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## GC-MS: THE SUPERIOR FORENSIC TOOL



In the wake of the recent dioxin poisoning scare in Europe that sparked import bans on some German farm products, GC-MS is emerging as the perfect analytical tool for forensic analysis. In fact, its simplicity, sensitivity and effectiveness in separating and identifying components, has made it one of the most important tools in analytical chemistry today.

Where other analytical techniques fundamentally determine the quantitative issues arising from analysis of a specific sample – answering the question “how much is present” – gas chromatography-mass spectrometry (GC-MS) is one of a very few techniques able to qualitatively identify the actual nature of chemicals in the sample. It answers the question, “What molecules are present?”

Of these two questions, in many analytical scenarios the “what?” is actually more important than the “how much?” Conducting quantitative work on a sample with unknown content is fruitless. The qualitative method is especially relevant to research applications and lays the correct foundation for the analysis. Only when it is known which chemicals are present, can the quantitative analysis be performed.

The GC principle is that molecules in a sample separate in the chromatography column because of differences in their chemical properties. The MS breaks components into ionised species and separates these based on their mass to charge ratio. This is the great advantage of the combination of GC as the first separation step and the MS as the qualitative detector.

“Amid heightened concerns about food safety in many parts of the world, GC-MS comes to the fore as a very important analytical technique,” says the Linde Group’s Stephen Harrison, Head of Specialty Gases and Specialty Equipment. “It is one of few techniques to determine exactly what is in a food sample. Characterised by its quick screening abilities, GC-MS has been widely heralded as the ‘gold standard’ for forensic substance identification.”

On the same standing with GC-MS, liquid chromatography-mass spectrometry (LC-MS) is also a qualitative analytical chemistry technique that combines the physical separation capabilities of high performance liquid chromatography with the qualitative analysis capabilities of mass spectrometry. Both techniques involve using a mass spectrometry detector, but GC-MS is used to screen a sample using a gaseous phase component separation process in the gas chromatography column, while LC-MS is able to detect and identify chemicals using a liquid phase component separation process in the liquid chromatography column.

The medium in which the sample exists and is most effectively separated in the chromatography column – gaseous or liquid – determines which technique is more appropriate. While many samples can be vaporised for GC-MS, other samples are better dissolved in a suitable solvent and examined using LC-MS. However, GC-MS is preferred when quick screening is required because the column separation is generally faster in the gaseous phase.

GC-MS is commonly used to screen for contaminants in food and drugs and we were reminded of its relevance in Europe recently after elevated levels of dioxins showed up in small amounts of German farm produce. This sophisticated scientific technique, which is routinely used by the Food and Drug Administration in the US to detect dioxin, could have been successfully employed to trace the source to a single fats manufacturer. European Union rules allow no more than a one part per trillion of dioxin in food for human consumption. Over a period of two weeks, batches of eggs and a shipment of pork with 1.5 trillionths were found. The German feed-mixing company supplied 110 egg farms, 651 piggeries and other farms in four German states, federal officials have said.



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Dioxin and chemically-related compounds (referred to collectively as dioxin-like compounds or DLCs) are a group of environmental contaminants found throughout the world. While German officials have said that eaten occasionally, the excess is unlikely to make anyone sick, the European Food Safety Authority suggests that exposure to DLCs may lead to a variety of adverse health effects including reproductive and developmental problems, cardiovascular disease, increased diabetes and increased cancer. Because DLCs tend to accumulate in the fat of food-producing animals, consumption of animal-derived foods, such as meat, poultry, eggs, fish, and dairy products, is considered to be the major route of human exposure to low levels of DLCs.

Harrison said: “Who found out about this dioxin problem and how? Someone was looking – thank goodness people are always looking at these processes, because things do go wrong. How did these analysts know they had found a problem? Using qualitative techniques like GC-MS they would be able to identify the presence of a specific chemical such as dioxin. They can then combine that with a quantitative technique to determine the extent of dioxin contamination. Immediately they realised that they had to track this chemical to its source as quickly as possible to safeguard public health.”

“Applying a qualitative technique in a detective-like fashion in this crisis situation was critical. Samples could be checked right back through the food chain until analysts reached a point where the chemical did not show up in the sample – indicating that this is where it entered.”

Similar scares have occurred in the beverage industry, one of the most notable being the benzene in soft drinks episode of the 1990s.

Benzene levels are regulated in drinking water nationally and internationally, and in bottled water in the United States, but only informally in soft drinks. Benzene in soft drinks is of some concern due to its carcinogenic nature. This contamination is a public health concern and has periodically caused outcry among environmental and health advocates. The benzene results from decarboxylation of the preservative benzoic acid in the presence of ascorbic acid (vitamin C), especially under heat and light.

The worst example found to date was a soft drink containing 87.9 parts per billion of benzene. Someone drinking a 350 ml (12 oz) can would ingest 31 micrograms of benzene. While there is no justification for a soft drink to contain such high levels of benzene, the casual consumption of such a drink is unlikely to pose a significant health hazard to a particular individual. However, spread out over millions of people consuming soft drinks each day, there is likely to be a broad-ranging impact on the number of small cancers caused by this exposure.

On the flip side of legitimate beverage production is the worldwide issue of distilled spirits made in unlicensed stills. As with all distilled spirits, yeast ferments a sugar source to produce ethanol,

then the alcohol is extracted through distillation using a still. Because of its illegal nature, these beverages are rarely aged in barrels like conventional spirits and sometimes contain impurities and off-flavours. On occasion, these beverages may contain toxic alcohols such as methanol, which can cause blindness and death.

Last year 89 people from south-west Uganda died after drinking a home-brewed beverage laced with methanol — and a further 100 were hospitalised, including a two-year-old child. A large number of Ugandans, with little disposable income, resort to drinking cheap, home-made alcoholic brews which often contain dangerous chemicals. In this instance local brewers had made waragi – a gin made from bananas – and added large amounts of methanol to increase the potency of the drink.

“In an ideal world, GC-MS or LC-MS in the hands of food safety officials and forensic policing teams would be able to prove the presence of the toxic molecules, identify intentional or unintentional malpractice and protect the consumers from the potentially lethal effects,” says Harrison.

Alongside the food and beverage industry, the pharmaceutical arena rates drug content safety high on its agenda to avoid contamination, check for appropriate active ingredients and to maintain customers’ trust in pharmaceutical authenticity. Alongside NMR and LC-MS, GC-MS is one of the most appropriate qualitative analytical techniques for this sector, because of its high analysis throughput that speeds up results.

“Doping in top level sporting events is another pharmaceutical scenario where GC-MS is a powerful forensic technique,” says Harrison. “How do we know athletes in a given sport are free of performance enhancing drugs? And how do we know what we’re looking for in this regard? If analysts are looking for specific performance enhancing drugs based on past experience, they can rely on quantitative techniques. However, an equally necessary response would be to run the blood and urine samples through a qualitative technique to see if any new or unexpected molecules are present. This will ensure that official sporting bodies keep abreast of any advances in the underworld of illegal performance enhancement.

“Another benefit of the qualitative route in sport doping cases is that these unbiased results can be presented as critical evidence in a court of law, because they prove exactly what substances were present. To back up this evidence, a quantitative analysis will reveal how much of the substance is present. The combination of qualitative and quantitative analysis is the key here.”

“But the application and benefits of GC-MS go way beyond food, beverage and pharmaceutical safety,” Harrison adds. “This technique can play a significant role in industrial production, analysing the composition of process streams. In industry you often find that even when you’ve successfully done the same things in the same way for a long time, suddenly one day something changes that puts the whole process at risk. An unknown molecule has infiltrated the process stream that wasn’t there before. GC-MS can identify the exact chemical causing the problem and is therefore perfect for troubleshooting in these cases.”

“Granted, this method is generally not required across the full spectrum of industrial processes, but when it comes to research, investigation, innovation, discovery and trouble-shooting, GC-MS can be an absolutely key approach, particularly at the beginning of a new process or during process changes and enhancements.”

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred McLafferty in the USA. These sensitive devices were bulky, fragile and originally limited to laboratory settings. The development of affordable and miniaturised computers and other parts of the instrument have helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyse a sample.

In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas a first-generation GC-MS would have required at least 16 minutes. This has led to their widespread adoption in a number of fields in the past two decades.

“The first gas chromatography mass spectrometer I ever saw filled a large room,” says Harrison. “It was bigger than a dining room table and cost a fortune. It was only one of few of its kind in the entire country where I lived at the time. Today, there is a big contrast. You can put a GC-MS into a small suitcase and carry it to any location on a plant or factory, or even a crime scene. Over the past 20 to 30 years, this technology has moved from being hallowed and rare, to still being hallowed and important, but very commonplace and portable. It’s still a relatively expensive analytical instrument, but nowhere near what it was in the past.”

“As a result of its rapid evolution as an increasingly accessible technique, applications for GC-MS are opening up in a big way. With such increased portability, today you can bring the mountain to Mohammed — analysing samples at source, which always makes for an optimum result.”

“As a supplier of gases for this equipment, the implications for us involve keeping up with the changing modes of supply. In the old days, when these were huge machines which resided in laboratories, we could supply the different gases that are essential to operate these instruments in standard 40 or 50 litre cylinders or liquid gas supply such as CRYOSPEED to the laboratory site.”

“When ‘micro-GC’s’ became small enough to be carried around in a suitcase, we were obliged to come up with an appropriate solution. And Linde did just this. Our design engineers solved this portability issue with a product called the HiQ MICROCAN. It’s the size of a beer bottle and contains enough gas to keep a GC-MS running for several months.”

HiQ MICROCAN is a high-pressure cylinder with a maximum of 200 bar (2,900 psi) filling pressure. It is therefore a miniature version of approximately one 250th in scale in comparison to the 50 litre high-pressure cylinders that are more common in the market. Due to its high filling pressure, the HiQ MICROCAN has more gas content than conventional small compressed gas cylinders which might typically be filled to 12 bar (174 psi) or 34 bar (493psi), while also being much smaller. The HiQ MICROCAN is part of Linde’s “Gases in Small Cylinders” range developed in response to universal gas application needs; wherever it is critical that light containers are used in a mobile way.

GC-MS requires high quality specialty gases for instrument operation and for calibration. Specialty gases are used as carrier gases (helium is most common), collision gases (nitrogen or helium) and can also include reagent gases. The carrier gas plays an important role by transporting the sample through the chromatography column into the mass spectrometer. The carrier gas must be inert or at least must not react with the stationary phase in the column. The choice depends on the sample, column, application and safety requirements.

The choice of carrier gas is also dependent on requirements in terms of separation efficiency and speed. Hydrogen has the lowest viscosity of all gases, thereby provides the highest mobile phase velocity and therefore the shortest analysis time. Helium, on the other hand, gives the best overall performance and peak resolutions for many applications, making it an optimum choice of carrier gas in those cases.

The purity of the carrier gas is a critical factor for the performance, maintenance and lifetime of the column. Impurities in the carrier gas, especially hydrocarbons, cause base line noise and reduced sensitivity and might increase detection limits. Traces of water and oxygen may also decompose the stationary phase, which leads to premature destruction of the column. Linde’s gases range in purity up to 7.0, which is the highest commercially available grade, being 99.99999% pure with 0.1 parts per million of total impurities. The gas company also provides certificates of analysis on its own gases to reassure customers of these exceptional quality attributes.

“While the reliability of analysis is only as good as the quality of gas being used, distribution systems and equipment for high-purity gases and specialty gas mixtures must also be able to meet increasing demands for high standards of performance and new analysing methods,” said Harrison. “Impurities occurring in concentrations as low as parts per billion can have serious consequences; particularly if the analyst is not sure which molecules are present in the sample.”

“Demands made on regulators and valves in these environments are extremely high. Components must be capable of dealing with high and low pressures, large and small flows. They must be suitable for high-purity inert gases as well as reactive, flammable, corrosive or toxic gases.”

REDLINE from Linde is a hi-tech range of regulators, modularly designed to slot into central gas supply systems, containing gas panels, points-of-use and cylinder regulators generally suitable for purities up to 6.0 (99.9999%). In addition to common models, REDLINE regulators also have models for vacuum dosage and low pressure precision adjustments. The most recent introduction to REDLINE is a range of regulators and gas supply panels with welded connectors and face-seal fittings to reduce the possibility of contaminant ingress. With this new range, launched in 2010, gases of 7.0 grade purity can be handled and supplied without purity deterioration.

The Linde BASELINE range provides alternative entry-level laboratory equipment with a high-tech range of specialty gases cylinder regulators for basic applications. Linde also have HiQ laboratory gas generators providing high purity hydrogen and nitrogen for carrier gas suitable for GC-MS.