewater by Ion Chromatography). In short, suppression reduces background conductivity to a minimum and decreases baseline noise. Both effects together improve the signal-to-noise ratio and increase the sensitivity of the measuring system. Thus, whenever the quantification of very low concentrations of cations is required, analysis with sequential suppression is the method of choice.

Analyses that benefit with the use of sequential suppression

Some typical examples of cation suppression are:

- Traces and ultratraces of Na in the presence of monoethanolamine at high concentrations (typical of sample matrices in nuclear power plants).
- Trace and ultratrace concentrations of alkali and alkaline earth metals such as Li, Na, K, Mg, or Ca, and NH_4+ in ultrapure water.
- Traces of transition metals, e.g. Co, Ni, Zn, Mn, and Cd in various types of water samples.
- Aliphatic and aromatic amines in pharmaceuticals, e.g. piperazine in cetirizine·HCl, tetrabutylammonium in atorvastatin, dimethylamine in meropenem, dimethylamine in imatinib mesylate, and meglumine in meglumine salts.

How does sequntial cation suppression work?

The term "sequential suppression" represents the combination of chemical suppression immediately followed by CO_2 suppression.

Chemical Suppression

The Metrohm Suppressor Module (MSM) is conditioned/regenerated using a carbonate buffer (**78698**). The anion exchange resin causes the conversion of all counterions into their respective hydrogen carbonate salts. Dissociated acids (e.g. nitric acid) are used as the eluent. In addition, trace amount of rubidium is added to stabilize the baseline in trace analysis (**78737**).

The eluent counterions are also replaced with hydrogen carbonate. The carbonic acid produced in this way is unstable and only weakly dissociated, resulting in the measurement of a lower background conductivity, in contrast with the non-suppressed eluent.

Sequential Suppression

For sequential suppression, chemical suppression is combined with subsequent CO_2 suppression. This is accomplished with a Metrohm CO_2 Suppressor (MCS). In the MCS, the eluent is passed through a capillary made of a gas-permeable membrane surrounded by a vacuum. This removes the carbon dioxide formed, and hence all the hydrogen carbonate from the flow path. All what remains in addition to the analyte is mainly water.

The sequential suppression configuration described above reduces background conductivity (< 0.2 µS/cm) and increases the detection sensitivity of the analytes. Suppression makes the injection peak very small. This means higher resolution between the injection peak and the early eluting cations, e.g. lithium, which makes the integration and quantification of these peaks easier.

Determination of ammonium in acidic absorption solution[2]

Ammonia scrubber systems are required by law to be used by manufacturers in the chemical industry to reduce or eliminate their ammonia gas emissions. The employed acidic scrubber solutions typically have a pH of 2 or lower. This pH value is too low for silica-based IC columns generally applied in direct conductivity detection of cations. The Metrosep C Supp 2 is polymer-based and allows the injection of low pH samples. As a proof of concept, in this work

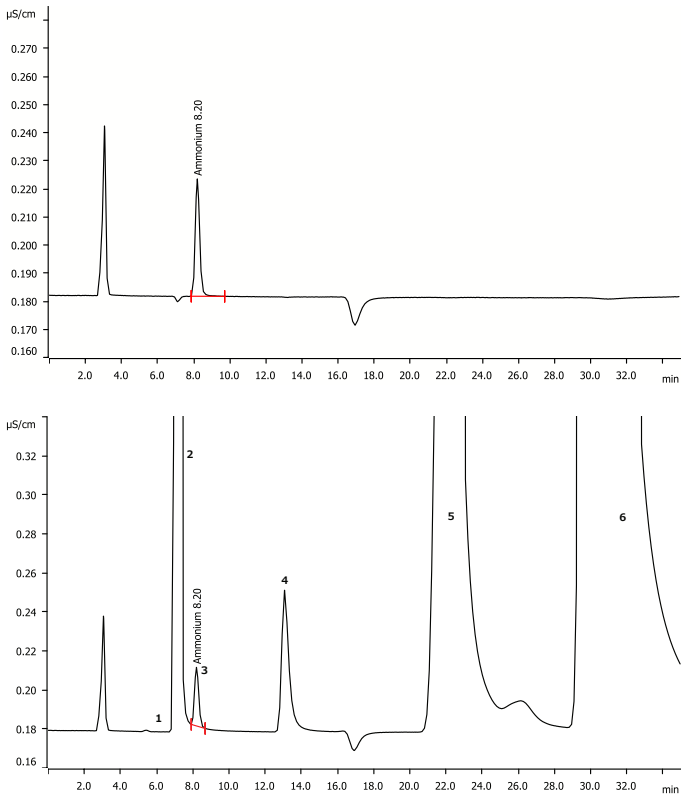

Figure 1. Chromatogram of an acidified standard (top) and acidified drinking water sample spiked with ammonium (bottom). Peak 3 is NH_4^+.

two acidified drinking water samples spiked with 0.15 and 0.4 mg/L of ammonium were analyzed. The results as shown in **Figure 1** and **Table 1**, indicate that the analysis of such acidic solutions by conductivity measurements can be carried out after sequential cation suppression.

The measured ammonia concentrations (Peak 3 in **Figure 1**) are given in **Table 1**. Li^+(1), Na^+(2), K^+(4), Mg^{2+}(5), Ca^{2+}(6) were not quantified. The peak between Mg and Ca might be Zn. Negative peak at 17 min corresponds to the Rb in the eluent.

Table 1. Ammonium concentrations using sequential suppression

Sample	Standard		Drinking water spiked		Drinking water spiked	
Cation	Conc [mg/L]	Recovery [%, N=2]	Conc [mg/L]	Recovery [%, N=2]	Conc [mg/L]	Recovery [%, N=2]
NH_4^+	0.2	100	0.15	96.7	0.4	97.5

Conditions

sample:	Drinking water acidified to pH 2, directly injected, no further sample prep
columns:	Metrosep C Supp 2 – 250/4.0
	Metrosep C Supp 2 Guard/4.0
Solutions	
eluent:	5.0 mM nitric acid
	50 µg/L rubidium
eluent concentrate:	100 mM nitric acid
	1 mg/L rubidium
suppressor regenerant:	70 mM $NaHCO_3$
	70 mM Na_2CO_3
rinsing solution:	STREAM
Parameters	
flow rate:	1.0 mL/min
injection volume:	10 µL
pmax:	25 MPa
recording time:	34 min
Column temperature:	40 °C
Analysis	
Conductivity detection after sequential suppression	
Instrumentation	
930 Compact IC Flex Oven/SeS/PP/Deg	
IC Conductivity Detector	
858 Professional Sample Processor	
941 Eluent Production Module	
MSM-HC Rotor C	

Conclusion

This article demonstrates that the acidic binary IC eluent nitric acid/rubidium nitrate concentrate and the sodium bicarbonate/sodium carbonate suppressor regenerant for use with Metrosep C Supp 2 column, allows for the accurate determination of ammonium in acidic solutions, such as those found in ammonia scrubbers. We also offer a representative range of inorganic cation standards as certified reference materials (CRMs) for IC.

References

1. Metrohm Literature 8.000.5163 “Brochure: Cation suppression in ion chromatography – Cation determination in the trace range”, https://www.metrohm.com/en/documents/80005163
2. Metrohm Literature AN-CS-017 “IC Application note: Metrosep C Supp 2 – 250/4.0: Ammonium in acidic absorption solution – proof of concept”, https://www.metrohm.com/en/applications/AN-CS-017

Featured Products

Description	Package Size	Cat. No.
IC Eluent Concentrates		
Nitric acid/Rubidium nitrate concentrate	1 L, 2.5 L	**78737**
Sodium carbonate regenerant	2.5 L	**78698**
IC Certified Reference Materials - *Trace*CERT®		
Ammonium Standard for IC	100 mL	**59755**
Lithium Standard for IC	100 mL	**59878**
Sodium Standard for IC	100 mL	**43492**
Magnesium Standard for IC	100 mL	**89441**
Potassium Standard for IC	100 mL	**53337**
Calcium Standard for IC	100 mL	**39865**
Zinc Standard for IC	100 mL	**67902**

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SPME Adsorbent Fibers on Nitinol Core for Improved Fiber Reproducibility

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Abstract

Adsorbent SPME fibers with Carboxen® or divinylbenzene (DVB) coatings on a Nitinol core have been introduced. In the provided study, the Nitinol core fibers show an improved reproducibility and mechanical stability vs. their predecessors on fused silica cores while maintaining inertness and analyte recovery, enabling direct implementation into existing methods.

Introduction

Late 2019, saw the introduction of Supelco® adsorbent fibers with a Nitinol core. Compared to absorbent ("film") type coatings, adsorbent ("particle") type coatings for SPME fibers facilitate multiple interaction/mechanisms between sample analytes and the coating, often improving analyte retention/sensitivity. However due to multiple materials used in particle type SPME fiber coatings, such as adsorbent and binder/adherent, variability between fibers can increase. Nitinol core coated fibers, contained in standard stainless steel (SS) assemblies, are not only more durable but also highly reproducible. In this article, the various factors likely to cause variability such as the coating process, testing process, inertness, raw materials, and durability are discussed by comparing coatings on fused silica cores and Nitinol cores.

Results of Study

Coating process

Nitinol core fibers are produced using new state-of-the-art coating equipment. The equipment allows reliable and reproducible application of coatings to the Nitinol core and monitors the entire process. **Table 1** shows the results of coated fiber diameter measurements from multiple coating lots. The resulting inter-lot and intra-lot variabilities are less than 1% RSD.

The new proprietary coating process also results in an even distribution of a highly bonded coating around the Nitinol core, further contributing to the improvement of fiber reproducibility. Further, the reproducibility of performance of newly made fibers with a Nitinol core was tested against historically available fibers with a fused silica core.

Table 1. Variability of Measurements of Multiple Fiber Coatings Lots

	Diameter Measurements	
Fiber Lot	µm	RSD
2049-10	282.5	0.37%
2049-11	283.3	0.39%
2049-13	278.7	0.26%
2049-46	277.0	0.19%
2049-47	276.2	0.24%
2049-48	282.2	0.13%
2049-57	281.7	0.38%
2049-74	277.3	0.39%
2049-75	277.8	0.39%
Average#	279.6	0.29%#
Std. Dev.	2.76	
RSD	0.99%*	

*Inter-lot Variability #Intra-lot Variability

Analytical Testing Results

Method Precision

The QC testing of a fiber should be a good measurement of the performance of the fiber coating. The components in the test mix should measure various characteristics of the adsorbents and the testing method must be reliable.

With every adsorbent it is a balance between extraction efficiency versus desorption efficiency, so the test mixture must contain difficult to retain analytes and analytes that are difficult to desorb. Different classes of analytes that are retained by different adsorption mechanisms are useful. A 4-component mixture that meets these criteria was formulated.

To validate the method, an autosampler with fiber exchange capability was used. This capability allowed for multiple extractions per fiber (4) and also the test of multiple fibers per lot, in an automated manner. The four extractions from four samples per fiber offered the advantage of establishing precision in area counts of four extractions, for each analyte.

Table 2 shows the average of the precision of repeated extractions from over 200 fibers (800 extractions). Fibers used had PDMS/DVB and Carboxen®/PDMS coatings on fused silica and Nitinol cores.

Table 2. Summary of Precision (as %RSD) of Repeated Extractions. Based on 200+ Fibers

Isobutyl acetate	Toluene	n-Butanol	o-Xylene	Average of 4
2.03%	2.04%	1.92%	1.80%	1.95%

The results indicate that the method is highly reproducible and reliable.

Comparison of Fiber Cores

Fourteen fiber coating lots of Carboxen®/PDMS phase on Nitinol core and fourteen coating lots on fused silica core were evaluated with the automated QC testing method. For each core, the RSD per analyte was determined based on the total extractions of the fibers per given lot. The overall RSD per given lot was the average value of the 4 analyte RSD values. The intra-lot RSD values for all the lots of each core type were averaged to obtain the overall intra-lot values per core type.

The inter-lot RSD values were obtained by determining the RSD values per analyte from all the extractions from the 14 lots per each core type. The RSD values of the 4 analytes were averaged to obtain the overall inter-lot variability per core type. **Figure 1** shows the results of the intra-lot and inter-lot variability of the 2 fiber cores with Carboxen®/PDMS coatings.

The results showed that there was a 43.8% reduction in intra-lot variability and a 68.1% reduction in inter-lot variability with fibers coated on a Nitinol core compared to fibers coated on a fused silica (FS) core. The formula used to calculate the % reduction was ((FS-Nitinol) ÷ FS) x 100.

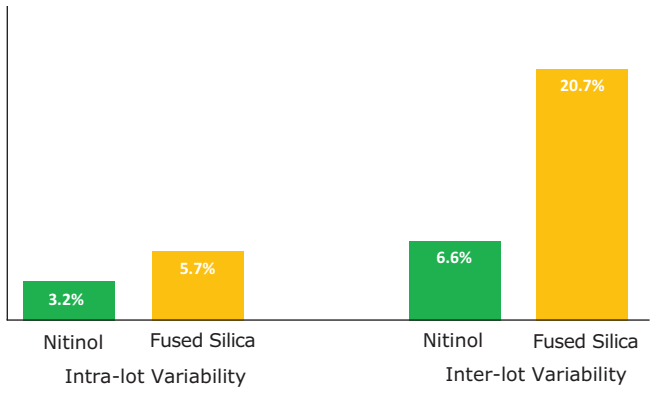

Figure 1. Comparison of Carboxen®/PDMS Coatings on Fused Silica and Nitinol Core Fibers (n=14 Fiber Coating Lots/Core)

Figure 2 shows the results of variability between the fiber cores for the PDMS/DVB coating. The calculations were obtained from eight fiber lots on fused silica and eight lots on Nitinol cores following the same procedures that were used to calculate the Carboxen®/PDMS coating variability.

The results showed that there was a 81.3% reduction in intra-lot variability and a 71.6% reduction in inter-lot variability for Nitinol core coated fibers compared to fused silica core coated fibers. Typically, intra-lot variability is found to be significantly lower than inter-lot variability, but for PDMS/DVB coated fused

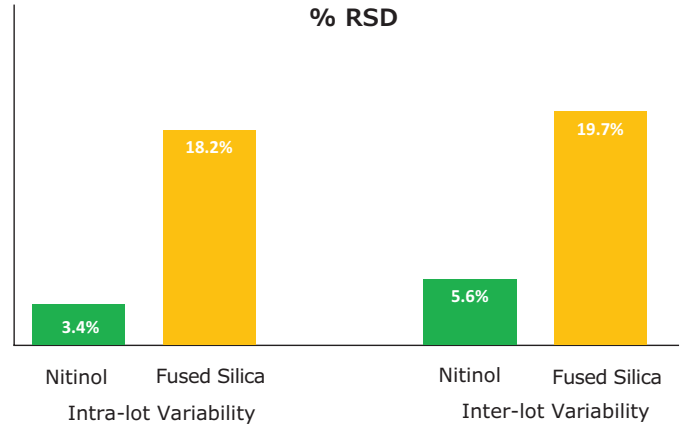

Figure 2. Comparison of PDMS/DVB Coatings on Fused Silica and Nitinol Fiber Cores (n=8 Lots per Fiber Core Type)

silica core fibers the intra-lot variability was similar to the inter-lot variability. This was not the case with the Carboxen® coatings on the fused silica core. The Carboxen® carbon molecular sieve is a much stronger adsorbent compared to DVB, with a higher percentage of micropores and a narrower average micropore diameter of 12 Å, than DVB's 18 Å. The coating thickness variability is less of a factor for Carboxen® coated fibers compared to DVB coated fibers. The retention of analytes on a weaker adsorbent is more dependent on the coating thickness than with the stronger adsorbent. Slight variations in the coating thickness are more easily observed with the weaker adsorbent. Since the coating process on Nitinol core is more tightly controlled than it is for fused silica core fibers, the latter ones show a variability of the coating thickness that is greater within a lot. This indicates the efficiency and reliability of the testing process for detecting variations in the coating, and subsequently to ensure high quality and reproducible fibers.

Inertness

For a fiber to deliver reliable, reproducible results, and exhibit only desired analyte-coating interactions, the core must be inert. An active core can catalyze the breakdown of some analytes during the desorption process. This can not only decrease the response of the analytes but can also create artifacts that were not present in the original sample. For example, alkyl halides tend to be susceptible to breakdown, especially when the desorption temperature is above 250 °C. The higher the temperature the greater the breakdown. Typically, the breakdown is further enhanced by the presence of iron that catalyzes the reaction.

Fused silica is typically used as a benchmark of high inertness, and thereby the coatings on fused silica cores

were compared to the coatings on the Nitinol core. Nitinol is a combination of titanium and nickel that makes up over 99.99% of the composition. There are small amounts of trace metals also present, but in low or sub ppm levels. The material is used in the medical industry to produce highly inert devices such as stents.

In this study, the comparison of cores was carried out for the extraction and desorption of haloalkanes. In most cases, the breakdown of an alkyl halide takes place with a loss of an HCl or HBr molecule, forming a non-saturated-haloalkene. Since there were no haloalkenes spiked in the samples, the presence of haloalkenes in the analysis were the result of breakdown of the haloalkanes. Both the Carboxen®/PDMS coating and the PDMS/DVB coating were compared on both fiber cores. **Figures 3** and **4** show the average percentage of breakdown by core type with Carboxen®/PDMS and PDMS/DVB coatings, respectively. The average is based on four extractions per fiber coating and core type.

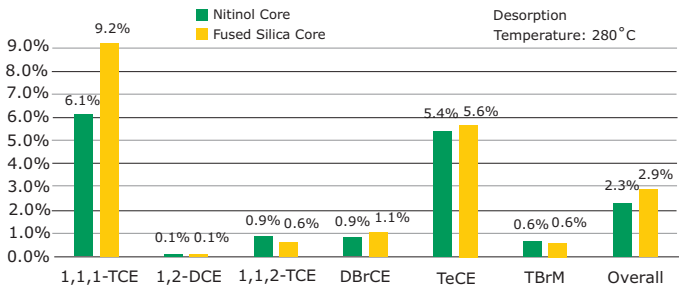

Figure 3. The Average Percentage of Breakdown of Haloalkanes by Core Type for Carboxen®/PDMS fibers (n=4). 1,1,1-TCE =1,1,1-trichloroethane; 1,2-DCE=1,2-dichloroethane; 1,1,2-TCE=1,1,2-trichloroethane; DBrCE=dibromochloroethane; TeCE=sum of 1,1,1,2- & 1,1,1,1-tetrachloroethane; TBrM=Bromoform; Overall=Average breakdown of all haloalkanes

The results show that both core types exhibit similar behavior and the primary cause of the breakdown of the analytes is the desorption temperature. Both core types appear to be inert.

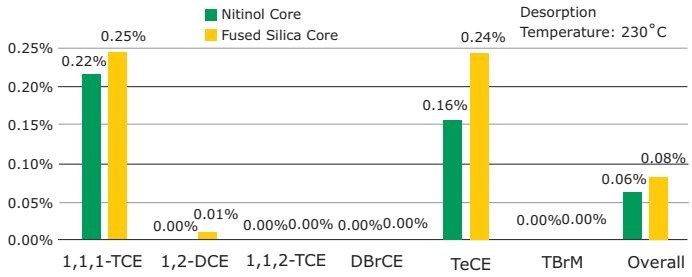

Figure 4. The Average Percentage of Breakdown of Haloalkanes by Core Type - PDMS/DVB (n=4) - For Compound IDs see **Figure 3**.

Raw Materials

The coating materials used for the Supelco® SPME fibers are manufactured in-house. This enables complete control of the materials used in the production of the coatings. When a manufacturer purchases particles or adsorbents from an outside vendor, they may have little control on the specifications and manufacturing process of the materials received. This could result in more variable coatings between lots of raw materials.

Since the introduction of the Nitinol core fibers, additional specifications have been added to the adsorbent particles and polymers. The additional specifications ensure that the raw materials are even more consistent between manufacturing lots and produce fibers with less inter-lot variability.

Analyte Recovery Performance

Those currently using adsorbent coatings on a fused silica core may have concerns about fiber performance when switching to Nitinol. Our testing has shown that there is little difference in analyte recovery between the two core types with the same coating. To demonstrate the similarity in recoveries, a group of alkyl halides was extracted four times with the two adsorbent coatings on both fiber cores. **Table 3** shows the average responses obtained and the percent difference between the fiber cores for each coating type.

Table 3. Comparison of Analyte Response (Peak Area) between Fiber Cores and Coatings

	PDMS/DVB Coating			Carboxen®/PDMS Coating		
Analyte	Nitinol	Fused silica	Percent Difference	Nitinol	Fused silica	Percent Difference
1,1,1-trichloroethane	8088154	7912758	2.2%	29939050	31422213	-5.0%
1,2-dichloroethane	4293384	4117080	4.1%	26590648	24668157	7.2%
1,1,2-trichloroethane	11273578	11725096	-4.0%	22329364	20896864	6.4%
dibromochloromethane	17196101	17801539	-3.5%	28040335	26462127	5.6%
1,1,1,2-tetrachloroethane	21794638	22258723	-2.1%	25229513	23347665	7.5%
tribromomethane	20050799	20789889	-3.7%	21482461	19892605	7.4%
1,1,2,2-tetrachloroethane	25324934	25937177	-2.4%	24819794	24055163	3.1%

Percent Difference is calculated by the following formula:

$$\frac{\text{Nitinol response} - \text{fused silica response}}{\text{Nitinol response}}$$

The difference in analyte responses between the two core types is small; similar to what would be expected between fibers on the same core type, but of different coating lots.

Durability

A more durable fiber made with a more rugged coating process, reduces the chance of breakage and damage, and increases the number of extractions that can be carried out. The Nitinol core fibers are very flexible and mechanically stable. **Figure 5** shows a picture of the fiber flexed at a 90° angle without breaking. The fibers have even been flexed 180° until they touched the inner rod without any breakage. A fused silica fiber will typically break when flexed between 30°-45°. However, irrespective of the flexibility of Nitinol core fibers, it is recommended to keep mechanical stress to a minimum.

Any damage to the fiber coating results in an increased fiber variability. The proprietary coating process creates an extremely well bonded coating, less prone to easy damage, and resulting in more reproducible results over time.

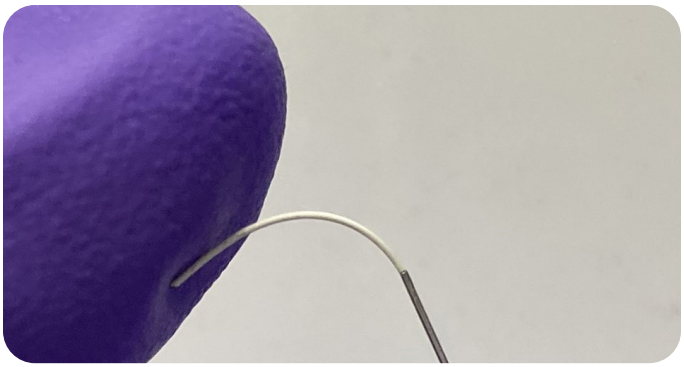

Figure 5. Flexing of a Nitinol Fiber at a 90° Angle

Conclusion

The adsorbent fiber coatings on the Nitinol core have been shown to be highly reproducible due to the following reasons:

- New state-of-the-art coating technology – variability in coated fiber diameter under 1% RSD
- Improved intra-lot reproducibility for Nitinol cores, as compared to the fused silica cores. 43.8% lesser variability for Carboxen® coatings and 81.3% lesser for DVB coatings
- Improved inter-lot reproducibility for Nitinol cores compared to the fused silica cores. 68.1% lesser variability for Carboxen® coatings and 71.7% lesser for DVB coatings
- New QC testing process
  - Repeatable extractions with fibers having less than 2% RSD
  - Testing probes that measure different adsorption mechanisms
- Good fiber inertness – similar or better than fused silica fiber cores
- Highly controlled raw materials – in-house made materials with additional specifications to have more tight control on variability between material lots
- High durability - nearly unbreakable flexible fibers with well bonded smooth coatings

The analyte recoveries between core types for the same coating are similar.

These results suggest that users of adsorbent fibers on fused silica cores can easily transfer their methods for the same coatings onto the Nitinol core versions and take advantage of the improvements.

Featured Products

Fiber Coating and Thickness	Fiber Core/ Assembly Type	Hub Description	Sampling Mode and Needle Size			
			Manual Holder/(w/spring)		Autosampler	
			23 Ga*	24 Ga*	23 Ga*	24 Ga*
Carboxen®/Polydimethylsiloxane (CAR/PDMS)						
75 µm CAR/PDMS	NIT/SS	Black Metallic	**57901-U**	**57904-U**	**57907-U**	**57908-U**
Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)						
65 µm PDMS/DVB	NIT/SS	Blue Metallic	**57916-U**	**57921-U**	**57923-U**	**57931-U**

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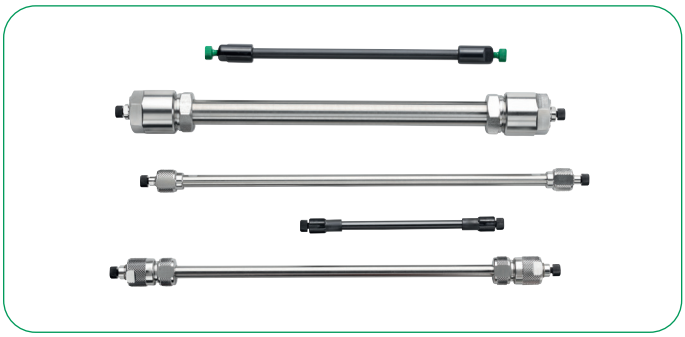


The sensitivity of a chromatographic method might be described by its limit of detection (LOD). LOD is usually defined as the minimum detectable amount or concentration of a component that can be reliably detected using a given analytical method. In other words, LOD is the lowest amount or concentration of an analyte in a test sample that can be reliably distinguished from zero. In reality, "zero" is obtained from the measurement of a sample not containing the component (blank sample). The usual estimation of LOD in chromatography involves the measurement of signal-to-noise ratio (S/N). The chromatographic signal value is determined by the height of the analyte peak and the noise value. The noise value can be derived from either the standard deviation of the noise or from the so called peak-to-peak value. Latter one is determined by injecting a blank sample and using the difference between the highest and lowest points in the baseline noise around the time where the peak of interest would elute. The globally accepted criteria for the detection of an analyte is a S/N ratio of equal to or above 3. Multiple (minimum 3) measurements are performed at the lowest concentration, with all the measurements still showing significant detection of the compound (S/N≥3) for the concentration to be taken as LOD. Limit of detection is the most important value that researchers look for when considering their method's validity.

So how to increase the sensitivity of your analytical method? Logically, it is quite simple: we must either increase the signal or/and decrease baseline noise.

Reduction of baseline noise

In many cases, the type of solvent and additives used and their purity in the eluent are strongly responsible for the noise of the baseline. Additives such as TEA or TFA might increase noise due to their relatively high UV absorbance. It is particularly important if the detection is done at low wavelengths of below 220 nm. First, check whether or not the detection can also be performed reliably at longer wavelengths. Doublecheck the eluent selection, for example, methanol exhibits a higher absorption at low wavelengths, which can make the detection of smaller peaks difficult. Uncontrolled temperature drifts during the day for detector and the solvents should also be avoided. Next, even minor impurities from the column after synthesis or because of column bleeding can greatly increase baseline noise. Therefore, it is best to first install a new guard column and, if necessary, also a new separation column and compare the obtained chromatograms. Another factor to be considered is the HPLC system itself. It can be checked for any contamination or air in the system, performance of pumps (pressure fluctuations), lifetime of UV lamp, and cleanliness of the detector cell - all of which can contribute to the baseline noise. Finally, the size of solvent mixing unit. A smaller size offers less contribution to the dead volume, but a higher baseline noise, usually because of less perfect mixing. A larger solvent mixing unit would facilitate a better mixing but would also contribute to larger dead volume. In general, regular maintenance, cleaning cycles, and good understanding of the system's individual components are prerequisites for a problem-free HPLC analysis.

Increasing the signal intensity

Decrease the column internal diameter (ID). The ID of the HPLC column affects the concentration of the sample in the column. Samples are diluted in proportion to the cross-sectional area of the column and therefore, smaller ID columns yield less dilution. Just a decrease by half of the diameter will result in a ~4 times higher concentration in the detector. Keep in mind that the column capacity is also reduced at the same time and hence the injection volume as well as the flow rate must be adjusted. However, above mentioned increase in sensitivity will be obtained even after adjusting/lowering the injection volume.

Increasing column efficiency. Reduction of particle size causes an increase in the sensitivity because of more narrow and higher peaks. Excellent choice is to use superficially porous particle (SPP)/Fused-Core® columns like Ascentis® Express. These will simulate a smaller diameter (more efficient) particle without a larger increase in backpressure. For example, replacing a fully porous 3 µm particle packed column with a superficially porous particles of 2.7 µm, would almost double the column efficiency. Since the efficiency is higher, the peak will be narrower and higher, and by that the sensitivity will increase.

System efficiency. In order to have a high-efficiency separation, it is important to minimize the instrument's dead volume. This can be achieved by optimizing the entire HPLC system using smaller I.D. and/or shorter connection capillaries, smaller injection unit, and smaller detector cell.

Optimal flow rate. The resulting peak height/efficiency is also influenced by the choice of the correct flow rate. A too high flow rate (very narrow peaks), at a too low detection frequency may result in losses during detection as the detector simply would not have enough "time" to reasonably detect the analyte (too few data points). Also, the van Deemter plot should be considered: try to operate at the optimum conditions, selecting an optimal flow rate where efficiency is at its maximum (minimum of theoretical plate height).

Column bleeding. The choice of the separation column can also affect the noise levels. Choose chromatographic conditions matching optimum temperature, pH, solvent compatibility, working ranges of the column to minimize potential bleeding effects.

Peak tailing. Select best column and chromatographic conditions to obtain symmetrical peaks. Optimization of method conditions must be performed to select the most suited buffer, pH, and if necessary, additives. For example, optimal pH value should be +/- 2 pH units of the analytes pKa value, in order to work with the non-ionized form. Use of a steep gradient can often yield a sharper peak than isocratic mode alone.

Conclusions:

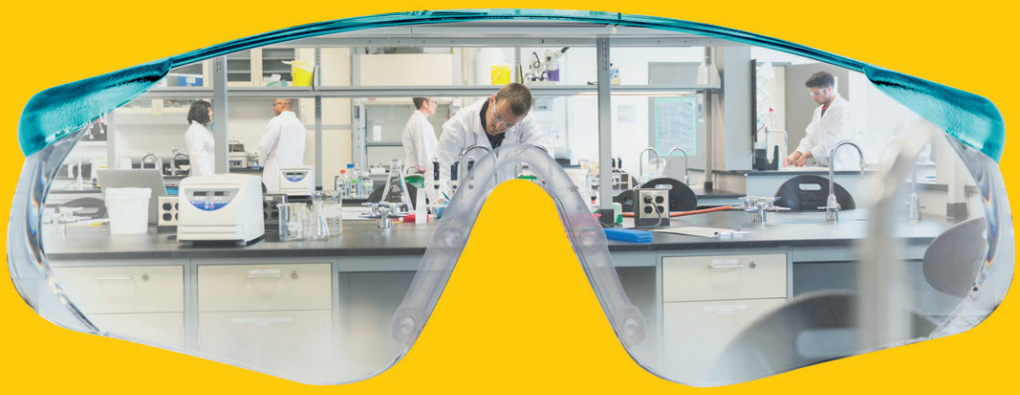
In order to increase your chromatographic method sensitivity, chose one or few options to reduce baseline noise and/or make suggested improvements to increase signal intensity.

I hope this is of use for you and your applications.

Regards

Egidijus Machtejevas

SAFETY



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