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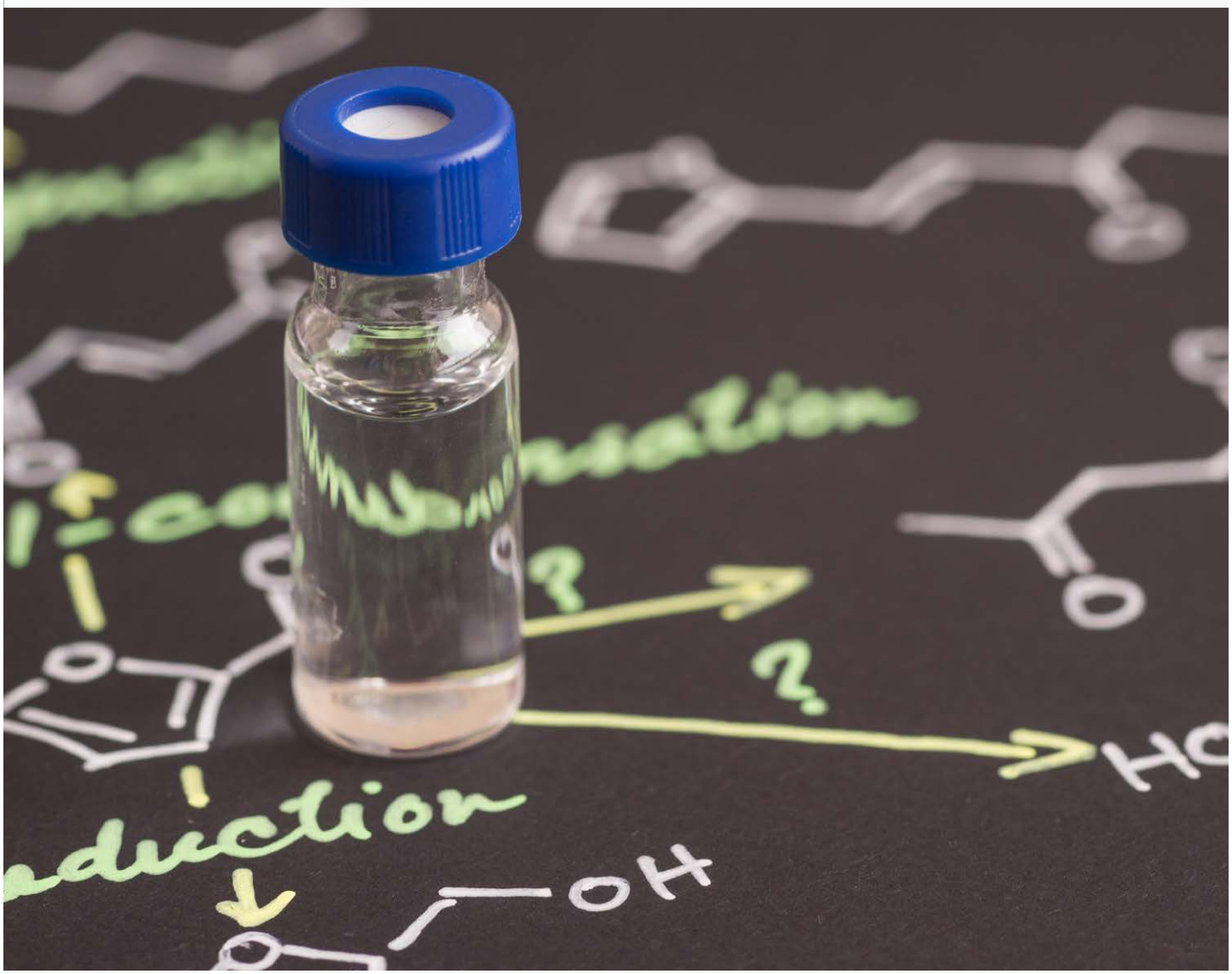
FOCUS: CHROMATOGRAPHY

Issue 1 2023



Welcome to the first edition of the FOCUS: Chromatography magazine 2023

In this latest edition of our FOCUS: Chromatography magazine, we review key application areas that are related to contamination, whether these are contaminants of concern such as nitrosamines or PFAS that are facing increasing and quickly evolving regulation, or methods to minimise the risk of contamination of a chromatographic sample. Our expert contributors look at specific challenges such as co-elution of NDMA with DMF, Perfluoroalkyl substances (PFAS) and the challenges associated with their analysis. In addition to other contaminants of chromatographic samples such as extractables and leachables that may be introduced via the analytical method.

There is more to find on vwr.com/chromatography, or why not watch some of our chromatography webinars at vwr.com/webinar and supplement this magazine with content from some of the leading experts in the industry.

If there is anything else we can do to support you please contact our industry-leading techniclal support team at chromsupport@avantorsciences.com

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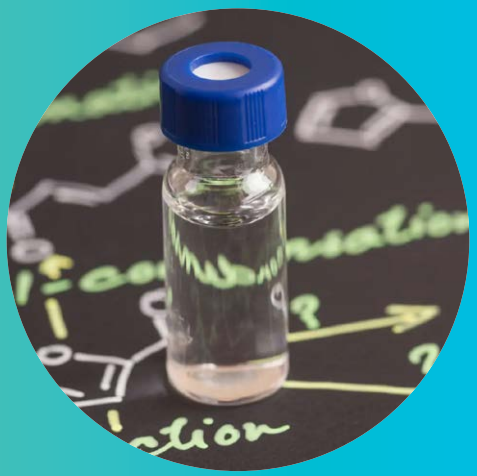

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Regulations in pharmaceutical analysis

Tony Edge, (R&D Leader and Scientific Advisor, Avantor) and Matt James (Senior Research Scientist, Avantor).

HISTORY OF REGULATION IN THE PHARMACEUTICAL INDUSTRY

Over the years, profound changes in the way the pharmaceutical industry is regulated has taken place. The US FDA (Food and Drug Administration) is a prime example of how regulation has evolved, having grown from one individual investigating the adulteration of food products, to an organisation employing nearly 18 000 employees with an annual budget exceeding $6 billion[1]. The increase in regulation has dramatically benefitted society by establishing the production of safer drug products and enforcing more ethical approaches to their development and manufacture. Much of this change has been driven through testing, the development of the stature of the regulatory authorities, and the establishment of chemical analysis as an essential tool in monitoring the quality and safety of manufactured drug products.

THE FDA

In 1862, Charles M. Wetherill, was appointed by President Lincoln as the first chemist in the US Department of Agriculture[2], which was the beginning of the Bureau of Chemistry, the predecessor of the FDA. Initially the task was overwhelming as adulteration was rife and the approach that was being taken was not objective enough. Thus, the Commissioner of Agriculture, George Loring, in 1882 replaced Wetherill's successor, Peter Collier, with Harvey Washington Wiley. Wiley had a practical knowledge of agriculture and a sympathetic understanding of the problems within the industry. The division expanded rapidly and during this time, introduced poison squads, whose role was to test deliberately adulterated food to determine the impact various illegal additives would have. In 1906, President Theodore Roosevelt issued the Pure Food and Drugs Act, which was effectively written by Wiley[3].

By the 1930s a campaign was launched for stronger regulatory authority by publicising a list of dangerous products that had been ruled permissible under the 1906 law. The list of products is incredulous by modern standards and included:

- Radioactive beverages
- A mascara that caused blindness
- Cures for diabetes and tuberculosis that did not work

Unfortunately, resulting legislation struggled to get through the United States Congress, and it was not until a major incident occurred that public opinion turned and Congress allowed the bill to pass. In 1937 a Tennessee drug company, S.E. Massengill Co., began marketing a product called Elixir Sulfanilamide[4]. Sulfanilamide is a drug used to treat streptococcal infections and had been shown to be effective and was safely used for some time

in both tablet and powder form. In June 1937, a salesman reported demand for the drug in liquid form, and so a new formulation was devised. The new formulation used diethylene glycol to dissolve the sulfanilamide, but was not tested for toxicity, since safety studies were not required for new formulations. Diethylene glycol is commonly used as antifreeze and is toxic. The new formulation resulted in over 100 deaths, mainly children, since it was seen as an easier method for administering the drug than powder or tablet formulations[5].

The resulting public outcry meant that the President Franklin Roosevelt was able to sign the Federal Food, Drug, and Cosmetic Act which came into law on June 24th 1938[6]. This significantly increased federal regulatory authority over drugs and mandated a pre-market safety review of all new drugs. False therapeutic claims in drug labelling were banned, and animal testing was introduced as part of drug development to ensure safety. By 1962, laws were passed which allowed for the inspection of drug manufacturing plants, and tighter controls on the manufacture and approval of drugs[7].

GOOD LABORATORY PRACTICE (GLP)

In subsequent decades, numerous high profile incidents highlighted some of the abhorrent practices that were employed in animal testing facilities[8,9], such as those involving Industrial BioTest Lab (IBT) and G.D. Searle & Co. In 1977, IBT was criminally implicated for producing fraudulent studies on a range of products, including Nemacur, Sencor, Naprosyn, and trichlorocarbanilide[8,10]. FDA audits determined that 618 of 867 (71%) studies performed by IBT were invalidated for having "numerous discrepancies between the study conduct and data"[11,12]. Three former company officials were eventually convicted, after one of the longest trials in US history, of fabricating key product safety tests[13].

Such cases contributed to the instigation of the concept of Good Laboratory Practice (GLP) into the US, with 600 new inspectors hired to ensure compliance within the industry. Other countries had already introduced this concept, most notably New Zealand. GLP aims to ensure the integrity of non clinical laboratory studies through planning, performing, monitoring, recording, archiving and reporting. This was very much an era of quality by inspection which was the prevailing philosophy for another 20 years. More recent decades have seen a change in direction with regulators favouring an industry move towards quality by design, where a greater emphasis is placed on process understanding. Although it is worth noting that the principles of GLP define a set of rules and criteria for a quality system, it does not 'per se' assume that the best scientific approach will be employed.

GLP is one facet of a series of systems designed to control quality within the pharmaceutical industry. In addition to GLP, GCP (Good Clinical Practices) and cGMP (current Good Manufacturing Processes) are also employed. These quality systems are also used in other industries such as food and beverage, cosmetics and medical devices, and are driven by regulatory authorities to improve quality standards.

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE (ICH)

The substantial increase in legislation throughout the 1960's and 1970's, relating to the reporting and evaluation of data on the safety, quality and efficacy of new medicinal products, generated a divergence in the technical requirements from one country to another. This was highlighted in the infamous thalidomide incident which drew attention to the need for harmonisation of the different regulators from a safety perspective[14]. This lack of harmonisation resulted in a great degree of duplication, in addition to time-consuming and expensive testing procedures. Increased administration and laboratory effort resulted in drugs being delayed and substantial increases in the costs of the drugs being sold.

Harmonisation of regulatory requirements was pioneered by European regulators under the guise of the newly formed European Community (EC). In the 1980s, Europe moved towards the development of a single market for pharmaceuticals. The success achieved demonstrated that harmonisation of the regulations was feasible and at the WHO Conference of Drug Regulatory Authorities (ICDRA), in Paris, in 1989, specific plans for the formation of a common set of standards began. The birth of ICH took place at a meeting in April 1990 in Brussels where representatives of the regulatory agencies and industry associations of Europe, Japan and the US met[15]. Since this meeting, the ICH process gradually evolved, with significant progress being made in the development of ICH Guidelines on safety, quality and efficacy in its first decade. Since then, the ICH has looked to expand its reach into other regions ensuring a common set of standards can be applied. This has had significant impact in reducing the costs of drugs, whilst ensuring that new approaches can be implemented in a timely manner.

The ICH has been instrumental in driving new ideas and approaches to interaction between regulators and the pharmaceutical industry. Until recently, the approach that many pharmaceutical companies applied for developing and validating assays involved ensuring the assay complied with the guidance stipulated by the regulators. This approach does not, however, encourage the adoption of better methods of testing, and the ICH have, therefore, launched guidelines (ICH Q14) to address this situation[16]. ICH Q14 specifically addresses analytical method development, and is based on the concept of quality by design, where analytical method robustness is evaluated as part of the method development process. It also inherently encourages scientists to investigate

approaches to making the analysis better, including the use of innovative technologies such as automated method development screening platforms and retention modelling software. This acceptance that innovative technologies should be actively encouraged is an exciting prospect for scientists within the pharmaceutical community, but also for the manufacturing world which has often struggled to move new ideas into the highly regulated environment of the pharmaceutical industry.

By applying the quality by design concept to LC method development, it is feasible to optimise a method by varying specific method parameters, such as flow rate, mobile phase composition etc using a multi-factorial approach. This approach can be readily incorporated into the method development process, and the use of DOE (Design of Experiments) ensures that robustness is inherently built into the analytical method. Analytical parameters that can be investigated would include:

- Stationary phase chemistry
- Mobile phase composition
- pH of mobile phase
- Column temperature
- Gradient profile
- System dwell volume
- Flow rate
- Column batches

Ultimately, this process of method development leads to the generation of robust methods which can be successfully validated according to the requirements stipulated by the regulatory authorities. For a more detailed discussion of these LC method parameters and approaches to LC method development, along with examples of LC and GC solutions to regulatory requirements, please refer to the following ebook:

CONCLUSION

Over the past 160 years, regulation of the pharmaceutical industry has evolved beyond recognition and has driven vast improvements in the safety and efficacy of pharmaceutical products. Continual evolution of analytical testing and the implementation of new analytical approaches and techniques has been instrumental in driving this change. Although enormous progress has been made, regulatory requirements and testing capabilities continue to advance and new analytical challenges emerge. This has, for example, recently been demonstrated by the detection of genotoxic nitrosamine impurities within certain pharmaceutical products. In response, rapidly evolving regulatory requirements for risk assessment and analytical testing have been implemented across the world to address the issue, requiring rapid development and deployment of highly sensitive analytical methods to ensure drug substance and product safety.

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Using stationary phase selectivity to address NDMA over-quantification, due to isobaric interference from DMF in the LC-MS/MS analysis of nitrosamines

Matt James (Senior Research Scientist, Avantor) and Tony Edge, (R&D Leader and Scientific Advisor, Avantor)

INTRODUCTION

In 2018, N-nitrosodimethylamine (NDMA) was detected in a batch of valsartan at levels exceeding acceptable intake limits for mutagenic impurities[1,2]. NDMA is an N-nitrosamine, a class of compound containing a nitroso group bonded to an amine (Figure 1), first reported by Barnes and Magee, who found that NDMA produced liver tumours in rats. Subsequent studies showed that of over 300 nitrosamines evaluated, nearly 90% were carcinogenic to a wide variety of animals[3].

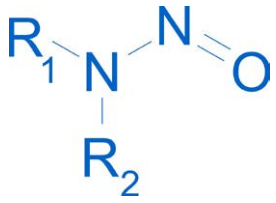


FIGURE 1: Chemical structure of N-nitrosamines.

Since 2018, the analysis of nitrosamines has become an intense focus point for the pharmaceutical industry. As summarised in Figure 2, the regulatory landscape has evolved very quickly since the first observation of NDMA in valsartan. In September 2020, the FDA released documentation related to controlling nitrosamine impurities in human drugs, which was recently updated in February 2021[4]. The FDA and EMA have highlighted several nitrosamines that could be generated during the production process and may potentially exist within drug products. These are highlighted in Table 1, with the designated daily acceptable intake (AI) limits[4,5,6].

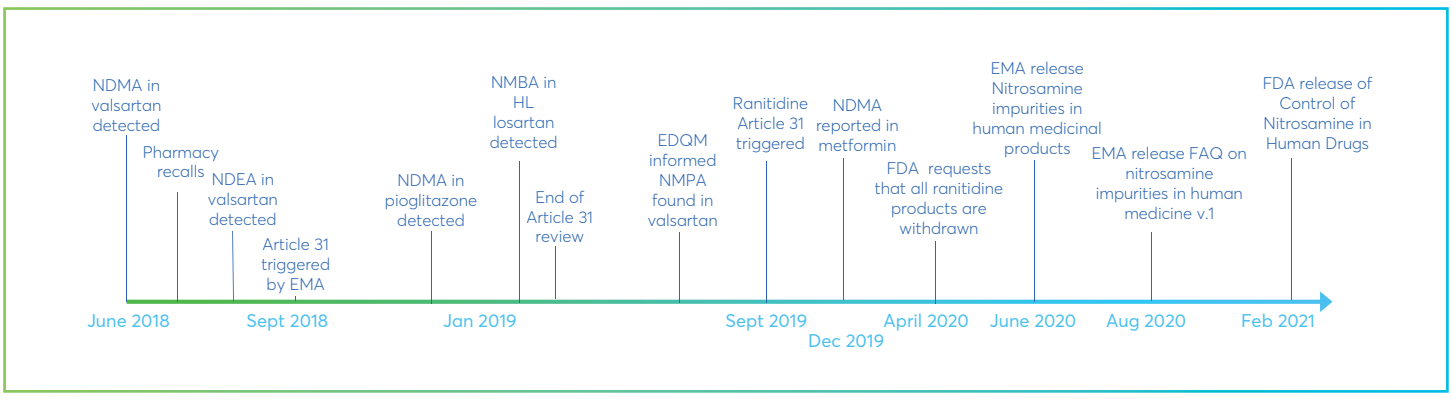


FIGURE 2: Timeline of main events in the evolution of regulatory requirements for nitrosamine analysis.

N-nitrosamine	Abbreviation	FDA limit ng/day	EMA limit ng/day
N-nitrosodimethylamine	NDMA	96.0	96.0
N-nitrosodiethylamine	NDEA	26.5	26.5
N-nitrosoethylisopropylamine	NEIPA	26.5	26.5
N-nitroso-diisopropylamine	NDIPA	26.5	26.5
N-nitroso-N-methyl-4-aminobutyric acid	NMBA	96.0	96.0
1-nitroso-4-methyl piperazine	MeNP	N/A	26.5
N-nitrosodibutylamine	NDBA	26.5	26.5
N-nitrosomethylphenylamine	NMPA	26.5	34.3

TABLE 1: List of eight nitrosamines that have daily exposure limits defined by the EMA and FDA. It should be noted that these limits are only applicable if the finished product contains a single N-nitrosamine. For multiple N-nitrosamines a different set of thresholds has been set.

Due to the high potential carcinogenicity of nitrosamines, the AIs for finished drug products are in the order of ng/day. The low level determination of nitrosamines is, therefore, challenging and requires the use of highly sensitive and selective detection systems. The analysis of finished drug product (i.e., drug substance and excipients) presents additional analytical challenges. The potential for interference from drug substance or excipients and the low detection limits required means that in some cases sample clean-up and concentration approaches, such as SPE, may need to be employed to mitigate the impact of the matrix[7,8].

Additionally, interference from other low molecular weight trace impurities could potentially result in inaccurate quantification. It has been reported that co-elution of N,N-dimethylformamide (DMF) with NDMA can result in over-quantification of NDMA. Yang et al[9] document a case in which a private testing laboratory reported that 16 of 38 metformin drug products tested by LC-High Resolution MS (LC-HRMS) contained quantities of NDMA above the AI limit of 96 ng/day. However, subsequent FDA testing of the same samples, reported overall lower values, with only eight samples determined to contain NDMA above the limit. It was postulated that interference from DMF, which co-eluted with NDMA, resulted in the over-estimation of NDMA content in the testing laboratory. Specifically, the ^{15}N DMF isotopic ion (which differs from the NDMA mono isotopic ion by just 0,0016 amu (21 ppm) could potentially be misidentified as NMDA, resulting in inaccurate quantification. Subsequent experiments recorded higher NDMA concentrations in samples containing DMF. It was concluded that if inappropriate mass accuracy and tolerance settings are applied, the 15N DMF isotopic ion can be misidentified as NDMA in the LC-HRMS analysis, resulting in over-quantification of NDMA.
Given the lower mass resolution of triple-quadrupole MS compared to HRMS, if residual DMF was present in API or drug product, then transitions from 13C and 15N DMF isotopic ions could potentially interfere with NDMA quantification if they are not sufficiently resolved chromatographically. In this article, the potential for interference from N,N-dimethylformamide (DMF) using an existing LC-MS/MS method is investigated, along with strategies for mitigating the risks of inaccurate quantification that arises.

NDMA OVER QUANTIFICATION DUE TO DMF COELUTION

A previously published LC-MS/MS method for the analysis of eight nitrosamines in drug substance, developed using an Avantor® ACE® UltraCore SuperC18 solid core column[10], was used to investigate the potential for NDMA over quantification. This was assessed by analysing a series of 1.0 ng/ml NDMA samples, spiked with varying concentrations of DMF (Table 2). The DMF concentrations selected are within the defined residual solvent limits specified in ICH Q3C(R8)[11]. Both NDMA and DMF showed very low retention on the solid core C18, with a retention factor (k) of just 0,3 and were found to co-elute. At this low level concentration, the presence of DMF detrimentally impacted the calculated accuracy (Table 2), leading to falsely high predicted NDMA concentrations. This could be particularly impactful in situations where multiple nitrosamines are detected, requiring lower level quantification limits[4,6,12]. It was also noted that the *m/z* 75,0 → 58,0 NDMA qualifier transition was affected to a lesser degree than the *m/z* 75,0 → 43,0 quantifier transition.

				Quantifier *m/z* 75.0 → 43.0		Qualifier *m/z* 75.0 → 58.0	
Spike level	NDMA (ng/ml)	DMF (ng/ml)	DMF (ppm)	Calculated NDMA conc. (ng/ml)	% Accuracy	Calculated NDMA conc. (ng/ml)	% Accuracy
0	1.0	0	0	1.03	102.9	1.07	106.7
1	1.0	83.3	1.25	1.03	103.3	1.04	103.6
2	1.0	833.3	12.5	1.37	137.0	1.15	114.6
3	1.0	1666.7	25	1.64	163.6	1.22	121.6
4	1.0	3333.3	50	2.20	220.0	1.42	141.9
5	1.0	6666.7	100	3.07	306.8	1.60	159.7

TABLE 2: Summary of spiking experiment used to assess potential interference from DMF on NDMA quantification.

From this data, chromatographic separation of DMF and NDMA would clearly be advantageous. The hydrophilic nature of both DMF and NDMA and the low starting percent organic used in the gradient makes obtaining better retention challenging. Varying column stationary phase is a powerful tool by which analyte selectivity and retention can be adjusted, therefore, a range of stationary phases were screened to assess whether better retention and separation was possible[13]. Fully

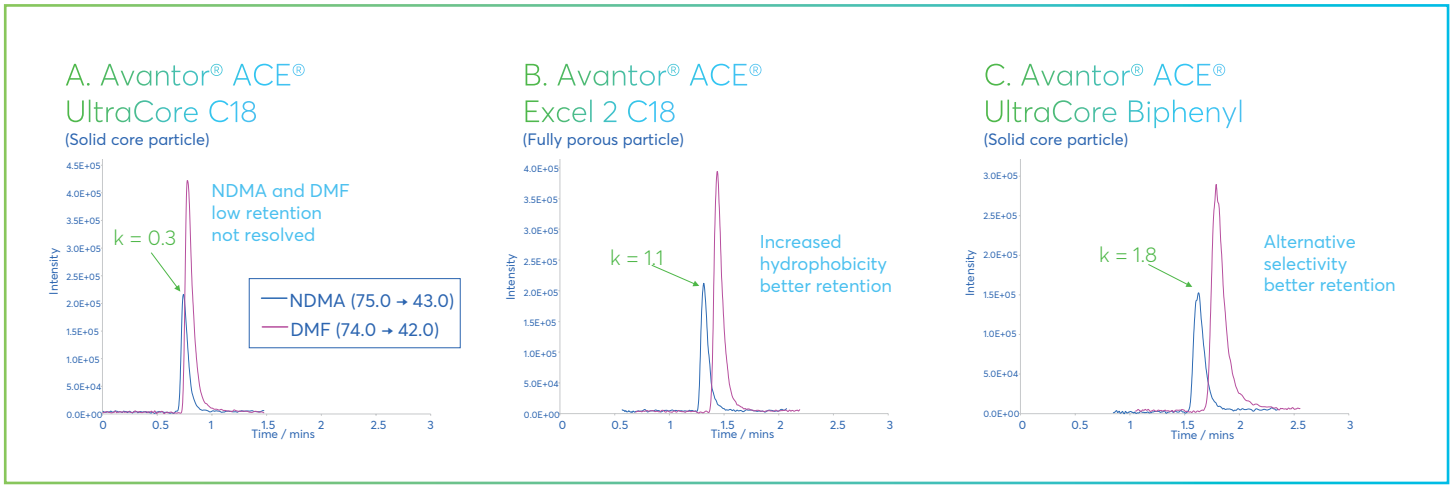
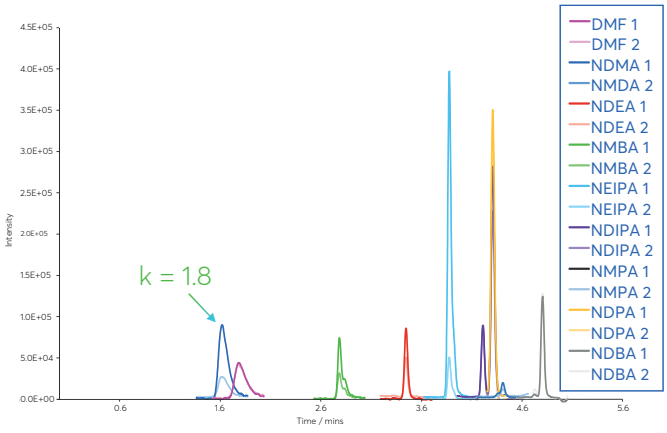


FIGURE 3: The chromatographic separation between NDMA and DMF on the three stationary phases tested.

porous columns are typically more retentive than their solid core counterparts, due to their increased porosity, and consequently, a larger surface area. By exchanging the solid core column with an Avantor® ACE® Excel® 2 C18 fully porous column, it was found that the increased hydrophobicity of this phase provided increased aliphatic interactions between the analytes and the stationary phase. This improved NDMA retention (k = 1,1) and provided additional separation of DMF from NDMA (Figure 3B).

As an alternative approach, the Avantor® ACE® UltraCore Biphenyl solid core stationary phase was assessed to determine whether an alternative stationary phase selectivity could provide better retention and separation. As shown in Figure 3C, π-π interactions with the Biphenyl phase provided enhanced retention for NDMA (k = 1,8) and DMF and a similar degree of separation to the C18 fully porous phase. The added retention offered by the Biphenyl phase could also prove useful for addressing ion suppression effects that may arise in the analysis of drug products containing hydrophilic APIs and/or excipients. The LC gradient conditions were optimised on both columns to provide maximum NDMA retention plus separation of the seven additional nitrosamines. The separation on the Biphenyl phase shown in Figure 4, for full method details on both columns, please refer to reference 14. Calibration curves and QC samples showed excellent linearity, accuracy and precision, whilst LOD and LOQ values were determined and found to be comparable to data obtained for the original method[14].

Both LC-MS/MS methods were then assessed using the spiking approach in Table 2, to determine whether they could be utilised to reduce NDMA quantification errors in the presence of DMF. The additional chromatographic



Column: Avantor® ACE® UltraCore Biphenyl
Dimensions: 100 x 2.1 mm i.d.
Particle size: 3.5 µm
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in methanol

Gradient:

Time (min)	% Mobile phase B
0	1
1	1
5	100
5.4	100
5.5	1
7.8	1

All other conditions as per Figure 4

FIGURE 4: Example LC-MS/MS separation of nitrosamines spiked into valsartan drug substance at 0,1 ng/ml on an Avantor® ACE® UltraCore Biphenyl column. Overlayed traces represent the quantifier and qualifier transitions for each nitrosamine and DMF. Please refer to reference 14 for full details of the MRM transitions.

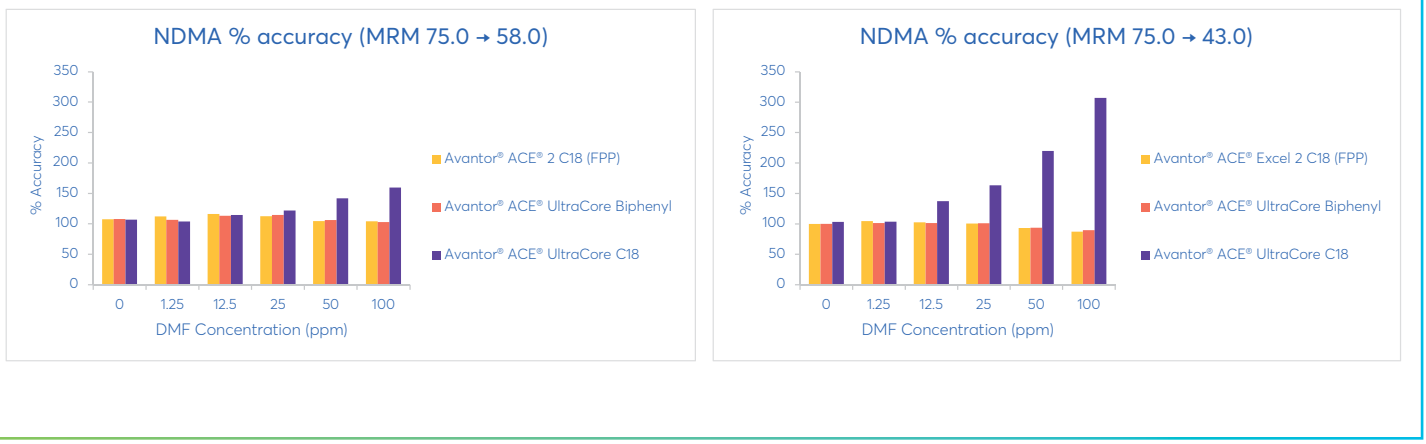


FIGURE 5: Percentage accuracy data for NDMA quantification in the DMF spiking experiment using original method (green) and the alternative approaches on the Avantor® ACE® Excel® 2 C18 and Avantor® ACE® UltraCore Biphenyl phases.

resolution of NDMA and DMF provided by both the Avantor® ACE® Excel® C18 and Avantor® ACE® UltraCore Biphenyl methods permitted accurate integration of NMDA in the presence of DMF, and significantly improved accuracy compared to the original method (Figure 5). Given that the *m/z* 75,0 → 58,0 NDMA transition was found to provide improved accuracy in the presence of DMF in the previous experiments (Table 2), it is recommended that this transition be assigned as the quantifier transition for NDMA.

Additionally, the ability to monitor drug product and substance for the presence of DMF, in the same analytical run to identify samples potentially at risk of NDMA over-quantification, would be beneficial. MRM transitions were, therefore, established and optimised for selective monitoring of DMF (Figure 6). The transitions were found to be highly selective in the presence of NDMA. Consequently, these DMF transitions can be used in any LC-MS/MS approach to monitor the DMF content of real life samples to screen for samples that may be prone to NDMA quantification issues. Figure 6 shows the NDMA and DMF transitions for a 30 ng/ml solution of NDMA. At this high NDMA concentration, no response is seen in either DMF transition, thereby demonstrating the applicability of these MRM transitions to monitor samples for residual DMF.

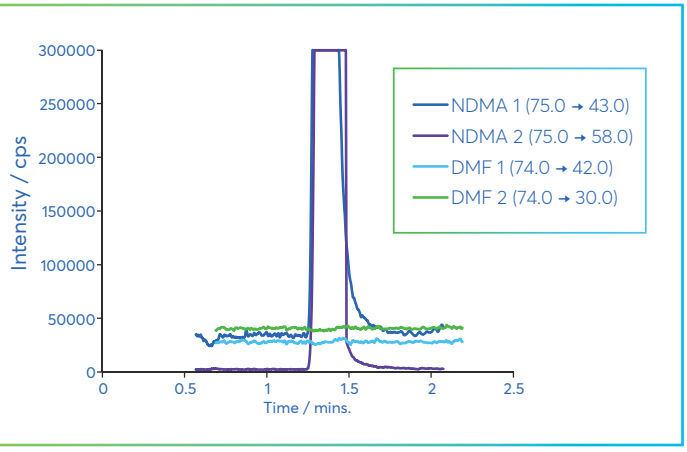
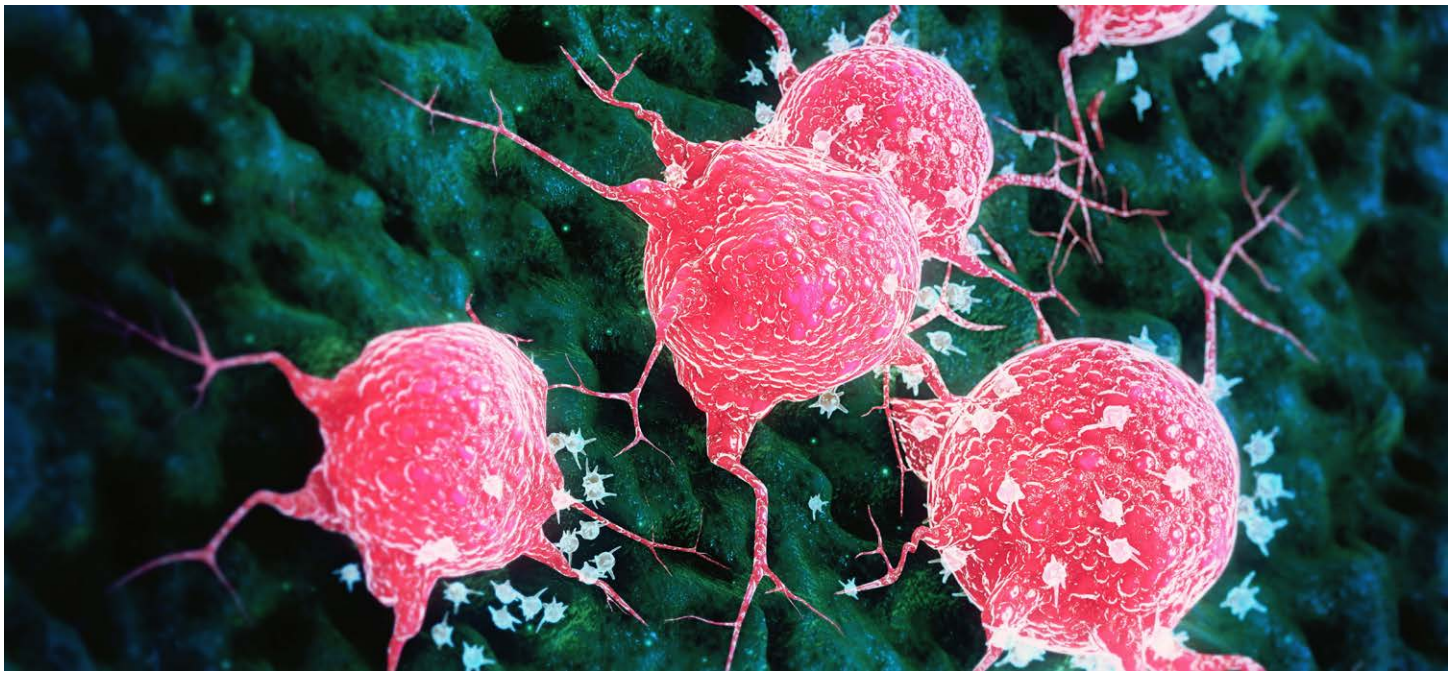


FIGURE 6: NDMA and DMF MRM transitions in a 30 ng/ml solution of NDMA, demonstrating high selectivity of the DMF transition in the presence of NDMA.

CONCLUSION

The combined approach of monitoring samples, using appropriate MRM transitions to identify residual DMF, and the use of a column stationary phase that provides at least partial resolution of NDMA and DMF, is recommended. The Avantor® ACE® Excel® 2 C18 and Avantor® ACE® UltraCore Biphenyl phases have both been demonstrated to achieve this separation and provide more accurate NDMA quantification at low concentrations by LC-MS/MS analysis. The chromatographic resolution provided reduces the risk of isobaric interference and guards against any potential for ion suppression or enhancement in the ionisation process that may result from co-elution of these two species. The improved retention provided by these phases could also aid in reducing the possibility for interference from other low retention matrix components. Provided suitable mass accuracy and tolerance settings are used, the chromatographic separation provided by these two stationary phases can provide additional safeguards against quantification errors for NDMA.



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Our current understanding of the human health and environmental risks of PFAS

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) ARE A GROUP OF MANUFACTURED CHEMICALS

PFAS are a group of manufactured chemicals that have been used in industry and consumer products since the 1940s because of their useful properties. There are thousands of different PFAS, some of which have been more widely used and studied than others.

Perfluorooctanoic acid (PFOA) and perfluorooctane sulphonate (PFOS), for example, are two of the most widely used and studied chemicals in the PFAS group. PFOA and PFOS have been replaced in the United States with other PFAS in recent years.

One common characteristic of concern of PFAS is that many break down very slowly and can build up in people, animals and the environment over time.

PFAS CAN BE FOUND IN MANY PLACES

PFAS can be present in our water, soil, air and food, as well as in materials found in our homes or workplaces, including:

- Drinking water – in public drinking water systems and private drinking water wells
- Soil and water at or near waste sites - at landfills, disposal sites and hazardous waste sites such as those that fall under the federal Superfund and Resource Conservation and Recovery Act programs
- Fire extinguishing foam - in aqueous film-forming foams (or AFFFs) used to extinguish flammable liquid-based fires. Such foams are used in training and emergency response events at airports, shipyards, military bases, firefighting training facilities, chemical plants and refineries
- Manufacturing or chemical production facilities that produce or use PFAS – for example, at chrome plating, electronics and certain textile and paper manufacturers
- Food – for example, in fish caught from water contaminated by PFAS and dairy products from livestock exposed to PFAS
- Food packaging – for example, in grease resistant paper, fast food containers/wrappers, microwave popcorn bags, pizza boxes and candy wrappers
- Household products and dust – for example. in stain and water repellent used on carpets, upholstery, clothing and other fabrics; cleaning products, non stick cookware, paints, varnishes and sealants
- Personal care products – for example, in certain shampoo, dental floss and cosmetics
- Biosolids – for example fertiliser from wastewater treatment plants that is used on agricultural lands can affect ground and surface water and animals that graze on the land

PEOPLE CAN BE EXPOSED TO PFAS IN A VARIETY OF WAYS

Due to their widespread production and use, as well as their ability to move and persist in the environment, surveys conducted by the Centers for Disease Control and Prevention (CDC) show that most people in the United States have been exposed to some PFAS. Most known exposures are relatively low, but some can be high, particularly when people are exposed to a concentrated source over long periods of time. Some PFAS chemicals can accumulate in the body over time.

Current research has shown that people can be exposed to PFAS by:

- Working in occupations such as firefighting or chemical manufacturing and processing
- Drinking water contaminated with PFAS
- Eating certain foods that may contain PFAS, including fish
- Swallowing contaminated soil or dust
- Breathing air containing PFAS
- Using products made with PFAS or that are packed in materials containing PFAS

EXPOSURE TO PFAS MAY BE HARMFUL TO HUMAN HEALTH

Current scientific research suggests that exposure to high levels of certain PFAS may lead to adverse health outcomes. However, research is still ongoing to determine how different levels of exposure to different PFAS can lead to a variety of health effects. Research is also underway to better understand the health effects associated with low levels of exposure to PFAS over long periods of time, especially in children.

What we know about the health effects

Current peer-reviewed scientific studies have shown that exposure to certain levels of PFAS may lead to:

- Reproductive effects such as decreased fertility or increased high blood pressure in pregnant women
- Developmental effects or delays in children, including low birth weight, accelerated puberty, bone variations, or behavioural changes
- Increased risk of some cancers, including prostate, kidney and testicular
- Reduced ability of the body's immune system to fight infections, including reduced vaccine response
- Interference with the body's natural hormones
- Increased cholesterol levels and/or risk of obesity

Additional health effects are difficult to determine Scientists at EPA, in other federal agencies, and in academia and industry are continuing to conduct and review the growing body of research about PFAS. However, health effects associated with exposure to PFAS are difficult to specify for many reasons, such as:

- There are thousands of PFAS with potentially varying effects and toxicity levels, yet most studies focus on a limited number of better known PFAS compounds
- People can be exposed to PFAS in different ways and at different stages of their life
- The types and uses of PFAS change over time, which makes it challenging to track and assess how exposure to these chemicals occurs and how they will affect human health

CERTAIN ADULTS & CHILDREN MAY HAVE HIGHER EXPOSURE TO PFAS

Adults

Some people have higher exposures to PFAS than others because of their occupations or where they live. For example:

- Industrial workers who are involved in making or processing PFAS or PFAS-containing materials, or people who live or recreate near PFAS-producing facilities, may have greater exposure
- Pregnant and lactating women tend to drink more water per pound of body weight than the average person, and as a result, they may have higher PFAS exposure compared to other people

Children

Because children are still developing, they may be more sensitive to the harmful effects of chemicals such as PFAS. They can also be exposed more than adults because:

- Children drink more water, eat more food, and breathe more air per pound of body weight than adults, which can increase their exposure to PFAS
- Young children crawl on floors and put things in their mouths which leads to a higher risk of exposure to PFAS in carpets, household dust, toys and cleaning products

Breast milk from mothers with PFAS in their blood and formula made with water containing PFAS can expose infants to PFAS, and it may also be possible for children to be exposed in utero during pregnancy. Scientists continue to do research in this area. Based on current science, the benefits of breast feeding appear to outweigh the risks for infants exposed to PFAS in breast milk. To weigh the risks and benefits of breast feeding, mothers should contact their doctors.

PFAS standards

Per- and polyfluoroalkyl substances (PFAS) are amongst the primary emerging contaminants of concern. The detection and quantification of known PFAS and the discovery of unknown PFAS substances has never been more important.

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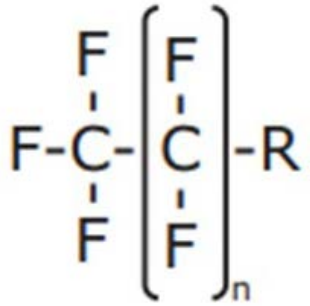
Fast and high resolution LC-MS separation of PFAS

Petra Lewits, Cory Muraco, Product Manager Liquid Separations, Johanna Simon, Scientist Central Analytics, Merck.

PFAS (Per- and poly-fluoroalkyl substances) are persistent, man-made organic compounds, widely found in the environment. Recent awareness about their toxicity has led the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA) to initiate actions against PFAS. Hence reliable and fast methods are needed for their determination.



PFASs are commonly measured using Liquid Chromatography–Mass Spectrometry (LC-MS). The column of choice for PFAS analysis by LC-MS/(MS) is a C18 column. Ascentis® Express PFAS columns are based on superficially porous silica particles (SPP) with C18 modification and are specifically tested using a PFAS compound mixture. This ensures the suitable and reliable performance of these columns for efficient PFAS analysis. PFAS compounds originating from the HPLC system and materials used for the analysis are a concern. Therefore, it is recommended that you place a delay column before the injection port in the system (Figure 1). The Ascentis® Express PFAS Delay column provides exceptionally high retention of PFAS compounds across various mobile phase conditions. It efficiently delays PFAS background contamination that originates from the instrument and, therefore, prevents co-elution with the PFAS compound present in the sample **(Figure 1).**

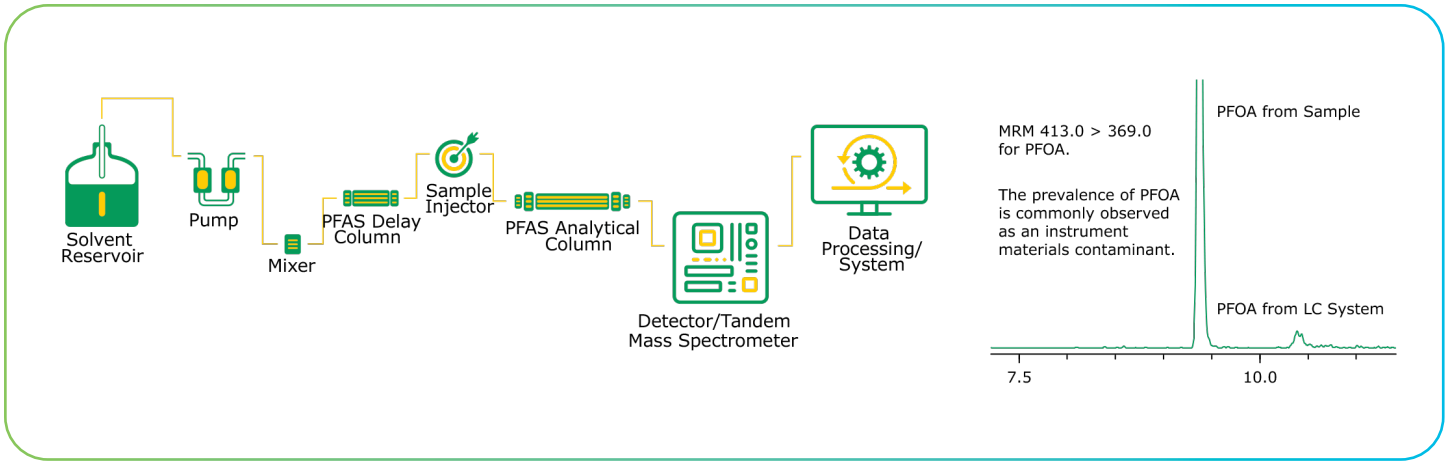


FIGURE 1: LC-MS instrumental set-up for PFAS analysis and results of perfluorooctanoic acid (PFOA) (MRM 413,0 >369,0) using a delay column.

LC-MS ANALYSIS OF PFAS - 33 COMPOUNDS IN 5 MINUTES

The rapid separation of 33 PFAS compounds found in EPA 537.1, EPA 533 and EPA 8327 demonstrates that the Fused-Core® technology of Ascentis® Express PFAS HPLC columns benefits the PFAS analysis for fast, efficient, and rugged separations—paramount to environmental analysis **(Figure 2).**

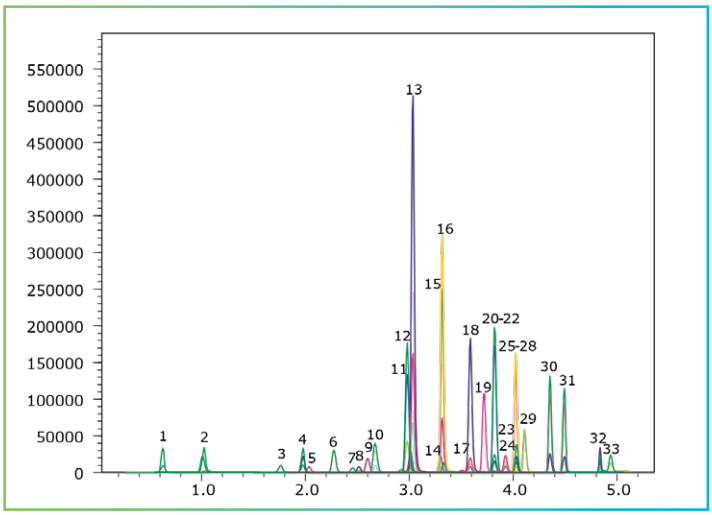


FIGURE 2: 33 PFAS compounds in 5 minutes.

LC CONDITIONS

Analytical column	Ascentis® Express 90Å PFAS, 10 cm x 2,1 mm, 2,7 µm (53559-U)	
Delay column	Ascentis® Express 90 Å PFAS delay, 5 cm x 3 mm, 2,7 µm (53572-U)	
Mobile phase	[A]10 mM ammonium acetate; [B] methanol	
Gradient	Time (min)	%B
	0,0	33,0
	4,0	98,0
	4,1	100,0
	6,0	100,0
	6,1	33,0
Flow rate	0,4 ml/min	
Pressure	479 bar (6947 psi)	
Temperature	35 °C	
Detection	ESI (-) MS/MS; ESI LCMS system: Shimadzu LCMS- 8040; spray voltage: -2,0 kV; nebulising gas: 2 L/min; drying gas: 15 L/min; DL temp.: 250 °C; heat block: 400 °C	
Injection volume	2,0 µl	
Sample solvent	Methanol (96%) water (4%)	

SAMPLE COMPOUNDS

Peak	Compound	Transition	Retention time (min)
1	PFBA	213,0>169,0	0,755
2	4:2FTS	229,0>85,0	1,031
3	PFPeA	263,0>219,0	1,762
4	PFBS	299,0>80,0	1,979
5	PFHpS	279,0>85,0	2,035
6	PFPeS	315,0>135,0	2,273
7	PFMPA	327,0>307,0	2,454
8	PFHxA	313,0>269,0	2,514
9	PFEESA	349,0>80,0	2,599
10	HFPO-DA	285,0>169,0	2,670
11	PFHxS	399,0>80,0	3,013
12	NaDONA	377,0>251,0	3,033
13	ADONA	377,0>250,9	3,034
14	FOSA	427,0>407,0	3,299
15	PFOA	413,0>369,0	3,316
16	PFMBA	449,0>80,0	3,328
17	PFHpA	363,0>319,0	3,388

Peak	Compound	Transition	Retention time (min)
18	PFOS	499,0>80,0	3,588
19	9Cl-PF3ONS	530,9>351,0	3,719
20	8:2FTS	549,0>80,0	3,816
21	PFNS	527,0>507,0	3,820
22	PFDA	513,0>469,0	3,822
23	N-MeFOSAA	570,0>419,0	3,925
24	PFNA	463,0>419,0	3,942
25	NFDHA	599,0>80,0	4,015
26	PFUnA	563,0>519,0	4,025
27	N-EtFOSAA	584,0>419,0	4,029
28	6:2FTS	498,0>78,0	4,033
29	11Cl-PF3OUdS	630,7>451,0	4,110
30	PFTrDA	663,0>619,0	4,355
31	PFDoA	613,0>569,0	4,496
32	PFTeDA	713,0>669,0	4,745
33	PFDS	295,0>201,0	4,921

FEATURED PRODUCTS

Description	Cat. No.
Ascentis® Express 90Å PFAS, 10 cm x 2,1 mm, 2,7 µm,	**53559-U**
Ascentis® Express 90Å PFAS Delay, 5 cm x 3 mm, 2,7 µm	**53572-U**
Solvents & Reagents	
Methanol for chromatography (LC-MS grade) LiChrosolv®	**1.06035**
Water for chromatography (LC-MS grade) LiChrosolv® or tap fresh from an appropriate Milli-Q® system	**1.15333**
Ammonium acetate suitable for mass spectrometry (MS), LiChropur™, eluent additive for LC-MS	**73594**

RELATED PRODUCTS

Description	Cat. No.
Perfluorobutanoic acid, neat	**68808-25MG**
Perfluoropentanoic acid, neat	**68542-25MG**
Perfluorohexanoic acid, neat	**43809-25MG**
Perfluorooctanoic acid, neat	**33824-100MG**
Perfluorononanoic acid, neat	**91977-50MG**
Perfluorodecanoic acid, neat	**43929-25MG**
Perfluorododecanoic acid, neat	**92291-50MG**
Perfluorotetradecanoic acid, neat	**80312-50MG**
Pentadecafluorooctanoic acid, 100 µg/ml in methanol	**33603-1ML**
Heptadecafluorooctanoic acid, 100 µg/ml in methanol	**33607**
Perfluorooctane sulfonic acid, neat	

Automated Solid Phase Extraction (SPE) of 10 Perfluorinated Compounds (PFAs) from tap water

This collaboration study was performed jointly by Gilson, Inc. and Affinisep

Perfluorinated compounds (PFAs) are a family of molecules consisting of varying lengths of fluorocarbon chains with a functional group such as carboxylic or sulphonic acid attached. Since 2009, PFOs have been classified as POPs (Persistent Organic Pollutants) and the International Agency for Research on Cancer (IARC) classified PFOA as a Group 2B possible carcinogen. The analysis of PFAs can be complex due to their presence in multiple items used in the analytical workflow.

This application note describes the automation of the isolation of 10 PFAs from tap water prior to their analysis by LC-MS using a specific SPE polymer AttractSPE™ PFAs and the Gilson ASPEC® 274 Large Volume (LV) system. The results show good reproducibility of the method without any contamination from the extraction system.

FIGURE 1: Gilson 274 ASPEC® Large Volume system.

INTRODUCTION

Perfluorinated compounds are a family of molecules consisting of varying lengths of fluorocarbon chains with a functional group such as carboxylic or sulphonic acid attached. To achieve the concentration limit defined by EU Water Framework Directive and the EPA for perfluorinated compounds in water, it is highly recommended to use Solid Phase Extraction (SPE) to concentrate the sample prior to mass spectrometry analysis. Due to the confusion created by the acronym "PFCs", and its two different meanings, namely perfluorinated compounds or fluorocarbons, the acronym "PFAs" (per-and polyfluoroalkyl substances), is now preferred to classify this family of compounds.

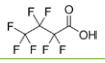
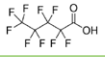
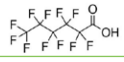
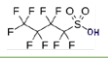
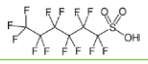
Compound	Chemical composition	CAS. No
Perfluorobutanoic acid (PFBA)		375-22-4
Perfluoropentanoic acid (PFPeA)		2706-90-3
Perfluorohexanoic acid (PFHxA)		307-24-4
Perfluoroheptanoic acid (PFHpA)		375-85-9
Perfluorooctanoic acid (PFOA)		335-67-1
Perfluorononanoic acid (PFNA)		375-95-1
Perfluorodecanoic acid (PFDA)		335-76-2
Perfluorobutanesulfonic acid (PFBS)		375-73-5
Perfluorohexane sulfonic acid (PFHxS)		355-46-4
Perfluorooctanesulfonic acid (PFOS)		1763-23-1

TABLE 1: List of the tested PFAs sorted from the smallest to the largest.

This application note describes the analysis of 10 perfluorinated compounds using a specific AttractSPE® PFA. This cartridge chemistry is dedicated specifically to perfluorinated compounds. The cartridges are used in the Gilson ASPEC® 274 Large Volume System (Figure 1) to purify and concentrate the sample prior to analysis by LC-MS/MS.

The analysis of PFAs can be challenging. They are common material components in analytical systems. Their presence in HPLC systems and solvents, sampling bottles, valves, transfer tubing, etc can lead to significant chance of sample contamination and high PFAs background, masking trace levels of PFAs from detection and/or leading to false positives.

This application ultimately shows the absence of potential PFAs contamination through the Gilson ASPEC® 274 Large Volume System with high recoveries and reproducibility.

PRECAUTIONS FOR THE ANALYSIS OF PFAS & RELIABILITY OF THE METHOD

PRECAUTIONS FOR SAMPLE HANDLING & PREPARATION

EPA 537-1[1] method describes numerous precautions for sample collection, preservation, storage, analysis and glassware treatment. It also prohibits the use of materials that can cause accidental contamination, ultimately resulting in cleaner analyses.

For this study, Gilson HDPE sample bottles and Gilson PIPETMAN® DIAMOND Tips were used for sample handling and for standard preparation. The Gilson ASPEC® 274 Large Volume System was equipped with PEEK tubing to avoid the use of any fluoropolymer, such as PTFE or FEP, to reduce contamination risk during the sample preparation process.

Cartridges, and the filters and resins contained in them, can also be sources of contamination. For this reason, AFFINISEP has developed a specific AttractSPE® PFAs dedicated to the isolation of perfluorinated compounds. AFFINISEP AttractSPE® PFAs cartridges will not contaminate any sample with additional PFAs.

To reduce contamination risk, best practice indicates that all sampling containers are discarded after use, nitrile gloves are preferred over other glove materials, nitrile gloves are often replaced, and all glass or fluoropolymer containers are avoided to prevent surface adsorption of PFAs or contamination from container material.

PRECAUTIONS TO IMPROVE HPLC METHOD RELIABILITY

HPLC devices often contain PTFE parts and tubing. This, coupled with potential traces of PFAs or other fluorine-containing material in solvents, can hinder analysis at low concentrations. The fluorine-containing materials that are released from the HPLC system tend to build-up at the front of the column creating interference. To minimise this interference, all PTFE or any other fluorinated or perfluorinated polymers are replaced with non fluorine-containing materials. Also, despite

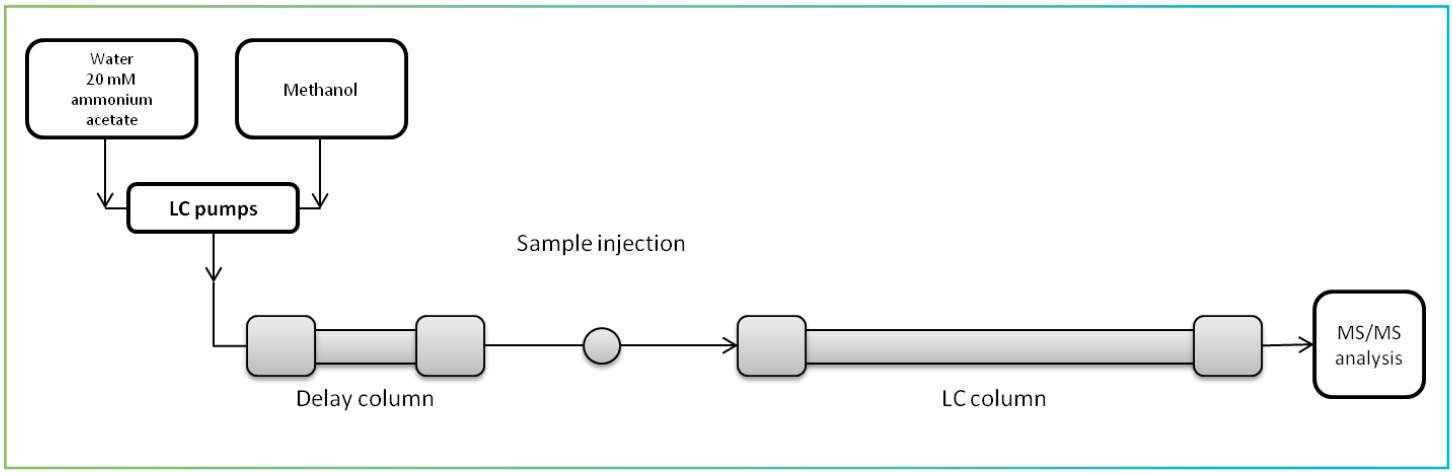


FIGURE 2: Diagram of delay column installation on HPLC.

the difficulty and added cost, all solvents used must be verifiably PFAs-free.

Another approach, which was used in this application note, is the installation of a delay column between the LC pumps and the injector. The diagram below (Figure 2) demonstrates the proper placement of the delay column in the HPLC fluid path. The interfering species from the solvents and LC pumps will concentrate at the front of the delay column resulting in a shift of retention time. This shift in retention time effectively separates interfering species from the PFAs analyte. This is a simple and cost-effective solution.

PFOA is an interfering species when analysing PFAs. Two solutions, a blank consisting of methanol and a 0,5 µg/L solution of PFOA in methanol were analysed (Figure 3) to demonstrate the efficiency of the method using a delay column. (Silact™ C18 LC-P 50x2,1 mm, 3 µm)

The injection of a 0,5 µg/L PFOA solution (Figure 3) shows two peaks. The first one at 16,69 min is PFOA in the injected solution at 0,5 µg/L, while the second one at 17,59 min corresponds to the delayed PFOA interference from HPLC lines and solvents. The injection of a blank methanol showed no interference at the expected retention time. The delay column allows the elimination of interfering PFOA at a concentration estimated between 0,05 and 0,1 µg/L.

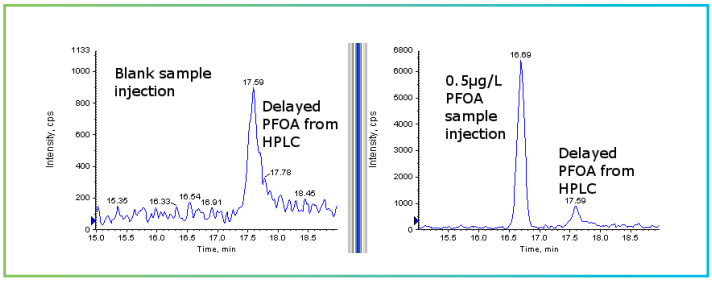


FIGURE 3: Injection of methanol blank (left) and injection of 0,5 µg/L PFOA in methanol (right).

ANALYSIS OF 10 PFAS IN 500 ML OF TAP WATER

The SPE protocol was carried out on the Gilson ASPEC® 274 Large Volume System in tap water using AttractSPE® PFAs 6 ml cartridges. The polymeric WAX phase contained in these cartridges undergoes a proprietary treatment at Affinisep to ensure it is entirely PFAs-free and a specific high loading capacity for PFAs.

The automation of PFAs analysis was carried out using the Gilson ASPEC® 274 Large Volume System equipped with segregated waste lines and VALVEMATE® II sample selection valve actuator. PEEK tubing was used to reduce risk of PFA adsorption and resulting contamination. The solvents and the 500 ml samples of tap water were contained in Gilson HDPE bottles.

SAMPLE PRE-TREATMENT

The tap water hardness was very high (Ca^{2+} ~300 mg/L, NO^- ~20 mg/L, Cl ~0,3 mg/L). For each sample, 475 ml of tap water was mixed with 25 ml of methanol. The pH was adjusted to ~4 with 100 µl of formic acid. The solution was then spiked with a mix of 10 PFAs at a concentration of 24 ng/L.

One non spiked sample blank and four spiked samples were processed and analysed. The blank was then analysed to verify non contamination.

The analyses were carried using a manual SPE manifold as a control, and on the automated system Gilson ASPEC® 274 Large Volume System to confirm the absence of adsorption and contamination potentially introduced by the automated system.

SPE PROTOCOL

Step	SPE protocol
Conditioning / Equilibration	1. 5 ml 0,1% NH_4OH in methanol 2. 9 ml methanol 3. 9 ml HPLC grade water with formic acid (pH = 4)
Loading	500 ml of loading solution (475 ml tap water + 25 ml methanol pH = 4)
Drying	20 ml air pushed through the cartridge
Elution	1. 2 ml methanol 2. 4 ml 0,1% NH_4OH in methanol

This protocol was easily transferred to TRILUTION® LH software for automation.

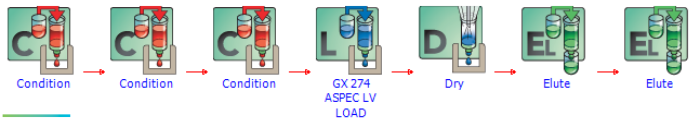


FIGURE 4: Gilson TRILUTION® LH SPE method.

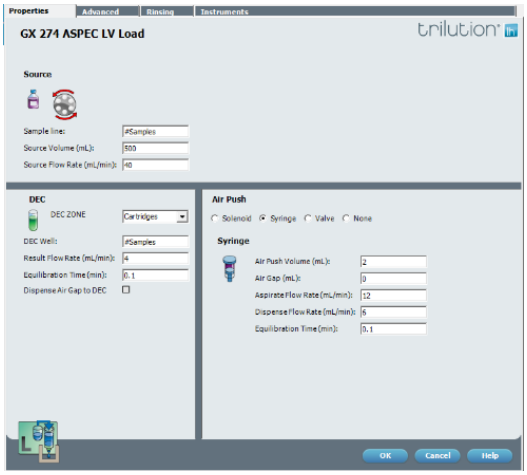


FIGURE 5: GX-274 ASPEC® IV load task.

The elution was collected in polypropylene vials, homogenised, and directly analysed by LC-MS/MS. To determine matrix effect, a fraction of the elution of the blank water sample was spiked at 2 µg/L and analysed.

HPLC/MSMS PROTOCOL

The analytical conditions are presented in the table below.

LC conditions	MS conditions
LC Dionex U3000	Qtrap 4000 ESI- MS/MS
Column: Silact C18 LC-P 150 x 2,1 mm, 3 µm and pre-column filter at 30 °C Delay column: Silact C18 LC-P 50 x 2,1 mm, 3 µm	Curtain gas: 30 CAD: High
Injection volume: 5 µl	IS: -4500 V
T° sampler: 10 °C	Temperature: 400 °C
Flow rate: 0,25 ml/min	GS1/GS2: 50/50

Time (min)	Solvent A	Solvent B	Analyte	Retention time (min)	Q1	Q3	CE (V)
0	60	40	PFBA	4,5	213,0	168,8	-14
1	60	40	PFPeA	8,6	263,0	218,8	-12
20	10	90	PFBS	9,4	299,0	79,8/98,9	-52/-44
30	10	90	PFHxA	12,2	313,0	268,9/119	-14/-28
31	60	40	PFHpA	14,8	363,0	318,8/168,8	-16/-26
35	60	40	PFHxS	14,9	399,0	79,9/98,9	-74/-56
Solvent A: 20 mM ammonium acetate (in water) Solvent B: Methanol			PFOA	16,7	413,1	368,9/168,8	-14/-26
			PFOS	18,3	499,0	80,1/98,9	-84/-70
			PFNA	18,3	463,0	418,9/219,0	-16/-24
			PFDA	19,6	513,0	469,0/218,8	-13/-11

TABLE 2: LC-MS/MS conditions for the analysis of the 10 PFAs.

Compound	Unspiked tap water	Spiked tap water at 24 ng/L	RSDr (n = 4)	Blank water eluate spiked at 2 µg/L
		Recovery		Observed
PFBA	ND*	99%	3%	+16%
PFPeA	ND*	99%	1%	+12%
PFBS	ND*	101%	3%	+10%
PFHxA	ND*	102%	3%	+17%
PFHpA	ND*	100%	4%	+8%
PFHxS	ND*	101%	2%	+1%
PFOA	ND*	102%	2%	-1%
PFOS	ND*	87%	2%	+8%
PFNA	ND*	97%	3%	+8%
PFDA	ND*	83%	2%	-1%

*ND: Not Detected

TABLE 3: Recovery of 10 PFAs in 500 ml of unspiked tap water and spiked tap water and observed matrix effect after purification with AttractSPE® PFAs on Gilson 274 ASPEC® Large Volume System.

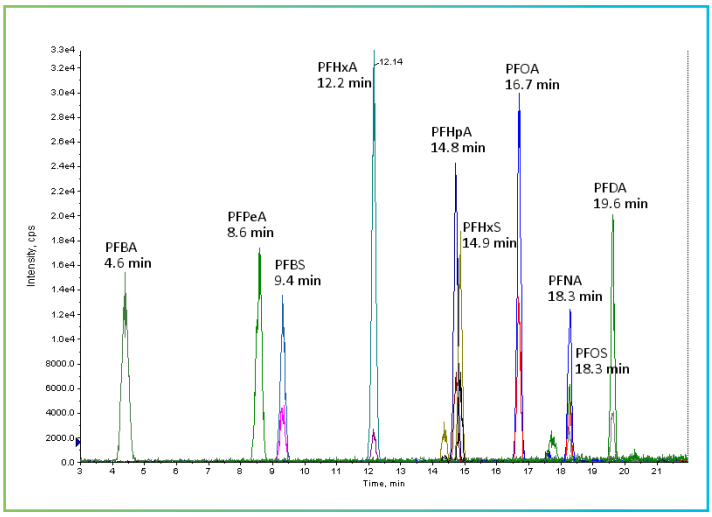
RESULTS

The method linearity was checked against a calibration curve with PFAs concentrations of 0,5; 1; 2; 4 and 6 µg/L. The R^2 value was found to be greater than to 0,998 for the 10 molecules, demonstrating linearity of the method over this concentration range.

As shown in Table 3, no detectable contamination was found using the AttractSPE® PFAs cartridges on the Gilson ASPEC® 274 Large Volume System.

The elution fraction of a blank sample was spiked at 2 µg/L showing no significant matrix effects, with a maximum of 16% signal enhancement for PFBA by comparison with the calibration curve. Without an additional concentration step after the SPE protocol, good values of LOQ (6 ng/L) and LOD (2 ng/L) were achieved.

Futhermore, AttractSPE® PFAs showed excellent recoveries from 83% to 102% and the Gilson 274 ASPEC® 274 Large Volume System allowed excellent relative standard deviation from 1% to 4%.



CONCLUSION

The automation of the isolation of 10 PFAs from tap water for analysis by LC-MS/MS, was found to be very effective with good recoveries from 83% to 102% without any contamination from the extraction system.

The AttractSPE® PFAs cartridges have enhanced selectivity and concentrated the sample more than 80 fold, allowing the direct analysis of the 10 PFAs with a LOQ at 6 ng/L without any evaporation step.

The combination of PFAs and the ASPEC® 274 Large Volume System allowed development of a robust method with excellent repeatability (RSD 1% to 4%) that helps eliminate environmental variables and effectively reduces chances of sample contamination.

Furthermore, the automation greatly reduces labour cost, reduces potential exposure to hazardous solvents, and increases overall efficiency for high throughput laboratories.

ORDERING TABLE

GILSON 274 ASPEC LARGE VOLUME SYSTEM

Description	Pk	Cat. No.
GX-274 ASPEC, Large Volume system with Z drive	1	GILI2614010LV

REFERENCE

1. Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/ Tandem Mass Spectrometry(LC/MS/MS). https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=343042&Lab=NERL

TRADEMARKS

NOTICE

This application note has been produced and edited using information that was available at the time of publication. This application note is subject to revision without prior notice.

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For over a century, professionals around the world have chosen the J.T.Baker® brand for quality and performance they can trust. J.T.Baker products consistently meet the needs of the most demanding applications, and their premium, high-performance syringe filters are no exception, providing efficient filtration with minimal extractables to maximize sample purity.

Every batch is delivered with the assurance of a chromatogram and a certificate of quality and is rigorously tested for burst pressure of housing, bubble point, flow rate performance and extractables.

These syringe filters are specifically designed for chromatography sample preparation applications and are optimized to provide the most consistent results with minimal extractables.

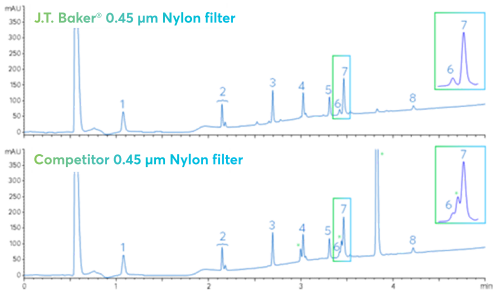

FIGURE 1: Comparison of chromatograms obtained from filtering a sample of valsartan spiked with eight nitrosamines with J.T.Baker® and competitor syringe filters.

Enhanced LC sample preparation with J.T.Baker® high performance syringe filters

J.T.Baker®

PFAS detection on Thermo Scientific™ Accucore™ RP-MS columns and SureSTART™ vials for EPA 8327

PFAS compounds are detected in different water matrices at both low and high spike concentrations with recoveries within the range required. All spiked water samples, in a variety of matrices, showed RSDs below 20% for most of the PFAS compounds, demonstrating the high robustness and reproducibility of the method. Thermo Scientific's Accucore RP-MS columns provide excellent chromatographic separation and maintain robustness in challenging water matrices. Thermo Scientific SureSTART polypropylene vials are recommended when analysing polar compounds/analytes, and dimensionally-verified/inspected by automated optical and gauges to ensure critical dimensions and tolerances.

Thermo Scientific PFAS workflow solution

Descrption	Cat. No.
Accucore RP-MS column: 80 Å 2,6 µm, 2,1 mm, 100 mm	554-1885
Accucore RP-MS guard cartridge: 80 Å, 2,6 µm, 2,10 mm, 10 mm	554-1945
Hypersil GOLD™ C18 selectivity column: 175 Å, 3 µm, 2,10 mm, 50 mm	554-4287
SureSTART polypropylene 2 ml micro vial screw top vial for <2 ml samples, performance level 1	HYPE6ESV9-1PP
Thermo Scientific 9 mm autosampler vial Screw, clear cap with integral polypropylene membrane	NSCAC5000-50

This workflow displays the newest recommended products

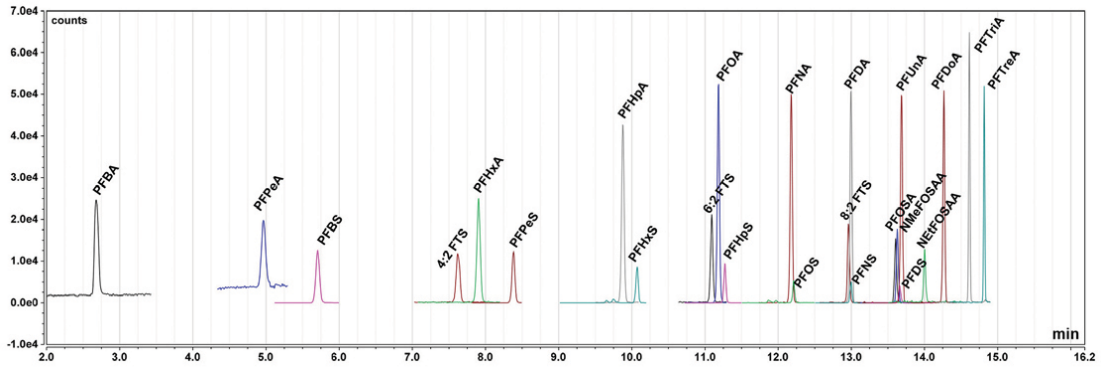

FIGURE 1. Overlaid chromatograms of all PFAS compounds included in this method. Direct analysis of selected PFAS in ground, surface and wastewater by LC-MS/MS.

PESTINORM® SUPRA TRACE solvents for pesticide and organic substance trace analysis

In recent years, the established regulations regarding maximum residue levels in commodities, (mainly for fruits and vegetables) have become more and more stringent.

The European Union (EU) has set new Directives for pesticides at low levels in vegetables in order to meet these health concerns. For example, new laws have increased the standards for human health, workers and environmental protection.

But of course, organic substances and pesticide residue are controlled in a lot of other different areas such as the environment, industry, pharmaceuticals or cosmetic companies.

Read more

As one of the world leaders in high purity solvents, we need to offer a complete range of products for every gas chromatography application in the laboratory, including, for example, highly sensitive pesticide, PAH, PCB, Furans and dioxin analyses.

PESTINORM SUPRA TRACE solvent qualities are ideal for all gas chromatography laboratory applications, such as highly sensitive pesticide and dioxin analysis. To ensure 'cutting edge' performance, we manufacture these solvents within special distillation cuts using the latest production processes. Only highly enriched solvents are used for suitability tests with various detection methods.

PESTINORM SUPRA TRACE – A UNIVERSAL SOLVENT FOR EVERY APPLICATION

These solvents are equally suited to the determination of components in the medium and high boiling range, even in the low boiling range. Our customers just need one solvent quality – independent of the sample (e.g., water or soil) and independent of the detection method (GC-ECD, GC-FID, GC-MS).

Our **PESTINORM SUPRA TRACE solvents** are designed for challenging sample preparation tasks in gas chromatography such as sensitive detection processes in residue and environmental analysis. Our range is a 'universal' solution.

Our specifications are even higher than our classical PESTINORM® range: The specified retention time range for ECD is larger (so even low boiling substances can be reliably detected), while the permissible concentration of interference signals within the retention time range for all detectors is lower. These high purity solvents can be used with the most relevant GC detectors (GC-ECD, GC-FID and GC-MS). Few other GC solvents on the market can give this advantage.

Using **PESTINORM SUPRA TRACE solvents**, you need only one solvent quality regarding samples (e.g., water or soil) or detection methods. Your advantages are more flexibility, better cost efficiency and high reliability.

Benefits

- Very high purity (>99,9%)
- Very low content on evaporation residues (<3 ppm)
- High reliability
- Bottled under inert gas and filtered at 0,2 µm
- One universal solvent for all samples and sensitive detection methods

Some GC-MS applications

- Dioxins and furans (PCDD/PCDF) in meat, fish and milk
- Polycyclic aromatic hydrocarbons (PAH) in vegetables, olive oil and broiled meat
- Pesticide analysis in fruits and vegetables
- Determination of drugs (cocaine, cannabis, ecstasy, heroine and alcohol) in human hair
- Analysis of phthalates in childcare products and toys

PESTINORM SUPRA TRACE solvents in 2,5 L packs

Descrption	Cat. No.
Acetone	85384.320
Cyclohexane	85385.320
Dichloromethane	85386.320
Methanol	85394.320
Ethyl acetate	85387.320
n-Pentane 99,5%	85852.320
n-Heptane 99%	85388.320
n-Hexane 95%	85389.320
n-Hexane 99%	85390.320
2 -Propanol	85391.320
Toluene	85393.320
2,2,4 Trimethylpentane	85801.320

Water is the most basic, and yet the most critical component of any experiment in your workflow - get familiar with the showstoppers and how to avoid them

These are the 'enemies' to reliable results in your workflow.

SUSPENDED PARTICLES, COLLOIDS & INORGANIC IONS

Particles 1 to 10 µm in size (sand, silt etc) can interfere with instrument operation, plug valves and other narrow flow paths as well as foul reverse osmosis membranes. Slightly smaller (0,01 to 1,0 µm) colloidal particles typically have a slightly net negative charge and clog filters, interfere with instrument operation, foul reverse osmosis membranes and can bypass ion exchange resins, resulting in lower resistivity in deionised water systems. Cations and anions adversely affect the results of inorganic analyses such as IC, AA, ICP/MS and may retard cell and tissue growth in biological research. They can also affect cartridge life in deionised water systems.

DISSOLVED GASES & ORGANICS

Water naturally contains dissolved gases that can alter its pH. While oxygen, the most common non ionised gas may cause corrosion of metal surfaces. Organics foul ion exchange resins and interfere with organic analyses including HPLC, gas chromatography and fluoroscopy, as well as hindering electrophoresis, tissue and cell culture. Typical organic impurities in tap water include proteins, pesticides residues, and more and more hormones as wastewater plants are unable to remove them.

TABLE 2: Efficiency of different methods at removing contamination from water.

	Reverse osmosis	Deionisation	Electro deionisation	Ultrafiltration (UF)	Ultraviolet oxidation (UV)	Combination UV/UF
Inorganic ions	++	+++	+++	+	+	+
Dissolved gases	+	+++	+++	+	+	+
Organics	++	+	+	+	+++	+++
Particles	+++	+	+	+++	+	+++
Bacteria	+++	+	+	+++	+++	+++
Pyrogens	+++	+	+	+++	+	+++
Nucleases	+	+	+	++	+	+++

Key: +++ Excellent, ++ Good, + Poor

VWR water purification systems can help you save time and costs so that the user can continue to concentrate on their daily research and development of innovative products. Ultrapure water provided meets or exceeds ASTM, CLSI, CAP and ISO Type I water standards, respectively.

BIOLOGICALS

Although chlorination eliminates harmful bacteria, tap water still contains live microorganisms along with pyrogens, viruses and nucleases that interfere with sterile applications, such as cell and tissue culture, and can have a devastating impact on many life science research protocols.

Concrete examples for poor performance in HPLC/LC-MS: Contaminants will have an effect on performance, from low column efficiency; decrease in resolution and peak tailing; ghost peaks and long-term troubleshooting including silicas from bottled purified water, can result in blockage of column preparing your mobile phase in HPLC and LC-MS.

Another application-driven issue are organics which may interfere with biochemical analysis – for instance, humic acids are known to affect PCR tests. Bacteria present in water used to produce culture media or buffers for cell culture will eat up the nutrients designed for cell growth which may result in less growth rates.

WHAT CAN YOU DO ABOUT IT?

Purification methods

To produce pure and ultrapure water, impurities need to be efficiently and effectively removed. Water purification systems employ multiple technologies, some synergistically, to remove impurities giving you consistently pure water. Water purification is a step-by-step process often requiring a combination of technologies.

Table 2 shows that the choice of methodology used depends on the application the water is being used for.

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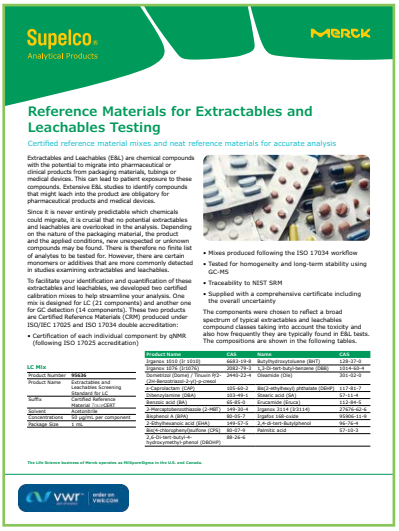
Extractables study with LC-UV/MS

Tim Mueller, Scientist Liquid Chromatography-Mass Spectrometry.
Marc Gemeinder, Project Manager for Extractables and Leachables Studies;
Saskia Haehn, Manager Extractables and Leachables Laboratory.
Matthias Nold, Product Manager Reference Materials.

Single-Use Systems (SUS) made of polymers are commonly used components in the manufacturing or handling of drugs. This direct contact can lead to the contamination of the drug by leaching of the polymeric material components into the product.

Single-use equipment



Read more

To manage this risk, it is crucial to understand the compounds that might potentially migrate from a material (extractable study) and also the quantities at which such a migration is occurring under certain conditions (leachable study).

As described in the BPOG (BioPhorum Operations Group) guidelines[1] and USP <665> guidelines for polymeric components and systems[2] (draft version), investigations regarding extractables should be performed using various solvents and incubation times with the analysis done using a variety of analytical methods applied to the extracts.

A well-suited method to analyse non volatile extractables such as additives, impurities, polymer components, or degradation products is Liquid Chromatography-UltraViolet Spectroscopy/Mass Spectrometry (LC-UV/MS). UV and MS are chosen to detect a wide range of extractables. As generally the exact composition of the polymeric material is unknown, a non targeted analysis is required that involves the detection and identification of any potential extractable.

To facilitate this type of analysis, we have developed a CRM mixture for 21 extractables typically found in LC-UV/MS studies. This CRM mix is not only helpful for a quick identification of unknown extractables but can also be used for quantification with traceability to a NIST SRM. Since the mix contains a wide variety of substance classes, it is also suitable to check the analytical method to reduce the risk of overlooking potential extractables. The 21 compounds in the mix are listed in Table 2. The corresponding single component reference materials are shown under 'Related Products' below.

Leachables: Chemical compounds that migrate into a drug formulation from any product contact material (e.g., single-use systems) because of direct contact under a typical process or storage conditions; leachables may affect the toxicity or efficiency of the drug product.

Extractables: Chemical compounds that are extracted from any product contact material usually under extreme conditions (harsh solvents, exaggerated time and temperature); an extractables profile represents a worst case leachables profile.

Single-Use Systems (SUS): Usually polymeric, disposable equipment for bioprocessing used in the manufacturing of pharmaceuticals.

- **Advantages:** Flexibility, no need for cleaning validation, low investment, no cross-contamination
- **Examples of SUS:** Bioreactors, disposable filters or tubing

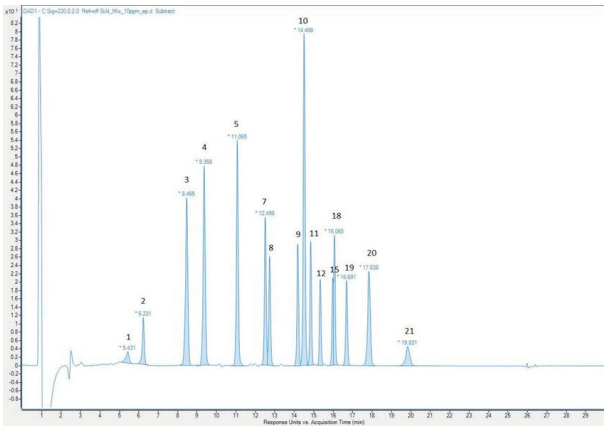


FIGURE 1: Extractables and Leachables Screening Standard for LC, UV (220 nm), 10 mg/L in acetonitrile.

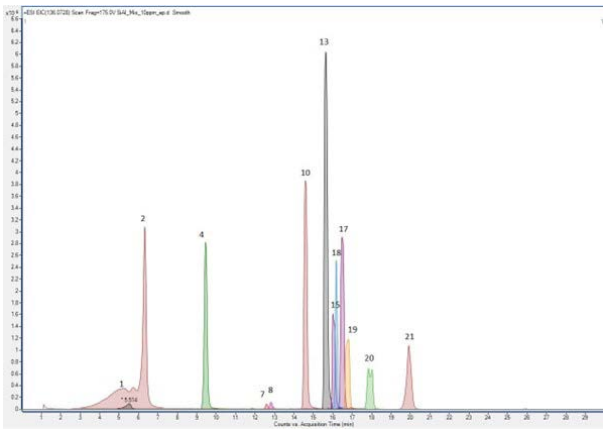


FIGURE 2: Extractables and Leachables Screening Standard for LC, ESI positive, 10 mg/L in acetonitrile.

In the following, an application is described using the Extractables and Leachables Screening Standard for LC mix, for the identification and quantification of the main extractables within an extractable study of a filter.

LC METHOD FOR EXTRACTABLES TESTING

The applied instrument parameters for an extractable study on single-use equipment (filter) are summarised in Table 1. According to the BPOG protocol[1], the separation was performed on a C18 column (Ascentis® C18 column: 15 cm x 2,1 mm, 3 µm). A representative sample was taken after 24 hours of extraction at 40 °C under orbital rotation with 50% ethanol. The sample and the standard mix were run in one sequence.

Instrument	Agilent Infinity II, QToF 6546		
Column	Ascentis® C18, 15 cm x 2,1 mm, 3 µm (581302-U)		
Mobile phases	[A] water; [B] methanol		
Gradient	**Time (min)**	**%A**	**%B**
	0	100	0
	1	100	0
	15	0	100
	25	0	100
	25,1	100	0
	30	100	0
Flow	0,5 ml/min		
Column temperature	40 °C		
Detector	DAD, 191 – 400 nm; MSD, full scan, m/z 75 - 1500		
Injection	5 µl		
Samples	1. Extract of a single-use filter (24 h extraction with 50% ethanol)		

TABLE 1: Experimental conditions.

RESULTS & DISCUSSION

The chromatograms of the Extractables and Leachables Screening Standard for LC are shown in Figures 1 to 3 (UV, ESI pos, ESI neg, peak IDs in Table 2). All 21 reference compounds were detected by the combination of UV-MS detector with almost complete separation. 16 reference compounds could be detected with UV (220 nm), 13 reference compounds with ESI positive, and 14 reference compounds with ESI negative ionisation. By matching of retention time and m/z ratio, Pentaerythritol tetrakis(3,5-di-tert- butyl-4-hydroxyhydrocinnamate) (Irganox 1010) was identified as the main extractable during the extraction of the single-use filter (Figure 4). A quantitative analysis against the Pentaerythritol tetrakis(3,5-ditert-butyl-4-hydroxyhydrocinnamate) (Irganox 1010) peak within the Extractables and Leachables Screening Standard for LC could be performed based on UV (220 nm), ESI positive or ESI negative chromatograms.

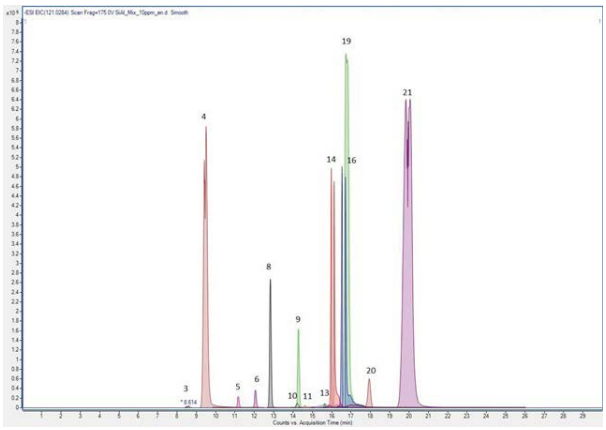


FIGURE 3: Extractables and Leachables Screening Standard for LC, ESI negative, 10 mg/L in acetonitrile.

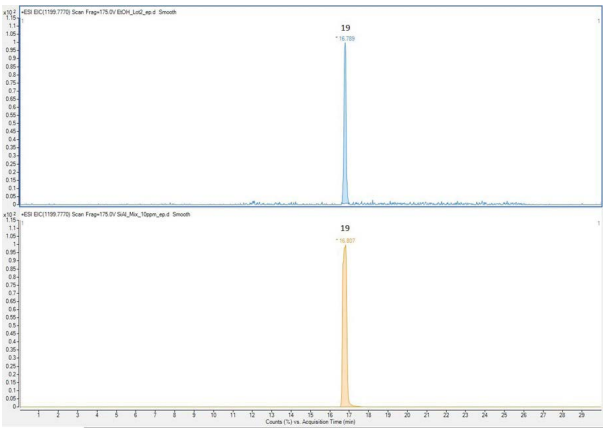


FIGURE 4: Representative sample of a single-use equipment extraction. Top: Extract sample of single-use filter with 50% ethanol, bottom: Extractables and Leachables Screening Standard for LC.

Peak	Compound	CAS	Molecular formula	m/z	UV absorption at 220 nm	Most prominent adducts		RT [min]
						ESI pos	ESI neg	
1	ε-Caprolactam	105-60-2	$C_6H_{11}NO$	136,0728	Very weak	[M+H]+	-	5,43
2	Oleamide	301-02-0	$C_{18}H_{35}NO$	282,2795 280,2643	Very weak	[M+H]+	- [M-H]-	15,6
3	Erucamide	112-84-5	$C_{22}H_{43}NO$	338,3417	Very weak	[M+H]+	-	16,5
4	Dibenzylamine	103-49-1	$C_{14}H_{15}N$	198,1279	Yes	[M+H]+	-	6,23
5	Benzoic acid	65-85-0	$C_7H_6O_2$	121,0284	Yes	-	[M-H]-	8,48
6	2-Mercaptobenzothiazole (2-MBT)	149-30-4	$C_7H_5NS_2$	167,9931 165,9788	Yes	[M+H]+	 [M-H]-	9,36
7	Bisphenol A (BPA)	80-05-7	$C_{15}H_{16}O_2$	227,1077	Yes	-	[M-H]-	11,1
8	Bis(4-chlorophenyl) sulfone	80-07-9	$C_{12}H_8Cl_2O_2S$	308,9519	Yes	[M+H]+	-	12,5
9	3,5-Di-tert-butyl-4-hydroxybenzyl alcohol	88-26-6	$C_{15}H_{24}O_2$	259,1672 235,1705	Yes	[M+Na]+	 [M-H]-	12,7
10	2,4-Di-tert-butylphenol	96-76-4	$C_{14}H_{22}O$	205,1597	Yes	-	[M-H]-	14,2
11	2-(2-Hydroxy-5-methylphenyl) benzotriazole	2440-22-4	$C_{13}H_{11}N_3O$	226,0972 224,0826	Yes	[M+H]+	 [M-H]-	14,5
12	Butylhydroxytoluene (BHT)	128-37-0	$C_{15}H_{24}O$	219,1754	Yes	-	[M-H]-	14,9
13	1,3-Di-tert-butylbenzene	1014-60-4	$C_{14}H_{22}$	-	Yes	-	-	15,3
14	Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	$C_{24}H_{38}O_4$	391,2839	Yes	[M+H]+	-	16,0
15	Tris(3,5-di-tert-butyl-4-hydroxybenzyl) isocyanurate	27676-62-6	$C_{48}H_{69}N_3O_6$	806,5084	Yes	[M+Na]+	-	16,1
16	Pentaerythritol tetrakis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate)	6683-19-8	$C_{73}H_{108}O_{12}$	1199,7770 1175,7726	Yes	[M+Na]+	 [M-H]-	16,7
17	Tris(2,4-di-tert-butylphenyl) phosphate	95906-11-9	$C_{42}H_{63}O_4P$	685,4365 661,4378	Yes	[M+Na]+	 [M-H]-	17,8
18	Octadecyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate	2082-79-3	$C_{35}H_{62}O_3$	553,4597 529,4624	Yes	[M+Na]+	 [M-H]-	19,8
19	Palmitic acid	57-10-3	$C_{16}H_{32}O_2$	255,2331	No	-	[M-H]-	16,1
20	2-Ethylhexanoic acid	149-57-5	$C_8H_{16}O_2$	143,1082	No	-	[M-H]-	12,1
21	Stearic acid	57-11-4	$C_{18}H_{36}O_2$	283,2644	No	-	[M-H]-	16,5

TABLE 2: Peak IDs, selected ions and retention times of compounds.

CONCLUSION

The example shown demonstrates the applicability and value of the Extractables and Leachables Screening Standard in LC analysis for repetitive wording since used in next sentence. Analysis of the most common extractables resulting from single-use equipment. The shown LC-UV/MS method using an Ascentis® C18 column provided a reliable identification and quantification of the 21 components in the mix.

References

1. Ding et al. Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing, Pharmaceutical Engineering,(2017). https://ispe.org/pharmaceutical-engineering/ispeak/standardized-extractables-testing-protocol-single-use-systems-0
2. USP <665> Plastic Materials, Components and Systems Used in the Manufacturing of Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products, third draft, published on March 1, 2019 by the USP.

FEATURED PRODUCTS

Description	Cat. No.
Extractables and Leachables Screening Standard for LC, certified reference material, 50 µg/ml per component, 1 ml or 5 ml	SUPL95636-1ML
Ascentis® C18, 15 cm x 2,1 mm, 3 µm	SUPL581302-U

RELATED PRODUCTS

Description	Pk	Cat. No.
Extractables and Leachables Screening Standard for GC, certified reference material, 50 µg/ml per compound in tert-butyl methyl ether	1 ml or 5 ml	SUPL01829-1ML

Description	Pk	Cat. No.
Single component Certified Reference Materials		
Benzoic acid	1 g	PHR1050-1G
Bis(2-ethylhexyl) phthalate	100 mg	67261-100MG
Bis(4-chlorophenyl)sulfone	100 mg	CRM96153-100MG
Bisphenol A	100 mg	42088-100MG
ε-Caprolactam	100 mg	CRM01483-100MG
Dibenzylamine (DBA)	100 mg	CRM95728-100MG
3,5-Di-tert-4-butyl-hydroxytoluene (BHT)	100 mg	CRM96857-100MG
1,3-Di-tert-butylbenzene	100 mg	CRM96659-100MG
2,4-Di-tert-butylphenol	100 mg	CRM00437-100MG
cis-13-Docosenoamide (Erucamide)	100 mg	CRM01374-100MG
2-Ethylhexanoic acid	3x1,2 ml	PHR19143X1.2ML
2-(2-Hydroxy-5-methylphenyl) benzotriazole (Drometrizole)	100 mg	CRM96697-100MG
2-Mercaptobenzothiazol	100 mg	CRM96051-100MG
Octadecyl-3-(3,5-di-tert-butyl-4- hydroxyphenyl)propionate (Irganox 1076)	100 mg	CRM00318-100MG
Palmitic acid	1 g	PHR1120-1G
Pentaerythritol tetrakis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate) (Irganox 1010)	100 mg	CRM96656-100MG
Stearic acid	1 g	PHR1114-1G
Tris(2,4-di-tert-butylphenyl)phosphate (Irgafos 168-oxide)	100 mg	CRM96839-100MG
Tris(3,5-di-tert-butyl-4-hydroxybenzyl) isocyanurate (Irganox 3114)	100 mg	CRM96737-100MG
Solvents		
Water for Chromatography (LC-MS grade) Lichrosolv® (or water tap fresh from a Milli-Q® IQ ultrapure water system)		1.15333.2500
Methanol, gradient grade for liquid chromatography LiChrosolv® Reag. Ph. Eur.		1.06007.2500

Exhaust filters in HPLC
Fact checking

Dominik Werner, BOHLENDER GmbH

In many labs, the collection of inflammable solvents (e.g., in waste containers of HPLC systems) presents a permanent threat of escaping hazardous vapours. By using high quality closing systems (e.g., b.safe waste caps), these sources of emission can be effectively closed.

To ensure pressure compensation when liquids are filled into containers, most systems are equipped with exhaust filters.

Ideally, suitable filling materials eliminate solvent vapours in containers. Exhaust filters are consumables and, therefore, it is no surprise that each supplier pays the highest attention to these systems.

Unfortunately, many half-truths circulate around exhaust filters and their function, often mixed with advertising slogans and even false statements.

Therefore, we would like to explain mandatory and possible functions of exhaust filters and the way we calculate their service.

WHAT ESCAPES FROM HPLC WASTE CONTAINERS?

- Vapours from hazardous organic solvents
- No acids in relevant quantities as they are bound in the buffer system
- No alkalis in relevant quantities as they are also bound in the buffer system
- Buffer solution does not escape because buffers are saline solutions that do not become gaseous!
- Sometimes traces of acetic acid and ammonia as well as special amines escape in the form of an unpleasant smell

WHICH UNPREDICTABLE FACTORS CAN INFLUENCE SERVICE LIFE?

- Extreme temperature variations in the lab
- Using an unpredictable variety of solvents
- Actual mixing ratio of different solvents in the canister
- Leaking systems

WHAT IS THE ADVANTAGE OF SERVICE LIFE CALCULATIONS COMPARED TO EXPERIMENTAL MEASUREMENT SET-UP?

- Activated carbon filters are highly complex systems, and with corresponding background knowledge, you can make reliable statements about their service life
- Any number of scenarios of different application conditions can be calculated and the corresponding service life can be checked
- We always calculate the worst case scenario and consider safety factors of all variables at the same time. Therefore, calculated service lives are more reliable than the collection of measurement data

HOW CAN YOU HOLD BACK SUBSTANCE SAFELY?

- Granulated activated carbon with suitable pore size and activation degree effectively adsorbs organic solvents
- To some extent, activated carbon also adsorbs substances that are not hazardous but have unpleasant smells (traces of acetic acid and ammonia

as well as special amines) unpleasant smells (traces of acetic acid and ammonia as well as special amines)

IS 'SPECIAL' CARBON OR 'HIGH PERFORMANCE' CARBON NECESSARY?

- Exhaust filters have to absorb highly saturated solvent mixtures. 'Normal' activated carbon with corresponding features can easily absorb them
- Using more and more specific carbons or carbons that are unnecessarily refined make filters more expensive and do not improve their efficiency. At worst, exchanging active carbon by other adsorbents can significantly reduce the total capacity of the filter

HOW DO WE CALCULATE THE SERVICE LIFE OF OUR FILTERS?

- Calculations are based on absolutely gas-tight systems (b.safe waste caps)
- We consider the filling quantities of the filter media we know
- Service life calculations are only valid for one pure solvent (no mixtures)
- Adsorption isotherms of the individual solvents are evaluated
- We consider typical flow rates that are currently used in analytic HPLC
- Full flow rate in usual operating hours per day and operating days per week
- As usual with safety products, a safety factor is included for all variables
- To cover all applications, we give praxis-oriented change intervals (every 3, 6 and 12 months) which include a safety reserve if a filter change has been forgotten or was not possible for logistic reasons

WHICH PREDICTABLE FACTORS CAN INFLUENCE SERVICE LIFE?

- Ambient conditions: Temperature, sea level and air

Restrictions

- In case you have severe deviations from the variables we mention (e.g., 24/7 continuous operation or significantly higher flow rates, several systems per waste container, semi or fully preparative HPLC) you should review your system and perhaps reduce service lives. Please contact us for any assistance
- Activated carbon filters can absorb hexane vapours only in small amounts because hexane has a high saturation concentration and activated carbon can adsorb hexane relatively poor. Heptane, which is often used as an alternative, also causes slightly reduced service lives. If your solvent mixture consists of a high percentage of hexane/ heptane, please contact us

We recommend

Filter size S (Cat. No. 590-0176): **Service life 3 months**
Filter size M (Cat. No. BOHLM506-02): **Service life 6 months**
Filter size L (Cat. No. 590-0178): **Service life 12 months**

There is no mystery around exhaust filters because the function they must fulfil is technically quite simple. Their main purpose is to remove solvent particles from air, and this has to be fulfilled reliably. As filters are consumables, their efficiency is their focus.

We at b.safe supply laboratories supply high quality filters at fair prices, directly from the manufacturer.

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