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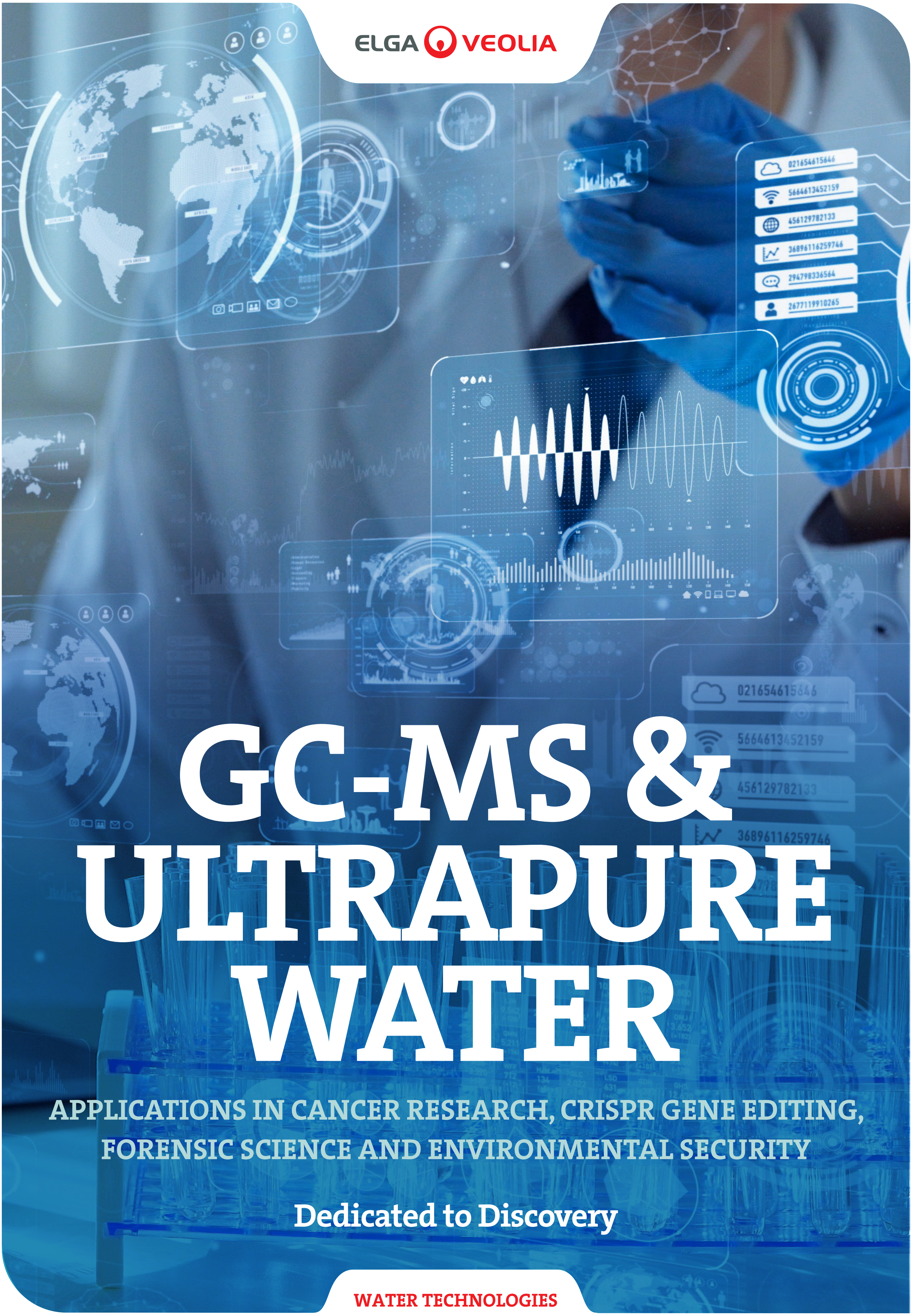


GC-MS & ULTRAPURE WATER

APPLICATIONS IN CANCER RESEARCH, CRISPR GENE EDITING, FORENSIC SCIENCE AND ENVIRONMENTAL SECURITY

Dedicated to Discovery

WATER TECHNOLOGIES

Inside

INTRODUCTION

Ultrapure water in GC-MS analyses

GC-MS (gas chromatography coupled with mass spectrometric analysis) is one of the most widely used analytical methods of all. It is used to determine the identity and concentration of different inorganic, organic and bio-organic substrates, especially volatile organic compounds (VOCs) and semi-volatile organic compounds (sVOCs).

As the name suggests, gas chromatography analyses compounds which can be transformed into a gas, i.e., vaporized – although it is sufficient if only a small portion of a compound is transferred into the gas phase. Coupling to mass spectrometry has the additional benefit of determining the mass of each GC fraction, which allows more precise statements about the molecular structure of a compound.

GC-MS is a powerful method that emerged in the late 1950's and has been continuously improved since that time.[1] Today, GC-MS is often used to separate complex mixtures of substances but can be used to quantify the concentration of different substances in a sample, even for trace amounts.

Although GC-MS is very versatile, it is sometimes eclipsed by a very similar method: HPLC-MS. This method offers some advantages over GC-MS, in particular the ability to separate and analyze larger molecules that would not withstand the temperatures required for evaporation in a GC column. However, GC-MS also has some of advantages over HPLC-MS:

- **Capillary GC have a higher resolving power and speed compared to HPLC. Therefore, GC is preferred (if possible) over HPLC for the analysis of complex samples.**
- **GC retention is based on volatility as opposed to polarity in HPLC. Therefore, GC is able to separate mixtures that HPLC cannot separate.**
- **There are no problems with the selection of different solvents or gradients, there is usually just one type of carrier gas.**
- **Column blockage is much rarer with GC.**

Since only light substances are volatile enough for GC, it is mainly used for the analysis of small (< 500 Da) molecules. Substance classes that are frequently analyzed with GC-MS are steroids, fatty acids and hormones due to their low polarity and therefore low boiling point.

In some cases, even such light molecules are not volatile enough to be used in GC, for example amino acids. However, there are strategies to convert such compounds into molecules that can be used in GC. This is usually achieved by derivatization with certain chemical derivatization reagents (e.g., ethyl chloroformate).

Compared to HPLC, relatively small amounts of water are used in GC, since the mobile phase for the separation is usually helium. It is, however, often involved in sample preparation and can have great influence on the results in GC and MS.

How water can affect your GC-MS results

ONLY USE ULTRAPURE TYPE I WATER TO GET IT RIGHT

Water is often used as the solvent of choice for the analysis of samples because it is non-toxic, inflammable and yet easily evaporated.

There are different types of GC, which differ mainly in how the sample is treated before being injected onto the GC column. A popular method is the so-called headspace GC, in which the gaseous headspace is analyzed over a liquid matrix. In many cases, this liquid matrix is water. Blanks and standard samples may then also be prepared with water. Any volatile impurities in the aqueous matrix can strongly influence the data quality of the GC-MS, since it is a very sensitive method capable of detecting substances even at low ppb concentrations. However, water impurities can also play a crucial role in GC types other than headspace GC, depending on the sample and its preparation method.

Since GC-MS is an extremely sensitive technique and places high demands on sample preparation methods, the solvents used must have a low total organic carbon (TOC) content. Organic compounds, especially volatile ones, contained in water used in sample preparation for GC-MS cause an increase in background noise, which leads to an increase in the sensitivity and selectivity of the corresponding method and to undesired or enlarged peaks.

As the graph illustrates, even the use of type II purified water (which has a TOC of < 50 ppb) has a significant negative impact on GC-MS results. Only the use of ultrapure type I water shows impurity levels below the detection limits of GC-MS analysis. It is therefore recommended to use only water with the highest purity standards for GC-MS.

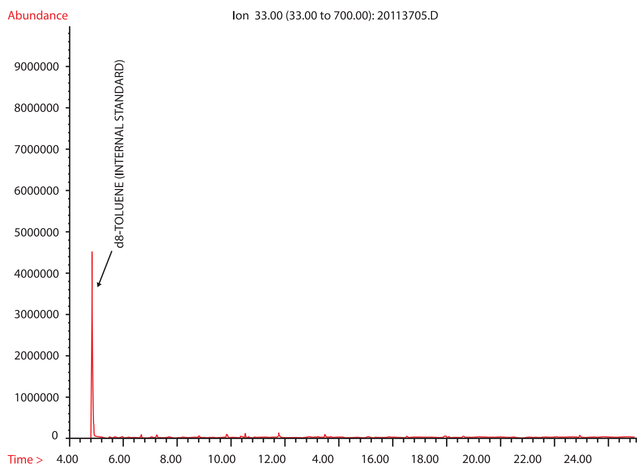


Figure 1: Analysis of SVOCs by GC-MS. Chromatogram of type I ultrapure water (ELGA's PURELAB Ultra Analytic)

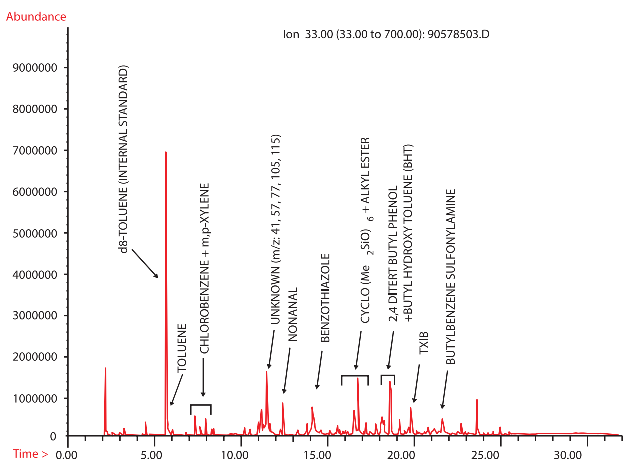


Figure 2: Analysis of SVOCs by GC-MS. Chromatogram of type II pure water (ELGA's PURELAB Pulse)

GC-MS can detect metabolic traces of cancer

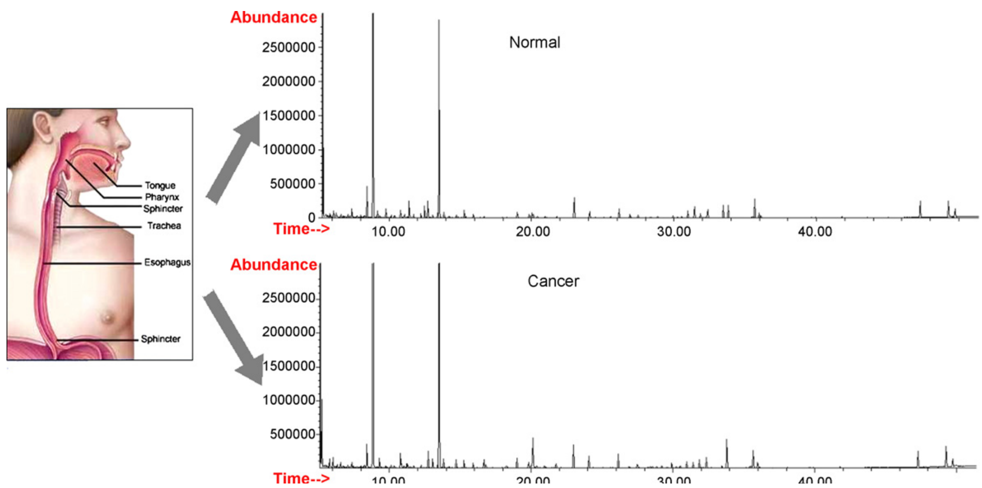
VOLATILE ORGANIC COMPOUNDS ARE KEY TO DIAGNOSIS

It has been discovered that there is a connection between cancer and certain volatile organic compounds (VOC) in the air breathed out by the patient.

These VOCs can be analyzed and quantified using GC-MS in the diagnostic process. In a particular study, VOCs in the respiratory air of patients with respiratory cancer were examined in comparison to patients with confirmed gastric cancer and a healthy control group.[2] The analysis showed that gas chromatography was indeed able to detect significant differences in VOC levels between these groups, suggesting that GC-MS can indeed be used to diagnose cancer. Similar studies have been conducted with esophageal cancer tissue, which also suggests that GC-MS may be used to diagnose cancer.[3]

In such scientific studies, the challenge of finding specifically elevated VOC biomarkers in cancer patients is quite high. Examples of these VOCs are cyclopentanone, 3-methylpyridine and ethylene carbonate.[4] The naturally occurring concentrations of these and other VOCs are quite low and the presence of cancer can only be detected by small changes in the levels of these markers, even if cancer is present. For this reason, it is essential that no impurities from contaminated water or other sources are introduced into the samples and blanks. It would be very difficult to obtain statistically significant results with contaminated samples.

Samples contaminated with bacteria or their waste products in particular pose a major problem, as many key metabolites are produced by both bacteria and humans. If highest purity of the water used for GC-MS cannot be guaranteed, the results of a metabolic GC analysis to detect cancer are never reliable. Corrupted samples may lead to wrong diagnoses and non-reproducible results, which in turn can lead to massive costs. It is therefore essential to maintain the integrity of all samples prior to testing by only adding the purest available solvents, especially water.



GC elution fractions from cancer patients and healthy controls.[3]

CRISPR gene editing and GC-MS

ANALYZE METABOLITES AFFECTED BY GENETIC ENGINEERING

In recent years, new techniques for the production of genetically modified organisms have attracted considerable attention in the scientific community and even in the general public.

The most remarkable of these techniques is the CRISPR/CAS method. It is based on a natural viral defense system of bacteria, which is able to excise viral DNA sequences from the DNA of a bacterium after it has been infected by the virus. This interrupts the natural replication cycle of the virus. The core of the bacterial defense mechanism is a special endonuclease called Cas9 and an RNA copy of the viral DNA. The best thing about this method is that it is very accurate and programmable.

Therefore, this bacterial defense system can easily be adapted to obtain a highly effective gene editing tool that is superior to other methods to enable the creation of transgenic organisms.

In many cases, gene editing is used to modify plants or bacteria to produce certain industrially useful chemicals in high quantities by reprogramming their natural biochemical machinery or to produce other kinds of useful crops or even animals. One such example is the creation of a new nicotine-free tobacco plant by removing a gene with CRISPR-Cas9.[4]

The success of the gene editing was confirmed by the absence of nicotine in a GC-MS analysis. In fact, GC-MS is well-suited to analyze many compounds of interest and key metabolites affected by genetic engineering.

Another example is the modification of the genome of E. coli to produce more and other types of fatty acids which the bacteria would normally not be able to do. As in the case of nicotine, the fatty acid composition was determined by GC-MS.[5]
In many other studies, volatile compounds responsible for the taste or smell of food are the target of genetic engineering experiments, for which analysis by GC-MS is perfectly suited.

Samples obtained from biological material naturally contain water. In addition, many methods for samples preparation use aqueous solutions or rinsing and washing of samples with water. The use of contaminated water endangers the integrity of all samples, can lead to inconclusive results and generally decreases the quality of the results.

GC-MS in forensic science

DETECTING NARCOTICS AND DRUGS

GC-MS is also frequently used in forensic science, mainly because it is able to quickly identify narcotics (such as barbiturates), illegal drugs (THC, GHB), poisons, alcohol and other compounds of interest for forensic analyses.

It enables quantitative analysis and a precise understanding of how much of a particular substance was present in a sample from suspects or victims. Results must be highly accurate in order to be used as evidence in court, which is why GC-MS is perfectly suited for this task. In addition, GC-MS can even be used in other areas of forensics, for example in the age determination of fingerprints.[6]

The challenge for scientists working in this field is to establish an efficient workflow and to ensure that even the smallest quantities of suspicious substances are detected.

In forensic science it is only natural that samples are taken from body fluids such as blood or pus, which are aqueous solutions. However, water or other aqueous solutions are also required for sample preparation in many other cases, for example for the determination of certain drugs in hair samples.[7] Ultrapure water can help forensic scientists by suppressing the noise of contaminations in the sample, thereby increasing the detecting limits for the other compounds. Therefore, the integrity and purity of this water is of crucial importance; the results may or may not lead to the resolution of a criminal case.

The role of GC-MS in environmental security

QUANTIFICATION OF CHEMICAL POLLUTANT LEVELS

The well-being and conservation of our environment is proving to be a growing challenge in industrialized nations.

Many efforts have been made to restore previously damaged ecosystems such as forests or rivers to their original state after they suffered from pollution that led to the temporary disappearance of various species. Scientists therefore routinely measure the level of contamination in environmental samples to determine the levels of chemical pollutants and their decomposition products.

For this purpose, water, soil and air are routinely analyzed for VOCs and SVOCs, usually by GC or GC-MS. This also includes a variety of crops and other foodstuff intended for human consumption to detect dangerous levels of toxic substances.[8] Additionally, Py-GC-MS (pyrolysis followed by analysis by GC-MS) can also be used for the analysis of micro plastics, which is hardly detectable with other analytic methods.[9]

As in forensic science, water is an important substance used in the preparation of environmental samples, and in the preparation of standards and blanks, especially for samples from drinking water, lakes, rivers or the sea. This measurement from aqueous environmental samples poses a unique challenge: The contaminations found in these samples (e.g., by plasticizers) may belong to the same substance group or even be identical to those compounds that could be introduced by impure water for rinsing, cleaning of equipment, sample preparation, etc. This can easily lead to false positive results if the measurement process is not carefully controlled. Using ultrapure water is therefore essential to ensure minimal introduction of impurities.

GC-MS: Analytical superpowers for unlimited applications

CONCLUSION

In addition to these areas, GC-MS is also widely used in other fields such as organic chemistry, detection of doping in sports [10] and the identification of microbes.[11]

As the accuracy of GC-MS and other analytic techniques is constantly increasing due to new technical innovations, so is the demand for representative samples that are free from exogenous contaminations. ELGA specializes in providing water purification systems to meet the highest standards for use in research and development and clinical laboratories.

Ultrapure water for GC-MS

If you make sure to use ultrapure water for your GC-MS systems you'll be well on your way to performing successful analyses for a wide variety of applications, ranging from metabolic trace detection in cancer to CRISPR gene editing to forensics and environmental safety.

To find your nearest ELGA representative, go to elgalabwater.com and select your country.

ELGA GLOBAL OPERATIONS CENTRE
t +44 (0) 203 567 7300
f +44 (0) 203 567 7205

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