Water Analysis in Food and Beverage

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From development to testing, Sigma-Aldrich® understands the importance of having the highest quality and most reliable products to make sure the water used in any application is safe and consistent. Our products have been quality-tested and are provided with easily accessible third-party validation to customers around the globe, and our expertise in supply chain management ensures the products to be delivered when you need them. With our breadth of products, quality and expertise, Sigma-Aldrich assures you as your trusted partner for safe and sustainable water, throughout the entire development process.

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DBPS AND POLLUTANTS

Disinfection of drinking water reduces risk of pathogenic infections, but the process can leave behind residues and/or byproducts (DBPs) that can pose risk to human health. Additionally, organic and inorganic pollutants may be naturally present in our water supply at its source. Identification of pollutants and DBPs is essential to make sure the end product is safe. Sigma-Aldrich offers certified reference materials and a wide variety of analytical products to help you pinpoint components in your source water.

Standards and CRMs

- Disinfection Byproducts
- Perfluoro Compounds
- Pesticides
- Semivolatile Organic Compounds
  – Polycyclic Aromatic Hydrocarbons
- Volatile Organic Compounds
- Phthalate Plasticizers

Volatiles Analysis by GC

- VOCOL GC Columns
- SPB-624 Columns
- Purge Traps
- Purge and Trap Accessories

PRODUCT HIGHLIGHTS

- *48045 SUPELCO* EPA 551A Halogenated Volatiles Mix, Certified Reference Material, 2000 µg/mL each component in acetone
  Sigma-Aldrich offers a suite of Certified Reference Materials based on the EPA methods for Drinking Water to help our customers decrease time in making their own mixes and ensure accuracy of the standard.
- VOCOL GC Columns
  This intermediate polarity column, designed for analyses of volatile organic compounds (VOCs), offers great retention and resolution of highly volatile compounds. Use this column in direct injection ports or coupled to purge and trap systems.

WATER QUALITY DETERMINATION OF VOCS IN WATER FOLLOWING ISO STANDARD 17943 – METHOD USING HS-SPME FOLLOWED BY GC-MS

Abstract

This work describes the details of the determination of more than 60 volatile organic compounds (VOCs) in different water matrices together with the required method optimizations. After extraction of the compounds in the headspace of the samples by SPME, the analysis was conducted by GC-MS. Additionally, the results from an interlaboratory trial for validation of this method as a new ISO standard will be presented. These demonstrate a very good performance of this method for the determination of VOCs in water.

Introduction

Volatile organic compounds (VOCs) are often used during the manufacturing of many different products, such as petroleum products, adhesives, pharmaceuticals, paints or refrigerants. Some are used as gasoline additives, solvents, hydraulic fluids, and dry-cleaning agents. This group of compounds belongs to the group of anthropogenic chemicals. VOC contamination of water resources is a human-health concern because many are toxic and are known or suspected human carcinogens.

Solid Phase Micro Extraction (SPME) was introduced in 1990.[1] Since then SPME has gained broad acceptance in environmental, pharmaceutical and food analysis demonstrated by the still growing number of publications on SPME developments and applications. The prevalence of this technique was additionally increased by the automation of SPME using GC autosamplers since 1993. Another indication of the broad acceptance is the use of SPME in official methods and standards.

- ASTM D 6520 (Volatiles and semivolatiles from water)
- ASTM E 2154 (Ignitable liquid residues from fire debris)
- ASTM D 6889 (VOCs in water)
- OENORM A 1117 (Volatiles in cellulose-based materials)
- ASTM D 6438 (Organics in paint and coatings)
- EPA Method 8272 (PAHs in sediment water)
- ISO 27108 (Pesticides in water)

VOCs in water can be determined by different methods,[2-4] but those were not sufficient in terms of LOD or automation.

Method

ISO 17943 covers the determination of 63 volatile compounds in different water matrices such as drinking, surface, ground and waste water. The SPME conditions can be found in **Table 1**.

Table 1: Chromatographic Conditions for SPME Extraction

Parameter	Setting (Supelco Lab,Trial participant)
Sample Volume	10 mL
HS-Vial	20 mL, addition of 3 g salt
SPME Fiber	DVB/CAR/PDMS, 24 gauge
Incubation Time	10 min @ 40 °C
Extraction Time	10 min @ 40 °C
Autosampler	CTC Combi PAL (agitated by circular motion of the vial, velocity: 250 rpm)
Desorption	10 min @ 270 °C

In **Figure 1**, a VOCOL Capillary GC column was used. This intermediate polarity column was designed for analyses of volatile organic compounds (VOCs), offers great retention and resolution of highly volatile compounds.

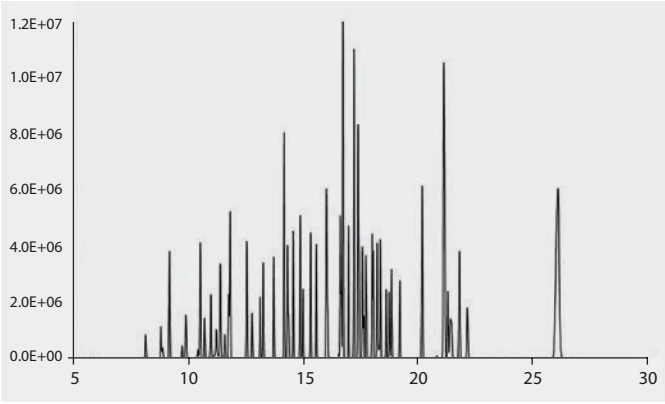

Figure 1. Chromatogram and GC conditions (Supelco Lab, participating in interlaboratory trial) for analysis of the compounds by ISO Standard 17943.

Results

Samples for the trial were surface water (river Ruhr in Muelheim, Germany) and municipal waste water (plant effluent). The samples were filtered, sterilized and stabilized using sodium azide. After spiking (surface water: 0,02 – 0,80 µg/l; waste water: 0,05 – 3,00 µg/l), the samples were tested for homogeneity and stability.

At the interlaboratory trial, 42 labs from 16 countries participated:

- No submission of results: 9 labs
- Significant deviation from the procedure prescribed: 6 labs
  - Calibration without internal standards (3x)
  - Other major deviations from draft ISO/CD 17943 (3x)
- A total of 27 labs reported results to be included in the evaluation process according ISO 5725-2
- All parameters analyzed: 10 labs
- Nearly all parameters analyzed: 9 labs
- Nearly each parameter had been analyzed by > 20 labs

The results from the interlaboratory trial after exclusion of outliers were evaluated for recovery rate (from assigned value), reproducibility standard deviation and repeatability standard deviation (**Figure 2**).

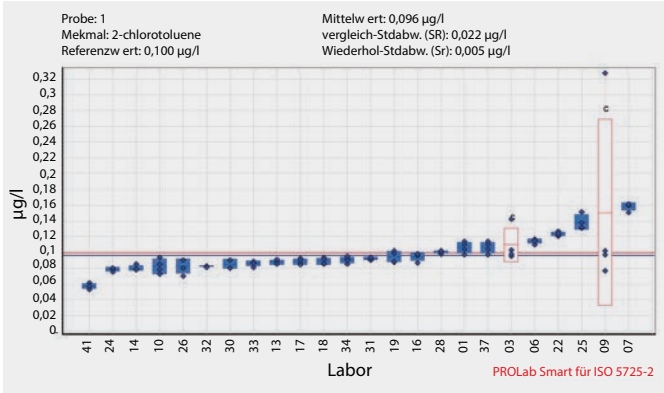

Figure 2: Evaluation of the interlaboratory trial (example compound 2-chlorotoluene). For this, compound results from 24 labs could be evaluated. The blue line is the assigned value, the red line is overall mean. Highlighted with the letter "C" are two outliers due to too high within-laboratory variance.

The evaluation of the interlaboratory trial demonstrates a very good performance of ISO Standard 17943. For more than 90% of the compounds:

- Recovery rate (from assigned value) is between 84 and 116% in surface water and 81 and 118% in waste water
- Reproducibility standard deviation is less than 31% in surface water and less than 35% in waste water
- Repeatability standard deviation is less than 10% in surface water and less than 8% in waste water

Conclusion

- Reliable and reproducible method for VOCs in water was developed
- Validation in ISO 17943 (and older DIN 38407-41)
- Successful interlaboratory trial showing high performance, accuracy and precision
- ISO 17943 will go live soon

Featured Products

Description	Cat. No.
Solid Phase Microextraction Fibers and Holders	
SPME Assembly, manual use	57330-U
SPME Assembly, autosampler use	57347-U
Stableflex SPME Fiber Carboxen/PDMS (85 µm), manual use, 24 gauge	57334-U
Stableflex SPME Fiber Carboxen/PDMS (85 µm), autosampler use, 23 gauge	57295-U
Stableflex SPME Fiber DVB/Carboxen/PDMS (50/30 µm), manual use, 24 gauge	57328-U
Stableflex SPME Fiber DVB/Carboxen/PDMS (50/30 µm), autosampler use, 23 gauge	57298-U
Columns	
VOCOL column	24154
Reference Standards	
VOC Reference Standard for ISO 17943	44926-U
Odor Reference Standard for ISO 17943	44923-U
VC Reference Standard for ISO 17943	48625
Equipment	
Vial	SU860097
	SU860098
Screw cap for vial*	SU860101

*Vial and Cap depend on the autosampler used. Listed here are for a CTC type.

References

1. Arthur, C. L., Pawliszyn, J., Analytical Chemistry (1990), 62(19), 2145-8.
2. ISO 10301:1997, Water quality – Determination of highly volatile halogenated hydrocarbons – Gas chromatographic methods.
3. ISO 11423-1:1997 and ISO 11423-2:1997, Water quality – Determination of benzene and some derivatives – Part 1: Head-space gas chromatographic method & Part 2: Method using extraction and GC.
4. ISO 15680:2003, Water quality – GC determination of a number of monocyclic aromatic hydrocarbons, naphthalene and several chlorinated compounds using purge-and-trap and thermal desorption.

MICROBIOLOGY

Water is essential to life, so keeping water free of microorganisms is key to provide safe water. Successful and consistent microbiological water analysis requires reliable standards, methods and tools. Sigma-Aldrich provides:

Microbiology CRMs

- Vitroids™
- LENTICULES®

Proficiency Testing

- Water Pollution/Waste Water
- Water Supply/Drinking Water

Identification

- Hybridization Kits
- ID Membranes

PRODUCT SPOTLIGHTS

- Vitroids™ and LENTICULE® discs are certified quantitative microorganism standards used as positive quality control strains to test and assure the performance of media and microbiological tests.
- Vitroids and LENTICULE discs contain viable microorganisms in a certified quantity (ISO 17025), produced under reproducible conditions (ISO Guide 34) with strains obtained from ATCC for Vitroids and NCTC & NCPF for LENTICULE discs. The discs consist of bacteria or fungi in a solid water soluble matrix. Microorganisms in this form are stable for at least one year and are in a viable stage (no lag phase or recovery time). Check out product RQC02008, Legionella pneumophila (serogroup 1) NCTC 12821 Vitroids 100000 CFU.

MEDIA

A clean water supply is needed for communities to survive. However, it can also be a source of pathogens, and without microbiological control, the supply system would spread and transmit diseases. It can be expensive and time consuming to check the water supply for all possible pathogens, so indicator organisms are used as assays to detect fecal and other contaminations. Coliforms, *E. coli* and *Klebsiella pneumoniae* are typically used for testing for fecal contamination, but it also depends on the source of water to decide a different indicator organism. For example, to check the surface water supply may also require the use of *Clostridium perfringens* or other organisms.

Types of Media

- Drinking Water
- Waste Water Control
- Legionella
- Vitamin Determination
- *Escherichia coli* and Coliforms

PRODUCT HIGHLIGHTS

- 09142 HiCrome™ ECD Agar with MUG (ECD Agar HiCrome with MUG) Application:
  For the detection of *Escherichia coli* in water and food samples by using a combination of chromogenic and fluorogenic substrate.
- 81938 HiCrome Coliform Agar Application:
  HiCrome Coliform Agar is recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples.
- 51489 HiCrome Rapid Coliform Broth (Rapid Coliform HiCrome Broth, Coliform Rapid HiCrome Broth) Application:
  Rapid HiColiform Broth is used for detection and conformation of *Escherichia coli* and coliforms on the basics of enzyme substrate reaction from water samples, using a combination of chromogenic and fluorogenic substrate.

RAPID DETECTION OF *CLOSTRIDIUM PERFRINGENS* BY A NEW CHROMOGENIC MEDIA

Abstract

CP *ChromoSelect* Agar is a selective chromogenic media for isolation and enumeration of *Clostridium perfringens* in water samples using membrane filtration. *C. perfringens* is an anaerobic, Gram-positive, spore-forming rodshaped bacteria. It is widespread in the environment and also found in the digestive systems of humans, and domestic and feral animals. Perfringens poisoning, usually from ingesting undercooked food, especially meat, is one of the most commonly reported foodborne illnesses. Early detection of *Clostridium* in food and water is important to control outbreaks. To facilitate detection, Sigma-Aldrich has developed a chromogenic medium, the CP *ChromoSelect Agar*, for enumeration and differentiation of *Clostridium sp.*, in particular *Clostridium perfringens*, in aqueous samples. This agar is more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*.

Introduction

Clostridium perfringens is found in undercooked or improperly sterilized canned foods (germination of endospores) and in water (surface water). The natural contamination source is human and animal faeces mainly transmitted into food by water. *C. perfringens* is an anaerobic, Gram-positive, spore-forming rod-shaped (**Figure 1**) bacteria.[1,2] *C. perfringens* produces an extensive range of invasins and exotoxins. The enterotoxins cause the undesirable, mostly meat-associated, food poisoning, and wound and surgical infections that lead to gas gangrene.

C. perfringens plays a subsidiary role in water examination.[3] Clostridia are spore builders and are resistant to heating, chlorination and other stress factors.

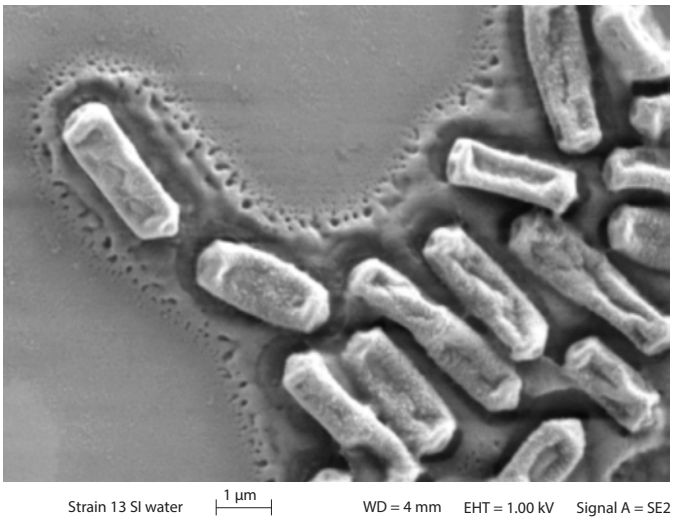

Figure 1. Scanning Electron Micrograph of *C. perfringens* grown on a silicon water (Source: S. Melville, Department of Biological Sciences, Virginia Tech University.)

In contrast to vegetative cells like coliforms (*E. coli, enterococci*), which are less resistant, *C. perfringens* has the advantage of surviving longer.[4] Therefore, while fecal contamination is detected mostly by coliforms as an indicator, which could disappear after a processing step, *C. perfringens* remains present. The organism is not a hazard in water; rather, it is problematic when the water comes in contact with food.

In consideration of the aforementioned facts, it is obvious that detection and identification of *C. perfringens* is an important step toward the control and eradication of this potent pathogen. Some characteristic enzymes of *C. perfringens* are: hemolysins (β-hemolysis), lecithinase, extracellular proteases, lipases (phospholipase-C), collagenase, hyaluronidase, saccharolytic, and enzymes to reduce sulphite to sulphide. These enzymes are also used as detection and differentiation targets. It is also notable that *C. perfringens* is a non-motile bacterium, and it is the most important of the sulphite reducing clostridia.

C. perfringens normally grows at 44 °C, whereas some other clostridia are inhibited at this temperature. This property is used in ISO methods to give the medium more selectivity.[5]

Results

Early detection of *Clostridium* in food is important to control outbreaks. To facilitate detection, we have introduced a new chromogenic media, CP *ChromoSelect* Agar, for enumeration and differentiation of *Clostridium sp.*, in particular *Clostridium perfringens,* in aqueous samples.

In the present study, three media types (m-CP, TSCF and CP *ChromoSelect* Agar) were evaluated for recovery of *C. perfringens* in different surface water samples. Using a membrane filtration technique on 139 water samples, 131 samples (94.2%) were found positive for *C. perfringens* in at least one of the culture media. Green colored colonies on CP *ChromoSelect* Agar (**Figure 2**) were counted as presumptive *C. perfringens* isolates.

For detection of *C. perfringens*, m-CP and TSC agar have been recommended.[3,6] However, there are problems associated with each of these media.

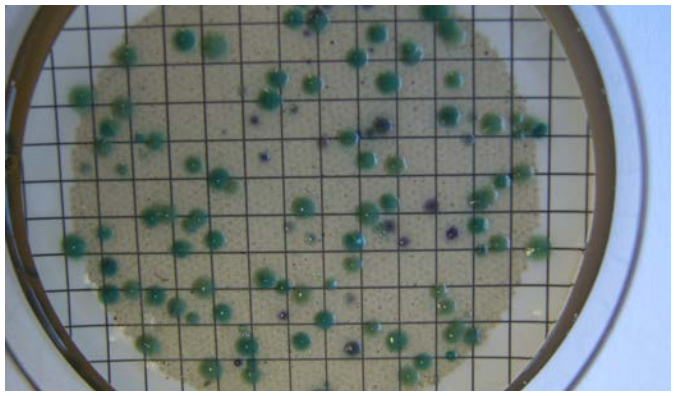

Figure 2. Drinking water sample cultured on CP *ChromoSelect* Agar. *C. perfringens* appears as distinct green colonies.

CP *ChromoSelect* Agar is more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*. In addition, the recovery of *C. perfringens* was rejected by ISO in favor of methods based on TSC agar[4,7] CP *ChromoSelect* Agar also eliminates the excessive and variable blackening of the peripheral colonies encountered with TSC agar, which makes colony counting at lower dilutions difficult and leads to false positives. It is also more reliable at high bacteria counts, where the TSC agar can produce false negatives because of interference with the other enzymatic mechanisms from acid production and oxygen contact (**Figure 3**). TSC detects not only *C. perfringens*, but also all sulphite-reducing clostridia.

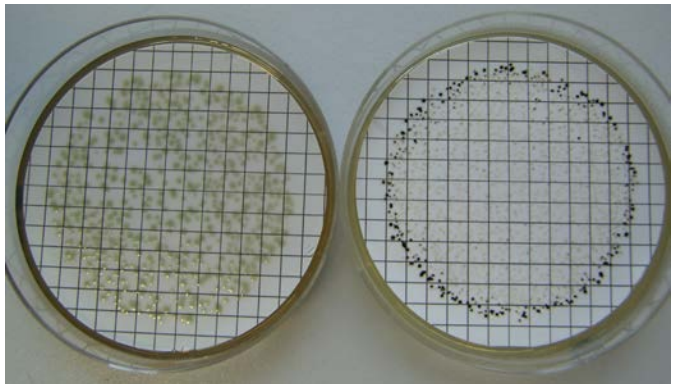

Figure 3. *C. perfringens* ATCC 10873 on CP *Chromoselect* Agar (left) and TSC agar (right) (Note the false negatives on the TSC agar).

Out of 483 green colonies on CP *ChromoSelect* Agar, 96.3% (465 strains, indole negative) were identified as *C. perfringens,* 15 strains (3.1%) were indole positive and were identified as C. *sordelli*, C. *bifermentans* or *C. tetani*. Only 3 strains (0.6 %) gave false positive results and were identified as *C. fallax*, *C. botulinum*, and *C. tertium* (**Table 1**). Variance analysis of the obtained data showed statistically no significant differences in the counts obtained between media used in this work (**Figure 5**).

In general, the identification of typical and atypical colonies isolated from all media demonstrated that CP *ChromoSelect* Agar was the most useful medium for *C. perfringens* recovery in water samples.[2]

CP *ChromoSelect* Agar avoids the disadvantages of m-CP agar, such as, the presence of ammonia that prevents subculturing the *C. perfringens* colonies, the too-selective nature of m-CP agar, and the evanescence of the red color of colonies after the addition of ammonia, which makes further confirmation impossible (**Figure 4**).

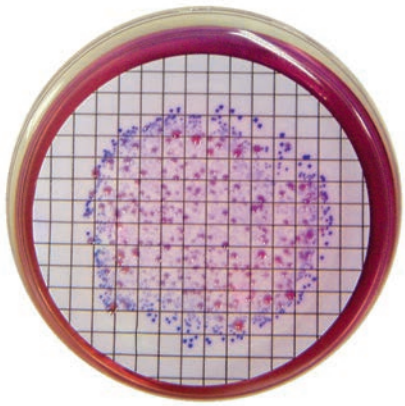

Figure 4. *C. perfringens* on mCP agar.

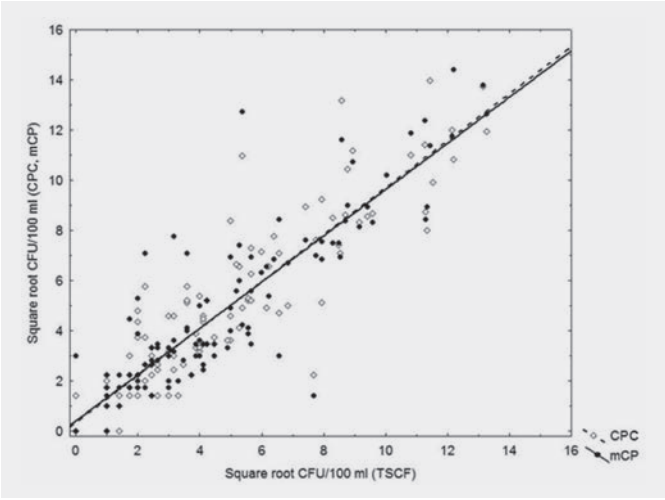

Figure 5. Comparison between TSCF agar and the mCP and CPC (CP *ChromoSelect* Agar) media for enumerating strains of *C. perfringeris* in water samples.

Besides its advantages over m-CP and TSC agars, CP *ChromoSelect* Agar is an ideal growth media. It contains only vegetable peptones and, together with yeast extract, it is an excellent source of nitrogen, carbon, amino acids and vitamin B complex. Sucrose acts as the fermentable carbohydrate, and reducing agents lower the redox potential of the media. Diverse salts provide the required ions for enzymatic reactions. Buffering agents stabilize the pH within the ideal growth range. Inhibitors Dcycloserine and polymyxin B give the medium its selectivity, while further selectivity is achieved by incubation under anaerobic conditions at 44 °C. Various promoters and substrates protect injured cells to improve recovery rate and enhance growth. The chromogenic enzyme substrates in the CP *ChromoSelect* Agar provide the differentiation, for *C. perfringens* in particular (**Table 2**). A negative indol reaction (Kovac's Reagent) is confirmatory for *C. perfringens.*

Table 1. All green colonies isolated from CP *ChromoSelect* Agar are identified with API system (n=483)

Strains	Indole Reaction	n
C. Perfringens	–	465 (96.93%)
C. Tertium	–	1 (0.2%)
C. Botulinum	–	1 (0.2%)
C. Fallax	–	1 (0.2%)
C. Bifermentans	+	2 (0.4%)
C. Sordelli	+	12 (2.5%)
C. Tetani	+	1 (0.2%)

Table 2. *Clostridium sp.* cultural characteristics in CP *ChromoSelect* Agar

Organisms (ATCC)	Growth	Colony Appearance
Clostridium perfringens (13124)	+++	Green
Clostridium bifermentans (638)	+++*	Dark blue with violet halo
Clostridium sporogenes (8534)	–	—
Clostridium sordelli (9714)	++	Dark green with halo (change to yellow with Kovac's Reagent)
Enterococcus faecalis (29212)	++	Violet
Escherichia coli (25922)	–	—
Pseudomonas aeruginosa (27853)	–	Coloreless
Staphylococcus aureus (25923)	–	—
Bacillus subtilis (6051)	–	—
Salmonella typhimurium (DSM 554)	++	Violet

*Growth at 40 °C, but no growth at 44 °C.

Conclusion

CP *ChromoSelect* Agar was the most useful medium for *C. perfringens* recovery in water samples. It is also more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*.

Featured Products

Description	Cat. No.
Chromogenic Media	
CP *ChromoSelect* Agar	12398
Perfringens T.S.C. Supplement	P9352
M-CP selective Supplement I	51962

References

1. Sherris Medical Microbiology, 4th ed., K.J. Ryan and C.G. Ray Editors, McGraw Hill, 2004.
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CHEMICAL AND PHYSICAL ANALYSIS

The chemical analysis of water provides considerable insight into our water supply. Water chemistry has helped scientists understand the interactions between water and geologic materials, giving insight into the impact of human activities on water bodies. It also provides understanding of the limits of a water body's ability to assimilate to pollution without harming the water system and its users. Sigma-Aldrich provides extensive products and methods to make sure your water source is free of contaminants that may cause harm or distaste to your drinking water.

Standards

- Cloud Point
- Color
- Hardness
- pH Values
- Odor Standards

UV/Vis Spectroscopy

- Photometers
- Chromoionophore

Metals Analysis

- Calibration Solutions
- Reagents
- Reference Books

PRODUCT HIGHLIGHTS

- SPME
  A simple and inexpensive sample extraction technique that can achieve very low levels of detection of trace organic compounds present in a variety of sample matrices. Sigma-Aldrich offers a variety of durable SPME fibers that meet all Food & Beverage needs.
- **Product Number THRD1000, Hardness, Total 1,000 mg/L Calibration Standard, certified reference material**
  Produced and certified in accordance with ISO Guide 34:2009 and ISO/IEC 17025:2005.

SOLID PHASE MICROEXTRACTION (SPME) METHOD FOR 4-METHYL-1-CYCLOHEXANEMETHANOL (MCHM) IN WATER

Introduction

4-Methylcyclohexanemethanol (MCHM) is a chemical used in the coal cleaning process. On January 9, 2014, there was an accidental release of an estimated 100,000 gallons of a chemical mixture containing MCHM into the Elk River in West Virginia. According to the owners of the tank, the mix contained 88.5% MCHM, 7.3% PPH stripped basic, and 4.2% water. PPH stripped basic consists of a mixture of propylene glycol phenyl ether (PPH) and dipropylene glycol phenyl ether. Consequently, drinking water for the local community of Charleston became contaminated, with the MCHM imparting a "licorice-like" odor to it. The Center for Disease Control (CDC) has set advisory limits in drinking water of 1 ppm maximum for MCHM and 1.2 ppm for PPH.[1,2] Consequently, this initiated an immediate need for water testing. Since there was no established test method for MCHM, labs had to develop their own within a short amount of time. Solid phase microextraction (SPME) is a widely accepted technique for trace level determination of a variety of compounds in many different matrices. Method development can often be more rapid than other techniques such as solid phase extraction (SPE) and liquid-liquid extraction (LLE). In direct comparison to these techniques, it is easier to automate and requires fewer laboratory consumables such as solvents and SPE cartridges. In this application, a rapid extraction method for MCHM from drinking water and surface water was developed. The method, which was a headspace extraction, required a total time of 10 minutes, including equilibration and extraction. Combined with GC/MS-SIM analysis, detection of MCHM was achieved at low part per billion levels. This method was used to analyze samples of surface and tap water collected locally, as well as a sample of tap water from Charleston, WV which was collected several months after the spill incident. PPH was included in the SPME method; however, as will be shown, there were stability issues for this compound in the presence of chlorine.

Experimental

The SPME and GC/MS analysis conditions are summarized in **Tables 1** and **2**. Quantitation was done using an external standard 5-point calibration curve from 0.3 µg/L to 10 µg/L, prepared in deionized water and analyzed with each set of samples. MCHM elutes as two isomers. The relative ratio of the isomers (1:3) was determined by liquid injection of the standard into the GC/MS system. This ratio was used to calculate the amounts of each isomer in the calibration standards. Quantitation was then done separately for each MCHM isomer. Calibration was linear for both MCHM isomers and PPH, with r2 values of >0.995.

For quantitation of MCHM, m/z=110 was used. The most abundant ions in the MS spectrum of MCHM are m/z=55 and m/z=97 (**Figure 1**); however, there was an interference coeluting with isomer #1 detected in blank samples, which contained these two ions.

For determination of accuracy and reproducibility, samples of drinking water and surface water were collected locally, and spiked with MCHM and PPH at 1 µg/L. A method detection limit study was conducted using deionized water spiked at 1 µg/L.

Table 1. SPME Conditions

Fiber	100 µm PDMS (57301)
Extraction Headspace	5 min equilibration, 5 min extraction @ 50 °C, agitation 250 rpm
Desorption Process	270 °C, 2 min (splitter open at 0.75 min)
Fiber Post Bake	5 min, 270 °C
Sample/Matrix	6 mL + 1.5 g NaCl (25%) in 10 mL headspace vial

Table 2. GC/MS Conditions

GC Column	VOCOL, 20 m × 0.18 mm I.D., 1.0 µm (28463-U)
Oven	50 °C (1 min), 15 °C/min to 220 °C (5 min)
Carrier Gas	helium, 1 mL/min
Liner	0.75 mm I.D. SPME liner
MS Conditions	aux at 220 °C, source at 230 °C, quads at 150 °C, SIM: m/z= 110 (quant), 97 for MCHM; m/z=94 (quant), 152 for PPH

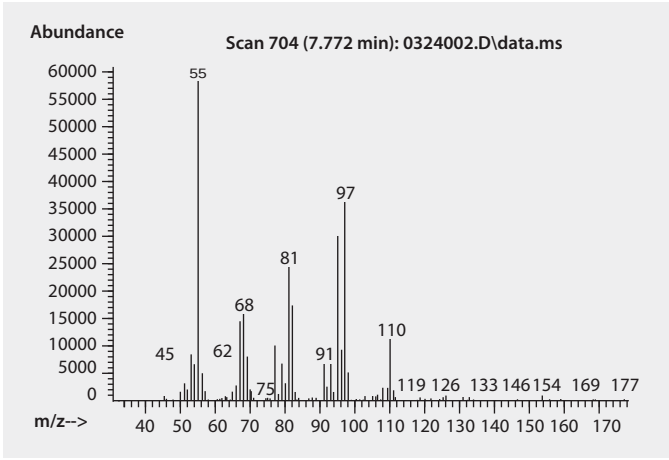


Figure 1. MS spectra of MCHM.

Results

Accuracy and Reproducibility. A summary of the amounts measured in spiked local water samples are presented in **Table 3**. No MCHM or PPH were detected in the unspiked samples. Accuracy and reproducibility (indicated by %RSD values in parentheses) were very good, with the exception of PPH in the tap water sample. Since this compound was detected easily in the spiked private well and creek water samples, it is believed that the presence of chlorine in the tap water may have degraded the PPH.

A representative chromatogram obtained from the GC/MS-SIM analysis of the creek water is presented in **Figure 2**. The MCHM and PPH peaks were easily detectable above the sample background. The addition of salt to the samples prior to extraction was found to increase response by 10 times, and thus, is critical to achieving a low detection level. However, data generated during analysis of the method detection limit samples indicates that it is detrimental to the determination of PPH if samples are prepared in advance of analysis.

Table 3. Accuracy and Reproducibility Results Using SPME Method, n=4

	Local Private Well Water	Local Creek Water	Local Municipal Tap Water
Spiking Level	1 µg/L*	1 µg/L*	1 µg/L*
Amt. measured MCHM isomer #1	0.27 (6)	0.30 (6)	0.25 (3)
Amt. measured MCHM isomer #2	0.84 (9)	0.91 (11)	0.84 (6)
MCHM total (two isomers)	1.11 µg/L	1.21 µg/L	1.12 µg/L
Amt. measured PPH	0.94 (1)	1.16 (6)	ND

*Total MCHM.

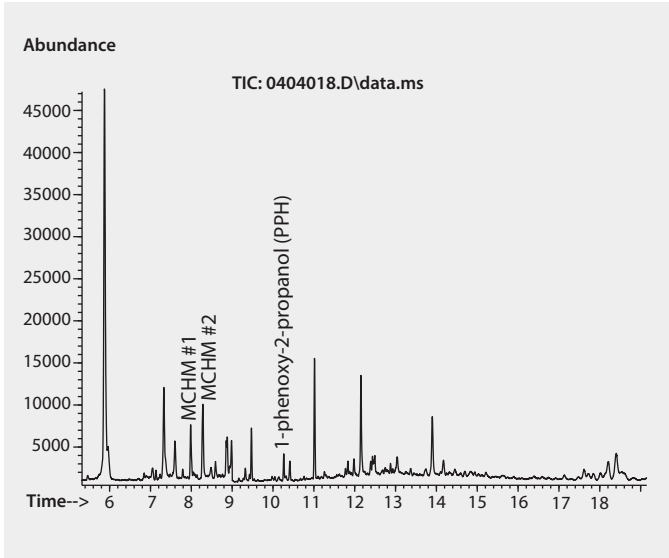


Figure 2. TIC of local creek water, spiked at 1 ppb; extracted using SPME.

Method Detection Limit Study. A set of 7 deionized water samples, spiked at 0.5 ug/L total MCHM, were analyzed and used to calculate a method detection limit (MDL). The method used for calculating MDL was as described by EPA in 40 CFR Part 136, Appendix B rev. The results of this study are summarized in **Table 4**. The MDL for MCHM was calculated to be 0.08 µg/L for isomer #1 and 0.33 µg/L for isomer #2. The limits of quantitation (LOQ) for each isomer were 0.25 and 1.0 µg/L, respectively. PPH results were low with regard to amount measured vs. spiked, and highly variable within the sample set, thus an MDL and LOQ could not be calculated. It is believed that the presence of the salt added for the SPME procedure generated chlorine over time, which resulted in decomposition of the PPH, similar to what was observed in the chlorinated tap water samples.

Table 4. Results of Method Detection Limit (MDL) Study (n=7); spiking level 0.5 µg/L total MCHM

	Mean	Std. Dev	MDL (µg/L)	LOQ (µg/L)
MCHM isomer #1	0.10 µg/L	0.03	0.08	0.25
MCHM isomer #2	0.34 µg/L	0.11	0.33	1.0
PPH	Not determined	—	—	—

WV Tap Water Sample. A sample of tap water from Charleston, WV collected approximately three months after the spill was tested using the SPME method. Three replicates of sample were analyzed without spiking. Two additional samples were spiked at 1 µg/L of total MCHM and run as matrix spikes (MS/MSD). Spiked deionized water was also analyzed as a laboratory control standard (LCS). The MS/MSD and LCS showed good accuracy for determination of the MCHM isomers at the spiking levels indicated (**Table 5**). There was no recovery of the PPH in the matrix spikes, confirming the findings of the effect of chlorination seen with the previous local tap water. A peak corresponding to MCHM isomer #1 was detected in the unspiked WV tap water samples (**Figure 3**); however, the level calculated was below the LOQ for the method. Isomer #2 was not detected above the MDL.

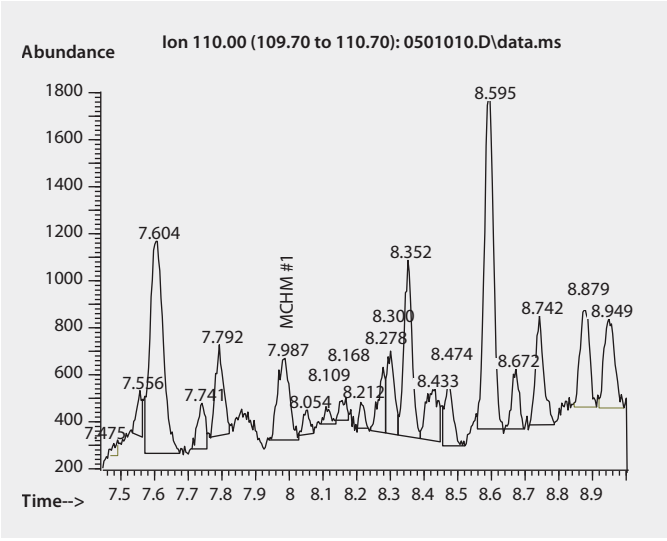


Figure 3. Peak corresponding to MCHM isomer #1 in municipal tap water collected in Charleston, WV. The peak was calculated to be above the method detection limit but below the limit of quantitation.

Table 5. Analysis Results of Charleston, WV Tap Water Sample

	WV Tap Water	MS	MSD	LCS
Spiking Level	unspiked	1 µg/L*	1 µg/L*	1 µg/L*
MCHM isomer #1; average of n=3	0.09 µg/L (9)	0.29	0.28	0.20
MCHM isomer #2	<0.33 µg/L	0.67	0.67	0.64
PPH	ND	ND	ND	ND

*Spiking levels of individual MCHM isomers: MCHM #1–0.25 µg/L and MCHM #2–0.75 µg/L.

Conclusion

Solid phase microextraction can be used to determine MCHM at low level from water. The detection level for the method described was calculated to be 0.08 µg/L for isomer #1 and 0.33 µg/L for isomer #2. The limits of quantitation were 0.25 and 1.0 µg/L, respectively, for the two isomers. Lower limits may be achievable with further optimization of the GC/MS instrument, allowing the use of the more abundant ions in the spectrum of MCHM for detection and quantitation.

PPH appears to be unstable in the presence of chlorine, making reliable detection difficult. It could not be recovered from chlorinated tap water, or deionized water that had been treated with sodium chloride and allowed to sit for a prolonged time prior to analysis.

Featured Products

Description	Cat. No.
Supel QuE QuEChERS Products	
SPME Assembly 100 µm, PDMS FS 24GA Auto (RED)	57301
VOCOL, 20 m × 0.18 mm I.D., 1.0 µm	28463-U

References

1. http://www.cnn.com/2014/01/09/us/west-virginia-contaminated-water/index.htmlCNN.com.
2. http://emergency.cdc.gov/chemical/MCHM/westvirginia2014/index.asp.
3. 40 CFR Appendix B to Part 136. Definition and Procedure for the Determination of the Method Detection Limit – Rev. 1.1.

ADDITIVES

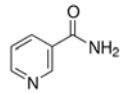
Water often loses naturally occurring components during the purification process, so many companies will add those components back before bottling. Due to the ever-changing consumer tastes, some bottled waters are being taken a step further by adding colors, vitamins, flavors and other nutritional additives. Sigma-Aldrich offers all the items you need to research your next water creation.

Types of Additives:

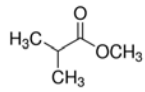
- Sugars
- Vitamins
- Flavor Ingredients

PRODUCT HIGHLIGHTS

- **Product Number N5535 Niacinamide**
  Meets USP testing specifications Synonym: Niacinamide, Nicotinamide, Nicotinic acid amide, Pyridine-3-carboxylic acid amide, Vitamin B_3,



- **Product number W269409 Methyl isobutyrate**
  Natural Occurrence: Apple, strawberry, banana, grape, papaya, pineapple, star fruit, dill herb, fried potato aroma. Taste Characteristics: Fruity, sweet, floral, creamy. Applications: Methyl isobutyrate is used in many food flavors, including frozen dairy and baked goods to gelatins and candies. Its fruity aroma and taste makes it one of the key ingredients in "Tutti-frutti".



HPLC ANALYSIS OF WATER-SOLUBLE VITAMINS USING TITAN™ C18 COLUMN

Introduction

Vitamins are important compounds and are essential for normal metabolism. They are naturally found in many foods and are often added to processed food products. Water soluble vitamins include compounds, such as thiamine (B_1), riboflavin (B_2), niacin (B_3), pyridoxine (B_6), pantothenic acid (B_5), biotin (B_7), folic acid (B_9), and cyanocobalamine (B_{12}). Qualitative and quantitative analysis of vitamins is a routine but challenging task since vitamins are relatively unstable and affected by a number of factors such as heat, light, air, and other food components. In this report a Titan C18 UHPLC column was used to analyze B vitamins in both standard mixture and in vitamin water.

Experimental

An Agilent® 1290 UHPLC system was used for separations. The UV detection wavelength was set to 220 nm. The column used in this study was a Titan C18, 5 cm × 2.1 mm I.D., packed with monodisperse 1.9 µm particles. The column temperature was controlled at 35 °C. The B vitamins are very hydrophilic, consequently a gradient with a low concentration of methanol and acidic mobile phase was used for elution under the reversed-phase conditions.

Water-soluble vitamins were obtained from Sigma-Aldrich. Most of the B vitamins were dissolved in water. Riboflavin and biotin were dissolved in 1 M KOH and their solutions were prepared daily.

Orange flavored vitamin water was purchased in the grocery store. It was filtered through the 0.2 µm syringe filter for sample preparation.

Results and Discussion

B vitamins can be eluted from reversed-phase columns using low concentrations of aqueous acetonitrile or methanol. At pH 3, few compounds (thiamine, pyridoxine, niacinamide) were very weakly retained on the C18 Titan column and required only 0.5% of methanol for elution. Biotin, cyanocobalamin, and riboflavin vitamins are more hydrophobic and were retained more strongly. Gradient elution required an increase of methanol concentration to 30%. The Titan C18 column exhibited good retention for all nine vitamins, and baseline separation for all vitamin peaks was easily achieved (**Figure 1**). All vitamins were eluted within 2.5 minutes with good peak shapes. Although the Titan C18 column contained sub-2 micron particles, the total back pressure was not excessive when using the aqueous methanol mobile phase, which is known to be less compressible and results in back pressures higher than those using aqueous acetonitrile mobile phases. The Titan column back pressure peaked at 550 bar during the separation. The smaller particle size, allowed for faster separation.

Standard solutions were made at 100 µg/mL except 250 µg/mL for calcium pantothenate, riboflavin, and biotin.

column: Titan C18, 5 cm x 2.1mm, 1.9 µm (577122-U)
mobile phase: [A] 20 mM potassium phosphate, pH 3.0; [B] methanol
gradient : 0.5% B for 0.5 min, increase to 30% B in 1.3 min, hold at 30% B for 1 min, re-equilibrate at 0.5% B for 0.8 min
flow rate: 0.5 mL/min (backpressure not more than 550 bar)
detector: UV, 220 nm
injection: 0.5 µL

1. Thiamine
2. Nicotinic acid
3. Pyridoxine
4. Niacinamide
5. Calcium pantothenate
6. Folic acid
7. Cyanocobalamin
8. Biotin
9. Riboflavin

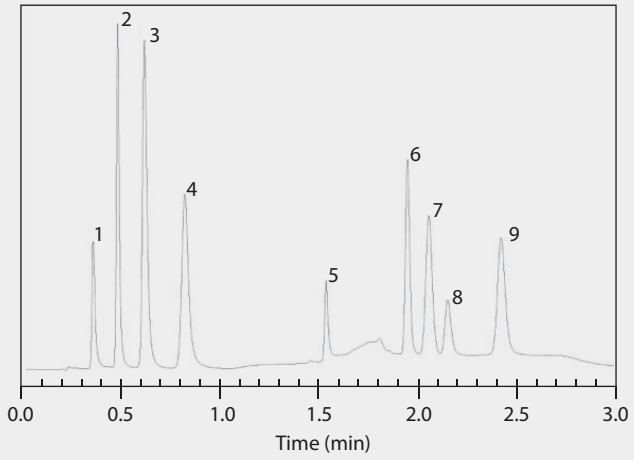


Figure 1. Separation of nine B vitamins using Titan C18 Column.

The developed HPLC method was applied to the analysis of B vitamins in vitamin water. The drink was filtered prior to injection to HPLC and no dilution was required. The peaks for four B vitamins were identified by comparison to the standard mixture (**Figure 2**).

column: Titan C18, 5 cm x 2.1 mm, I.D., 1.9 µm (577122-U)
mobile phase: [A] 20 mM potassium phosphate, pH 3.0; [B] methanol
gradient : 0.5% B for 0.5 min, increase to 30% B in 1.3 min, hold at 30% B for 1 min, re-equilibrate at 0.5% B for 0.8 min
flow rate: 0.5 mL/min (backpressure not more than 550 bar)
detector: UV, 220 nm
injection: 0.5 µL

1. Pyridoxine
2. Niacinamide
3. Calcium pantothenate
4. Cyanocobalamin

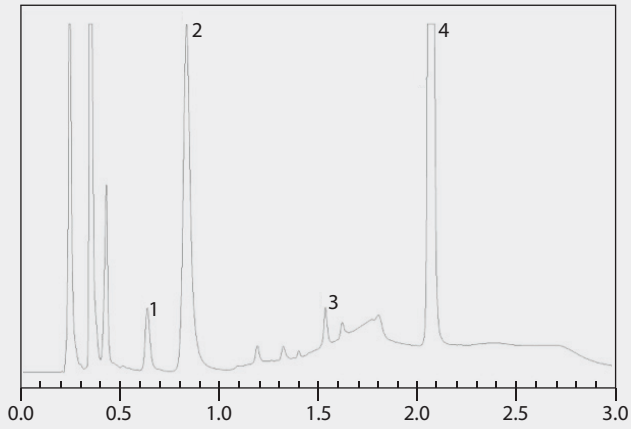


Figure 2. Analysis of vitamin water using Titan C18 Column.

Conclusion

The use of a Titan C18 column for analysis resulted in excellent resolution of the mixture of nine water soluble vitamins in 2.5 minutes. The retention of polar compounds was adequate on the C18 column when using an aqueous methanol gradient and an acidic pH of the mobile phase. The method was applied to the analysis of the vitamin water and four B vitamins added to the water were identified.

Featured Products

Description	Cat. No.
Titan C18 Columns, 1.9 µm particle size (quantity 1 ea.)	
2 cm × 2.1 mm I.D.	577120-U
3 cm × 2.1 mm I.D.	577121-U
5 cm × 2.1 mm I.D	577122-U
7.5 cm × 2.1 mm I.D.	577123-U
10 cm × 2.1 mm I.D.	577124-U
3 cm × 3.0 mm I.D.	577125-U
5 cm × 3.0 mm I.D.	577126-U
Titan C18 Guard Cartridges, 1.9 µm particle size (quantity 3 ea.)	
2.1 mm I.D.	577127-U
3.0 mm I.D.	577128-U
Titan Guard Cartridge Holder (quantity 1 ea.)	
Holder w/EXP Titanium Hybrid Ferrule (cartridge not included)	577133-U

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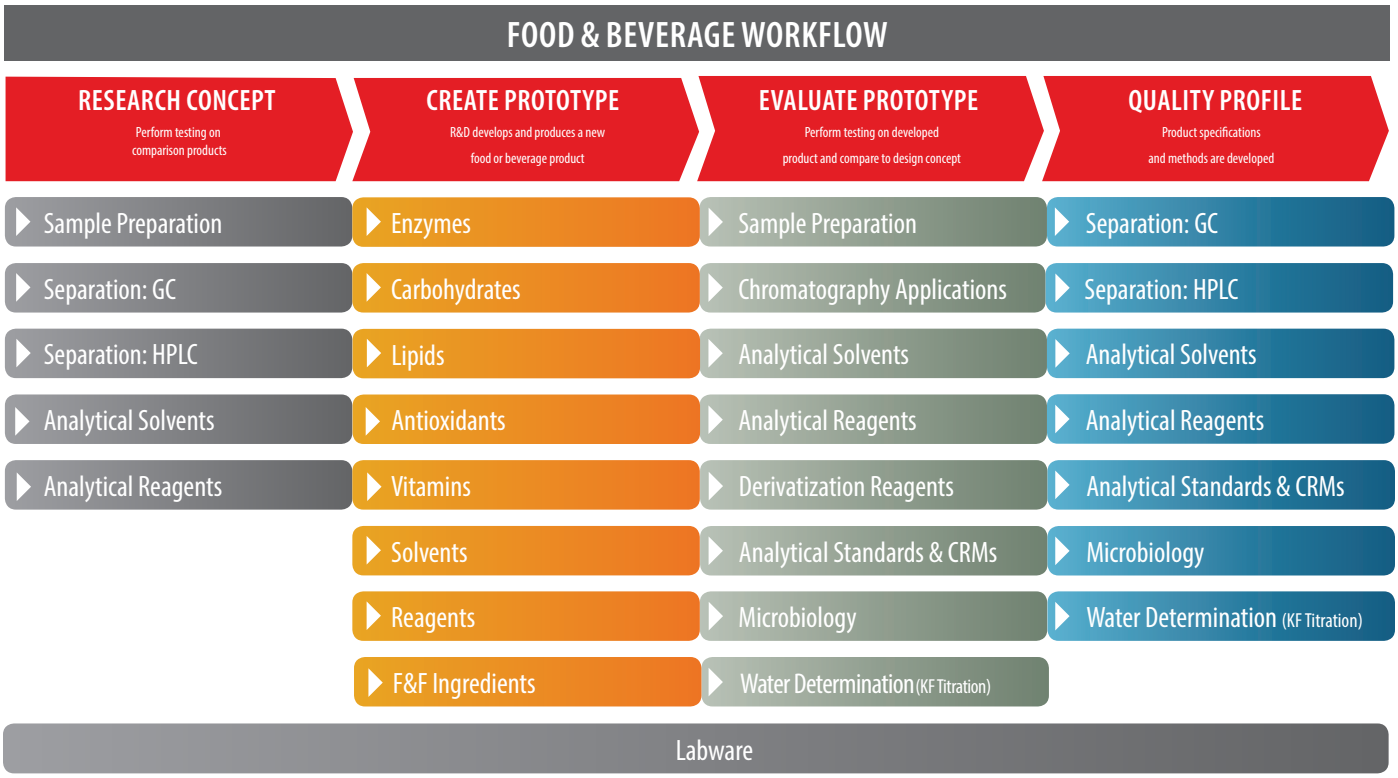

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