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A Review on Headspace Chromatography for Analysis of Volatile Oils

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Abstract

Headspace gas chromatography combines sampling in a headspace of a closed container, for example, the space occupied by gases or vapour in a sample vial, with analysis of the sampled gases or vapour by gas chromatography. The advantage of the headspace sampling is that direct liquid or solid probing is avoided and complex sample matrix in a liquid or solid sample can be simplified or even eliminated in its vapour phase. The basic principle of headspace gas chromatography (HSGC) and many useful techniques can be found in textbooks and review articles. The key in HSGC is the sampling and transfer of the samples in headspace to GC. A headspace sample is normally prepared in a vial containing the sample, the dilution solvent, a matrix modifier, and the headspace. Volatile components from complex sample mixtures can be extracted from non-volatile sample components and isolated in the headspace or vapour portion of a sample vial. An aliquot of the vapour in the headspace is delivered to a GC system for separation of all of the volatile components. This review article presents some recent developments in exploration of HSGC potential. The objective of this article is to encourage further innovations in HSGC. Headspace gas chromatography is a mature and reliable technology. Many HSGC techniques can be implemented in commercial systems. Because of the unique isothermal environment of the headspace and the headspace sampling process along with chromatographic separation of species, HSGC has great potential for many laboratory and industrial applications other than quantitative analysis of volatile species, which was the initial intended purpose of its development.

Key-Words: Headspace chromatography, Volatile oils, Pressure-Loop System, Basic Principle

Introduction

Headspace gas chromatography (GC) is a technique used for the concentration and analysis of volatile organic compounds. This technique is relatively simple and can provide sensitivity similar to dynamic purge and trap analysis. The popularity of this technique has grown and has gained worldwide acceptance for analysis of alcohol in blood and residual solvents in pharmaceutical products. Other common applications include industrial analyses of monomers in polymers and plastic, flavour compounds in beverages and food products, and fragrances in perfumes and cosmetics.

Sample matrices like blood, plastic, and cosmetics contain high molecular weight, non-volatile material that can remain in the GC system and result in poor analytical performance. Many laboratory analysts use extensive sample preparation techniques to extract and concentrate the compounds of interest from this unwanted non-volatile material. These extraction and concentration techniques can become time consuming and costly.

Static headspace analysis avoids this time and cost by directly sampling the volatile headspace from the container in which the sample is placed. Because of the diversities in the industry and related products, this guide attempts to cover only the basic principles of static headspace and demonstrate how to apply them to achieve optimum chromatographic results. With an understanding of these principles, various instrumentations will then be reviewed to help build upon this knowledge and identify the benefits and potential problems associated with each mode of sample transfer. Information from the *Basic* Principles and Instrumentation sections of this guide can then be brought together and applied to the conditions and methodology of common analyses. Like most applications, a variety of problems may arise in which the System Optimization section will help to identify these problems and offer techniques to help resolve them [1].

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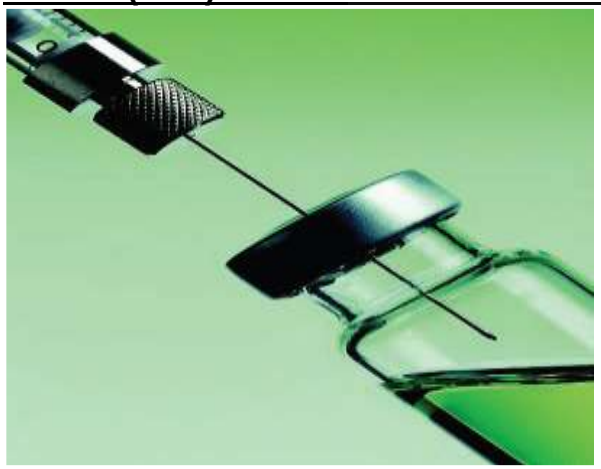

Fig. 1: Headspace sampler

Basic Principle of Head space chromatography:

Most consumer products and biological samples are composed of a wide variety of compounds that differ in molecular weight, polarity, and volatility. For complex samples like these, headspace sampling is the fastest and cleanest method for analyzing volatile organic compounds. A headspace sample is normally prepared in a vial containing the sample, the dilution solvent, a matrix modifier, and the headspace (see Figure 2). Volatile components from complex sample mixtures can be extracted from non-volatile sample components and isolated in the headspace or vapour portion of a sample vial. An aliquot of the vapour in the headspace is delivered to a GC system for separation of all of the volatile components [1, 2].

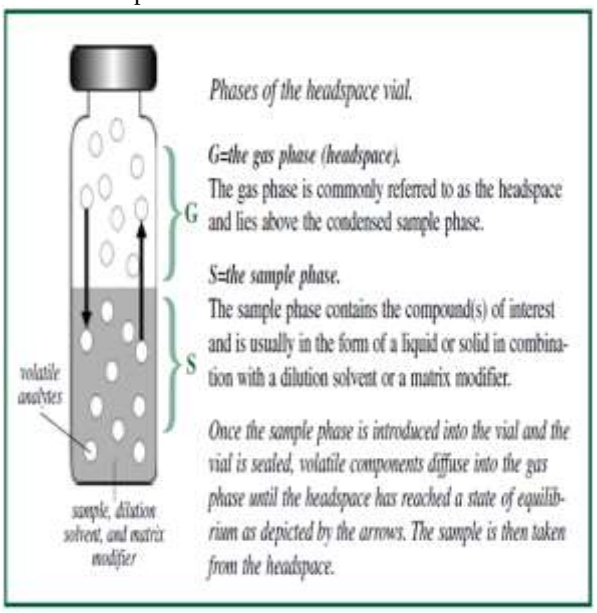

Fig. 2: Phases of Headspace vial

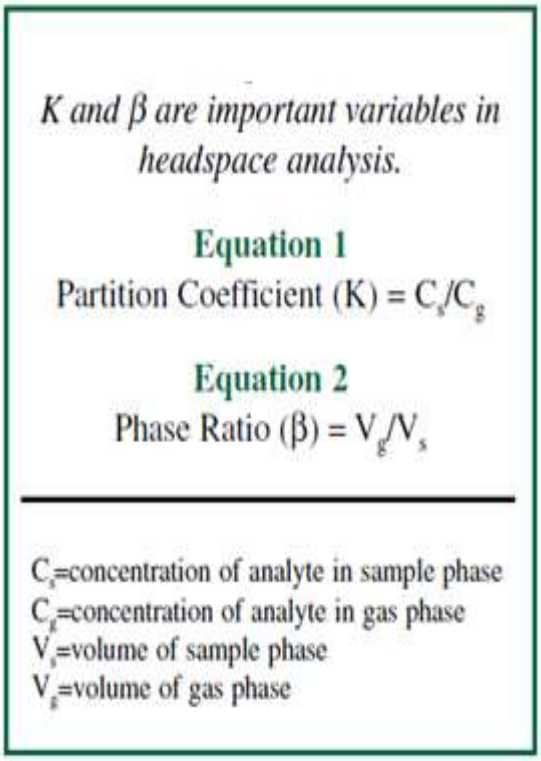

Fig. 3: Processing Variables

Partition Coefficient

Samples must be prepared to maximize the concentration of the volatile components in the headspace, and minimize unwanted contamination from other compounds in the sample matrix. To help determine the concentration of an analyte in the headspace, you will need to calculate the partition coefficient (K), which is defined as the equilibrium distribution of an analyte between the sample phase and the gas phase.

Phase Ratio

The phase ratio is defined as the relative volume of the headspace compared to volume of the sample in the sample vial (Figure 3). Lower values for (i.e., larger sample size) will yield higher responses for volatile compounds (Figure 4). However, decreasing the value will not always yield the increase in response needed to improve sensitivity. When is decreased by increasing the sample size, compounds with high K values partition less into the headspace compared to compounds with low K values, and yield correspondingly smaller changes in Cg. Samples that contain compounds with high K values need to be optimized to provide the lowest K value before changes are made in the phase ratio.

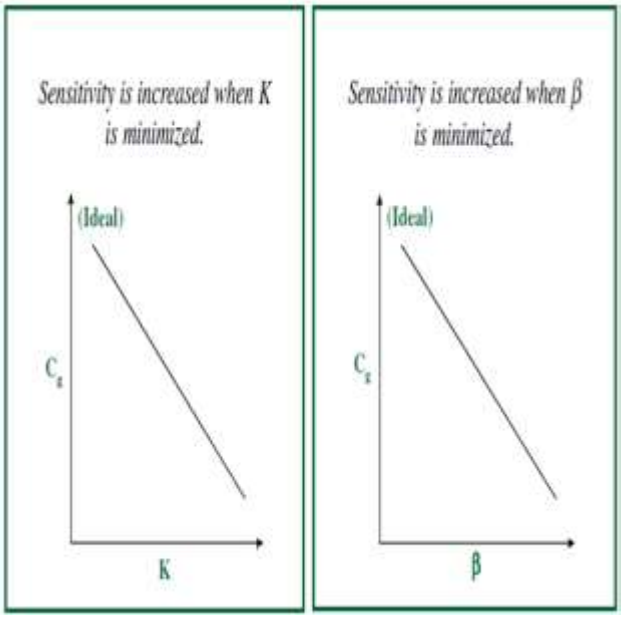

Fig. 4: Partition coefficient and Phase ratio

Partition coefficients and phase ratios work together to determine the final concentration of volatile compounds in the headspace of sample vials. The concentration of volatile compounds in the gas phase can be expressed as Cg=Co/(K+□) (where Cg is the concentration of volatile analytes in the gas phase and Co is the original concentration of volatile analytes in the sample). Striving for the lowest values for both K and B will result in higher concentrations of volatile analytes in the gas phase and, therefore, better sensitivity (Figure 5)[1, 2].

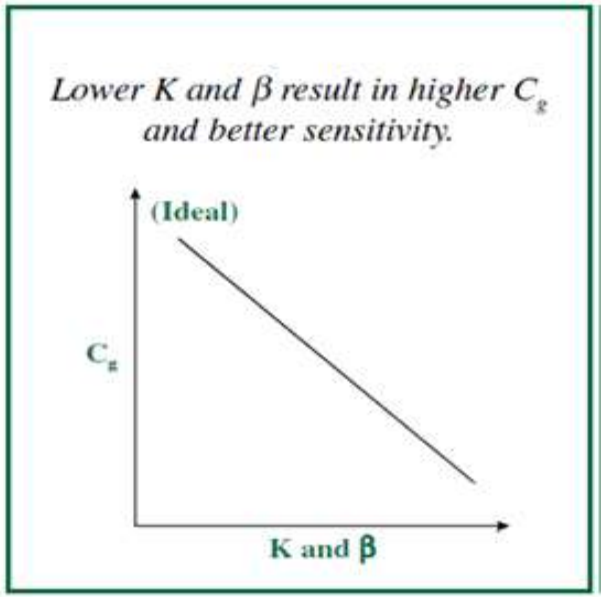

Fig. 5: Combinations of K and B

Instrumentation

Gas-Tight Syringe Injection

Use of a gas-tight syringe autosampling system is one of three common techniques (gas-tight syringe, balanced pressure, and pressure loop) used to transfer a headspace sample. Most of the autosampling units can retrofit to a standard GC with a split/splitless injection port, making them relatively simple to use and understand. These systems do not require the use of special configurations or special instrumentation other than the autosampler itself. The gas-tight syringe autosampler is beneficial for use with diverse samples because of the variety of sampler configurations and method options available.

The gas-tight syringe technique operates by initially thermostatting the sample in an incubation oven at a given temperature and for a given time until it has reached a state of equilibrium (Figure 6, Step 1). Once the sample has reached equilibrium, an aliquot is taken from the headspace using the gas-tight syringe (Figure 6, Step 2), and the aliquot is injected into the GC as if it were a liquid sample injection[3].

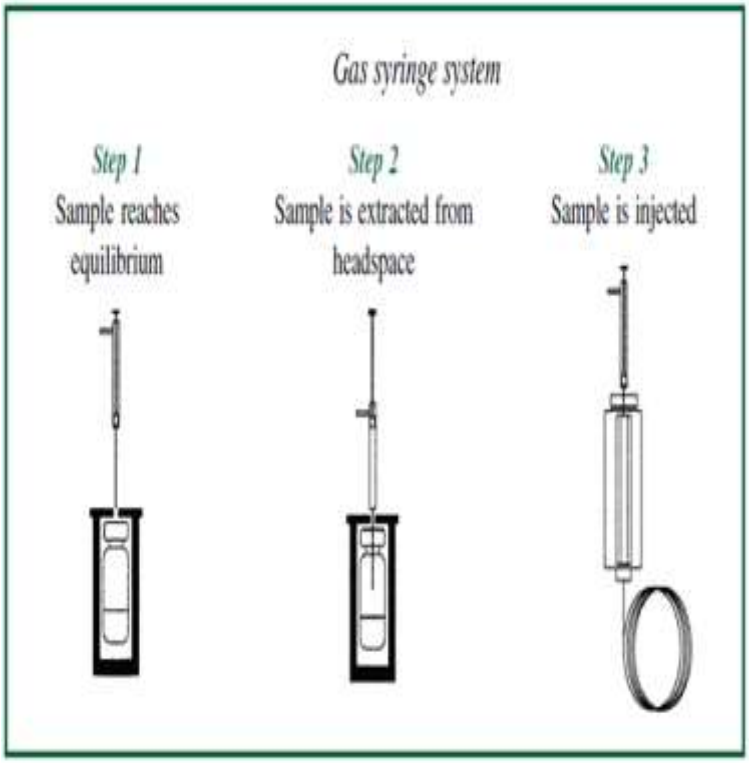

Fig. 6: Gas syringe System

Examples of manufacturers and models of the gas-tight syringe units are: the ThermoQuest TRACE™ HS2000 and HS850 (Figure 7) Headspace Autosamplers and the Leap Technologies CTC COMBI PAL Sampler.

Fig. 7: Gas tight Syringe

Balanced-Pressure System

Another common technique is the balanced-pressure system, which is capable of generating results with a high degree of repeatability. It uses a seamless injection directly from the vial into the carrier gas stream without additional moving parts other than a valve and a needle. The balanced-pressure system, like other techniques, uses an incubation oven to thermostat the vial so the sample reaches equilibrium (Figure 8, Step 1). During these initial steps, a needle is inserted into the vial and then is pressurized with a carrier gas (Figure 8, Step 2). After the vial is pressurized and equilibrium has been reached, the valve is switched for a specific amount of time to redirect the sample into the transfer line and onto the column (Figure 8, Step 3)[3, 4]. An example of a balanced pressure system is the HS 40XL manufactured by Perkin-Elmer (Figure 9).

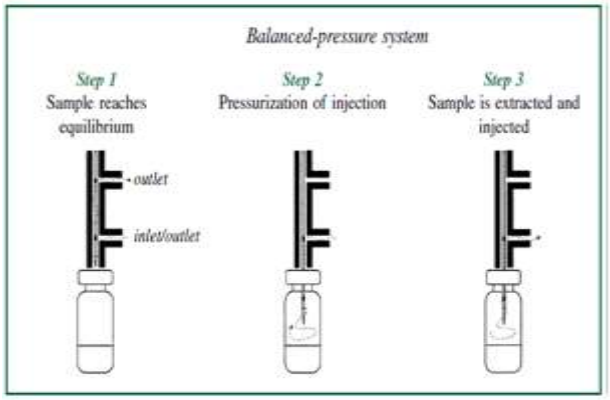

Fig. 8: Balanced Pressure System

Fig. 9: Pressure Autosampler

Pressure-Loop System

The last common injection technique discussed in this guide is the pressure-loop system. Unlike balanced-pressure, the pressure-loop system uses a known amount of sample. This technique typically uses a six-port valve, and initially thermostats and pressurizes the vial as in the previously described techniques (Figure 10, Step 1). After pressurization, the valve is turned and the loop is filled with the sample (Figure 10, Step 2). After the loop has been filled, the valve is turned again to redirect the gas flow and flush the sample into the transfer line leading to the analytical column (Figure 10, Step 3). Several makes and models of pressure-loop systems include the OI Model 4632 (**Figure 11**)[4].

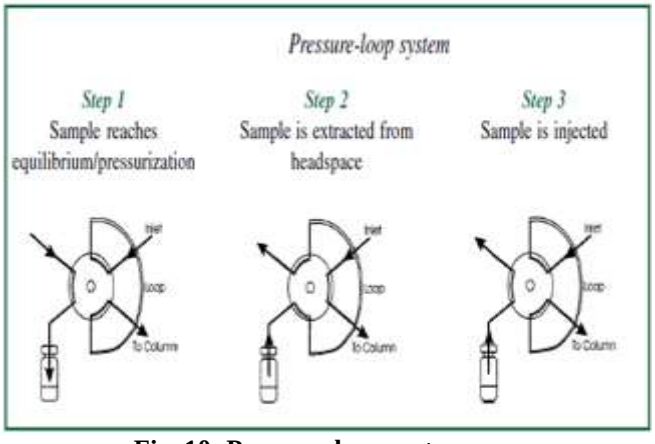

Fig. 10: Pressure-loop system

Fig. 11: Pressure-Loop system

System Optimization

Chromatographic performance in Headspace/GC is greatly influenced by how the sample is introduced into the analytical column. Variables that affect sample preparation and transfer of the sample from the headspace unit to the analytical column must be optimized to obtain reproducible and efficient separations. Key issues to address when setting up headspace/GC systems include minimizing system dead volume, maintaining inert sample flow paths, and achieving efficient sample transfer. This section will explain how to optimize areas that are critical in addressing these issues and providing good chromatographic performance [1, 5].

Sample Preparation

Samples for headspace/GC must be prepared in such a manner as to maximize the concentration of the volatile sample components in the headspace while minimizing unwanted contamination from other compounds in the sample matrix. Sample matrices such as biological samples, plastics, and cosmetics can contain high molecular weight, volatile material that can be transferred to the GC system. Water from the sample matrix also can cause problems by recondensing in the transfer line. High-concentration samples need to be prepared appropriately to obtain optimal chromatography. High-concentration samples can produce ghost peaks in subsequent analyses due to carryover of sample from previous injections. Sample carryover can be minimized by using higher transfer line and injection port temperatures, but some samples may need to be diluted and re-analyzed to obtain reliable results. Additionally, we recommend injecting standards and samples in order from low to high concentrations to help minimize carryover. When sample carryover or ghost peaks are evident, you may need to bake-out the column at its maximum operating temperature and elevate the transfer line temperature in order to remove the entire residual sample [1, 6].

Sample Vial

Sample vials should be selected to match the type and size of the sample being analyzed. Always use pre-cleaned vials for sample preparation and storage. Vials that are not properly cleaned prior to packaging or that absorb contaminants during shipping can produce unknown chromatographic peaks, or "ghost peaks." Ghost peaks that are the result of vial contamination can be identified by running method blanks and zero standards during the system calibration sequence[1,7].

Sample Vial Heater and Mixer

Once the sample is placed inside a clean, non-contaminating vial and the vial is sealed, volatile compounds from the sample will partition into the headspace until a state of equilibrium is reached. The rate at which volatile compounds partition out of the sample matrix and into the headspace, as well as the equilibrium concentration of volatile compounds in the headspace depends on several parameters.

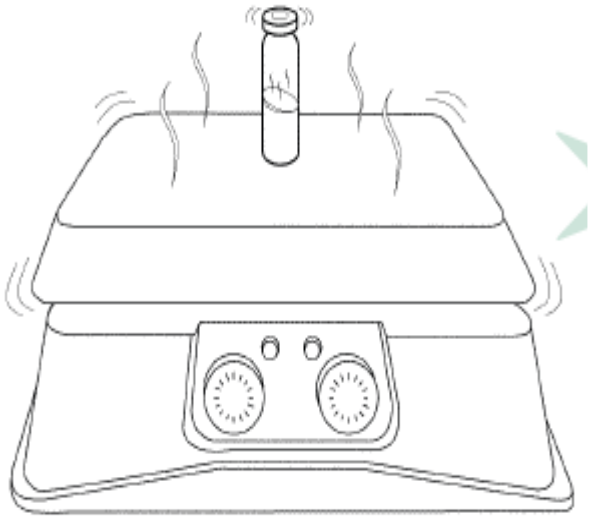

Fig.12: Heater and Mixture

Temperature, time, and mixing can be used to improve the transfer of volatile analytes from the sample into the headspace of the vial. Adjusting the temperature of the sample will change the solubility of the analyte in the sample matrix and can be used to drive the equilibrium in favor of the gas phase. Sufficient time

must be built into the sample cycle in order to achieve a constant state of equilibrium [1, 6, 7].

Sampling

There are several techniques used to transfer samples from the vial to the GC. When using a *gas-tight syringe* for sampling, heat the syringe to a temperature comparable to the sample vial temperature. This minimizes pressure differences and condensation problems. To prevent carryover from inside the syringe, flush the syringe after each injection. Because gas-tight syringe samplers inject through the GC injection port septum, ensure the septum is well maintained to decrease the possibility of a leak.

Transfer Line

After the headspace sample is withdrawn from the vial, it ready to be transferred to the GC. In balanced-pressure and pressure-loop systems a short piece of tubing called a transfer line is used to transfer the sample from the auto sampler to the GC.

Transfer line material must be chosen that suits the sample analytes. Many different materials can be used as transfer line tubing, including stainless steel, nickel, fused silica, and Silcosteel®- or Siltek™-coated tubing. Stainless steel provides a strong, flexible tubing material, but can be adsorptive towards more active analytes such as alcohols, diols, and amines. Nickel and Silcosteel® tubing are highly inert towards active compounds and provide ruggedness similar to stainless steel. Fused silica and Siltek™ tubing are extremely inert towards active compounds; however they are not as rugged as nickel or Silcosteel® tubing [1, 8].

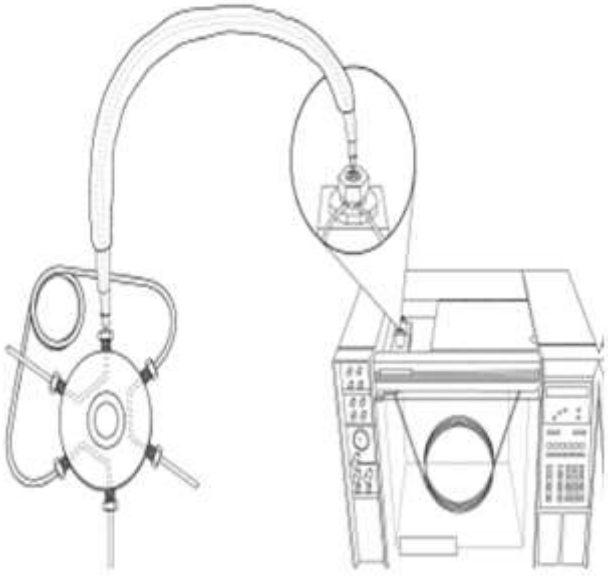

Fig. 13: Transfer Line

Injection Port Interface

The quality of the connection of the transfer line to the analytical column greatly affects sample bandwidth. In most cases, the transfer line has a smaller internal diameter than the injection port liner, and the vaporized headspace sample carrying the compounds of interest will be diluted into a larger volume of carrier gas when the sample elutes from the transfer line into the inlet liner. This can lead to broader peaks, tailing peaks, lower sensitivity, and loss of resolution. Because headspace samples are already in a gaseous state (vapor cloud) when they enter the injection port, there is no need to use a large buffer volume in the liner to allow for sample expansion as when analyzing liquid samples. Using injection port liners that have smaller internal diameters and lower buffer volumes will help maintain a narrow bandwidth as samples move from the end of the transfer line to the head of the analytical column. 1.0mm ID deactivated injection port liners are recommended for most headspace applications to achieve the lowest injection port dead volume [1, 9, 10].

Conclusion

Static headspace gas chromatography is a mature and reliable technique; it is considered the technique of choice for the analysis of ethanol in biological samples, and is therefore present in the vast majority of forensic laboratories around the world with the qualified personnel to operate it; however, the applicability of this technique is not limited to this test and can be used for the analysis of various substances with minimal modifications, providing proper calibration and proper handling of matrix effects, excellent validation parameters, along with a clean injection. So, with this technique, various substances can be analyzed without the need of additional methods, and that would allow forensic laboratories to expand the number of cases they can take care of, with a minimal investment. To accomplish that, it is necessary to know the fundamentals of this technique, the different chemical and physical phenomena involved, and the potential occurrences in the analysis of a particular substance, in order to develop a method with the required sensitivity, specificity and reproducibility.

Headspace gas chromatography is a mature and reliable technology. Many HSGC techniques can be implemented in commercial systems. Because of the unique isothermal environment of the headspace and the headspace sampling process along with chromatographic separation of species, HSGC has great potential for many laboratory and industrial applications other than quantitative analysis of volatile species, which was the initial intended purpose of its

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