Sample Introduction Method in Gas Chromatography

Kazutoshi Sugita*† and Hiroshi Sato**

*Laboratory of Veterinary Public Health-1, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Chuo, Sagamihara, Kanagawa 252-5201, Japan
**Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch-cho, Sasebo, Nagasaki 859-3298, Japan

In this review, we summarize the methods of sample introduction into a gas chromatograph. For volatile organic compounds, headspace measurements and purge-trap methods have been used traditionally. Recently, the trapped headspace method has been used in water quality testing. In addition, various solid-state adsorption methods have been developed, including a method in which the adsorbent is placed inside a needle, while new adsorbents and their applications have also been introduced.

Keywords Thermal desorption (TD), solid-phase micro extraction (SPME), needle trap device (NTD), headspace method

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1 Introduction

Gas chromatography (GC), especially capillary column-based gas chromatography, is widely used for multicomponent simultaneous analysis of dioxins, polychlorinated biphenyls (PCBs), pesticides, volatile organic compounds (VOCs), and other compounds due to its high separation capability. Typically, the target constituents are extracted and purified from environmental samples such as soil, water, airborne dust, or food, and a concentrated solution is injected into a gas chromatograph (GC). Alternatively, a gas sample can be directly introduced into the GC. However, in recent years, high sensitivity and rapid analysis are required, which has led to the development of detectors, such as mass spectrometers (MS) and mass injections of samples. Various injection methods are also

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Kazutoshi Sugita received his degree from Tokyo University of Science in 2004, and has been working at a contract environmental analysis laboratory for about 28 years, where he has been involved in the measurement of dioxins and polycyclic aromatic hydrocarbons in the environment. He started his current position in 2014 and has been conducting research on the behavior of toxic components in the environment and in living organisms using gas and liquid chromatographs.

Hiroshi Sato received his Master degree in 1983 and received a PhD degree in Pharmaceutical Sciences (2003) at Kyushu University in Japan. He had worked in Kao Corporation during 1985 - 1987 and then had worked in TOTO LTD. during 1987 – 2006. He is currently a professor of Nagasaki International University. His research is focused on the environmental analysis and bioremediation.

† To whom correspondence should be addressed.
E-mail: sugita@azabu-u.ac.jp
being developed and explored to answer this requirement. In headspace analysis, which has conventionally been adopted as the official method for drinking-water quality testing in Japan, the method of increasing injection volume by concentrating the volatilized sample in a trap tube has also been adopted. In addition, instead of injecting a gas itself, gaseous components are collected and concentrated by adsorbents, and then the adsorbents are introduced into the GC system, which makes it possible to perform simple and sensitive measurements. Furthermore, combining of the solid-phase adsorption method and the headspace method, which separates the target component from the interfering component, has been widely used for measuring limited amounts of samples, such as biological samples. Therefore, recent information on sample introduction methods and solid-phase adsorption methods are introduced.

2 Injection Methods to the Gas Chromatograph

2-1 Direct injection method (liquid samples and gas samples)

In general, samples are injected to the GC through an inlet. There are many textbooks for GC methods including the split method, splitless method, on-column method, and programed temperature vaporization method. The details can be researched in textbooks. The increasing solvent volume is a focus in this paper. The split/splitless injector is shown in Fig. 1, which is known to be the most useful injector. The sample solution is injected and vaporized in the glass insert. If too much sample solution is injected, the volume of the vaporizing sample can exceed the glass insert volume. In such cases, not only does it worsen the quantification, but it also causes inlet pollution. When the liquid sample evaporates, the volume significantly increases depending on the pressure and temperature of the inlet and types of solutions. An example of the volumes of commonly used solvents when they evaporate is shown in Table 1, and the method for calculating this volume is shown in Fig. 2. It also shows the actual case of n-hexane vaporization as a sample calculation. Although the volume of the glass insert of GC differs among manufacturers, the volumes range from 300 to 800 μL. Generally, there are no problems as long as the volume after vaporization is about half of these values. Similarly, the injection of sample gas in excess of the volume of the insert should be avoided.

2-2 Headspace injection method

2-2-1 Static headspace injection method

The headspace method is used to measure the VOC in a liquid sample. The sample is placed in a sealed vial and kept warm for a certain amount of time. After achieving gas-liquid equilibrium, a portion of the upper gas phase is injected into the GC (Fig. 3). This method has been adopted as an official ones such as environmental and water quality standards. It is suitable for high-sensitivity measurements of VOCs in wastewater and seawater because it eliminates the influence of non-volatile components, such as suspended solids and salts in water. To increase the quantified accuracy, it is necessary to add internal standards, as well as to standardize the vial volume, sample and

| Solvent         | Structural formula | Volume vaporized/μL |
|-----------------|--------------------|---------------------|
| iso-Octane      | C8H18              | 110                 |
| n-Hexane        | C6H14              | 140                 |
| Toluene         | C6H5CH3            | 170                 |
| Ethyl acetate   | CH3COOC2CH3        | 185                 |
| Acetone         | CH3OCH3            | 245                 |
| Dichloromethane | CH2Cl2             | 285                 |
| Carbon disulfide| CS2                | 300                 |
| Acetonitrile    | CH3CN              | 350                 |
| Methanol        | CH3OH              | 450                 |
| Water           | H2O                | 1010                |

water consists of 1 μL of solvent evaporated at a temperature of 250°C and pressure of 140 kPa.

V (μL) = V0 (μL) x D (g/cm³) x MW (g/mol) x (T (K)) x (P (kPa))

V : Gas volume in insert (μL for gas)
V0 : Injection volume of sample solution (μL of Liquid)
D : Density of solvent (g/cm³)
MW : Molecular Weight of solvent (g/mol)
T : Temperature of inlet (K)
P : Head pressure in inlet (kPa)

In the case of to inject n-Hexane 1 μL in inlet at 250°C, 140 kPa

MW of n-Hexane : 86.2 g/mol
D of n-Hexane : 0.659 g/cm³
Gas volume-1 (μL) x 0.659 (g/cm³) x 22.4 (L/mol) x 523 (K) x 1013 (kPa)

= 1.0677 mL 140 (μL)

Fig. 1 The split/splitless injector.

Table 1 Volume of various solvents at the evaporation

Fig. 2 Calculation of the volume of a liquid sample upon evaporation after injection and examples of calculations.

Fig. 3 Diagram of sample measurement using the headspace method.
reagent volumes, and heating temperature as well as the heating time. In addition, the salinity and other conditions of the standard and the sample should be adjusted to the same or similar state. Specifically, this can be done by adding a supersaturated amount of sodium chloride or something similar to unify the salt concentration in the sample water. By the official method, 3 g of sodium chloride is added to 10 mL of water (sample + diluted water). For measuring VOCs in water, special reagent grade sodium chloride is used. It has been confirmed by a preliminary blank test that the reagent to be used does not interfere with the VOCs measurement. For example, when sodium chloride, a reagent used to test for pesticide residues, is used, residues of dichloromethane and other substances used to clean the reagent may be observed.

2-2-2 Trap-type headspace injection method (Dynamic headspace injection method)

In the trap-type headspace method, volatile components are maintained in a gas-liquid equilibrium in a closed vessel, and inert gas is introduced into the vessel to extract a portion of the gas phase, the sample is adsorbed in a trap, heated and desorbed, and then introduced into the GC. In the headspace method, only a part of the gas phase with a single injection can be introduced into the GC, whereas the trap tube concentrates and collects several injections, this is allowing more target components to be extracted from the sample water. This “trap-headspace-gas chromatography-mass spectrometry” was formally approved by the Water Quality Testing Act Review Council in 2011. Figure 4 shows an example of a conceptual diagram (flow path) of the trapped headspace method. The headspace gas in the vial is enriched in a loop by pressurizing the vial with inert gas and releasing the valve on the loop fill line. This operation can be repeated several times for higher concentrations. Then, the six-port valve is switch and the loop is heated to desorb the adsorbed components, thereby introducing the concentrated components into the GC. It is expected to be several times more sensitive than the usual headspace method.

2-3 Purge-trap injection method

The purge-trap method is applied to measuring VOCs in water as well as the headspace method, and it is a method used to purge the VOCs contained in water by bubbling a water sample with inert gas, such as nitrogen gas (purge), collect them in a trap (trap), heat the trap, and introduce them into GC (Fig. 5). Since VOC components are forced out by the inert gas, the amounts of VOCs introduced into the GC will increase, and high-sensitivity measurements are expected. Samples with high concentrations of VOCs or high salinity may cause contamination of the lines through which the sample passes or clogging due to salt precipitation. This method is thus suitable for low-concentration, high-cleanliness samples, which are common in drinking-water quality testing. In addition, if the system is automated, care must be taken to avoid clogging of the wastewater line due to suspension components. This method is also recognized as an official method for measuring wastewater and drinking water, and many manufacturers supply automatic analyzers with attached autosamplers.

2-4 Solid-phase micro extraction method (SPME method)

In the solid-phase microextraction method, a fiber is inserted into the needle of the microsyringe and the fiber is modified with an adsorbent, the fiber part can be inserted and removed from the tip of the needle by plunger operation. During gaseous component collection, the plunger is pushed in to release the adsorbent resin from the needle tip, and when collection is complete, the plunger is withdrawn and the adsorbent resin is placed into the needle. It is necessary to submit to analysis as soon as possible after collection. In GC analysis, as in the case of normal analysis, SPME is inserted through the injection port and a plunger is pushed in to insert a fiber into the heated part of the injector, and the adsorbed component is heated and desorbed (Fig. 6). It is very easy to operate, since it requires no other equipment. Ten types of fibers are available depending on the adsorbent and thickness of the film, and can be selected according to the components to be measured. Table 2 shows the types of fibers in SPME. While convenient, the fibers must be quenched before use because they adsorb gaseous components from the surrounding air, even when the fibers are contained in the needle, and they must be measured as soon as after sampling. In addition, depending on the history of use and other factors, the adsorption capacity of the fiber may vary, making it
unsuitable for accurate quantitative analysis. Moreover, the adsorption fibers at the tip of the stainless-steel wire are easily dislodged, and the fibers may come off when touching an object with the fibers out or when pulling the SPME out of the injection port after measurement, so careful handling is required.

Various applications have been reported with the combination of the head spacing method and multidimensional GC. Due to the ease of operation and high concentration rate, a study on the diagnosis of prostate cancer by measuring 2,6-dimethyl-7-octen-2-ol, pentanal, 3-octanone, and 2-octanone in urine using SPME has been reported.8 In combination with multidimensional GC, 28 VOCs were detected in the lion’s marking fluid, among which 2,5-dimethylpyrazine, 4-methylphenol, and 3-methylcyclopentanone were reported to be involved in the lion’s odor9 and also used to assess the odor of cat urine on carpets.10 Another study has measured cocaine odor by SPME for training drug detection dogs.11 In the environmental field, semi-quantitative determinations of semi-volatile organic compounds (SVOCs) in sewage sludge were performed using a combination of the headspace method and SPME to detect methanetol, dimethyl polysulfide, octa atomic sulfur, phthalic anhydride, and indoles.12 Furthermore, it is possible to detect toluene, α-pinene, 1-butanol, 2-ethyl-1-hexanol, etc. by measuring VOCs in a room, thought it has been reported that it is impossible to know the volume of the collected air.13 A study of odor components from humans in indoor air identified C_{2-9} fatty acids, C_{8-10} aldehydes, and methyl ketones from the air in a bedroom, and it was speculated that the actual odor is a compound of the oxidative decomposition of unsaturated lipids from humans.14 In addition, MVOCs (Microbial Volatile Organic Compounds) emitted from carpets in headspace vials have been measured and reported to vary with the type of mold occurring in the room.15

### 2-5 Thermal desorption method

In the thermal desorption (TD) method, the target component is adsorbed on an adsorbent such as activated carbon or porous polymer beads (Tenax®) in air or water, and then heated in a thermal desorption apparatus to desorb the target component and introduce it into the GC. Depending on the type of adsorbent, the target components can be selected, and high-sensitivity measurements can be made by desorbing and introducing the adsorbent with a heat desorption device. Commonly used solid-phase adsorption tubes, star bars, and MonoTrap® are introduced here.

### 2-6 Solid-phase adsorption tubes

This is the most common process in the TD method. Glass tubes (or inert glass inserts) or stainless-steel tubes are filled with an adsorbent, and a sampling pump is used to suction air so as to adsorb gaseous components from the air (Fig. 7). As an adsorbent, Tenax TA (a polar resin based on 2,6-diphenyl-p-phenylene oxide), graphite carbon, carbon molecular sieves, etc. are used, and some of them have two-layered structures (Multibed type).16 There are two main types: one for PerkinElmer, Markes, and Shimadzu and the other for Gestel, and there are two types of glass and stainless-steel tubes depending on the shape (length and outer diameter) of the heating/desorption equipment. When using these solid-phase adsorption tubes, be sure to bake immediately before using them to ensure that the blank is as low as possible. When using it for the first time, check the suction speed, suction time, suction gas humidity, and breakthrough capacity of the suction gas, and use it within its non-breakthrough range. For VOCs, such as benzene, toluene, xylene, and styrene, a solid adsorption-thermal desorption-gas chromatography method is employed in indoor air17 or ambient air. This method is characterized by its ease of use, large volume sampling, and high quantitative capability. However, a heated desorption device with an attached autosampler is needed to increase the quantification accuracy.

### 2-7 Stir Bar Sorptive Extraction method (SBSE method)

Stir Bar Sorptive Extraction (SBSE) is a method for the
selective and efficient extraction and concentration of volatile compounds in a sample in which a magnetic stirrer encased in glass is coated with polydimethylsiloxane (PDMS), which is a non-polar liquid phase. It can be used in both gas and liquid phases. When organic components in water are collected, wash the stir bar with purified water, wipe the water with clean paper, etc., and introduce it into the GC by thermal desorption (Fig. 8). The PDMS stirrer is expected to provide easy and sensitive microanalysis because it can introduce a large number of analytes transferred to the PDMS phase into GC/MS with a thermal desorption device.

In the case of a gel or solid sample form, the HSSE (Headspace Sorptive Extraction) method, in which a sample is placed in a vial and a PDMS agitator is placed in the headspace and left in the vial to transfer the target component, which has been volatilized in the gas phase, into the PDMS phase, can also be applied. Currently, the PDMS stirrer is marketed by Gestel as Twister. The principle is based on the partitioning of water and PDMS, which is basically the same as that of SPME, but the recovery of the compound, which has even a little K(o/w) is improved when compared to SPME.\(^8\) Gasses generated by *Staphylococcus aureus* in a culture are collected by SBSE and was comparable, but the reported flavors were different showed that SBSE was superior in terms of the LOD (limit of detection) of 0.01 to 1.17 ng/L and a lower limit of quantification of 0.042 to 7.24 ng/L. It is expected to be used for clinical diagnosis and condition monitoring.\(^23\) In addition, the gas generated from cells in culture is collected by pumping the gas in the upper space of the cell flask with a syringe and measured by GC/MS for profiling analysis.\(^24\) Measurements using the disc-shaped MonoTrap\(^8\) have been used to study the early detection of cancer in the urine and skin gases (gases emitted from the skin) of cancer patients.\(^25\) The non-invasiveness, simplicity, etc. of the MonoTrap\(^8\) make it suitable for measuring odor of a baby’s head, and has been reported for common compounds with a maternal amniotic fluid.\(^26\) Other simple screening methods for toxic chemicals in health hazard management, etc., have used MonoTrap\(^8\) for 1,4-butanediol and gamma-butyrolactone (GBL)\(^27\) in water, and the development of highly sensitive and simple methods is expected. When researching the food industry, it is widely used in conjunction with the headspace method to analyze the constituents of apples,\(^28\) odor components in fish,\(^29\) and aromatic components in alcoholic beverages.\(^30\) In addition, measurements in combination with GC/MS-olfactometry (GC/MS-O) have also measured such things as the odor of Commercial fragrant rapeseed oil (CFRO)\(^11\) and fish.\(^32\) In the environmental field, it is also used for emission testing of building materials.\(^32\)

2-9 Needle trap device (NTD)

A needle trap device (NTD) is a device in which a syringe needle is filled with an adsorbent, through which sample air is aspirated to collect gas components (Fig. 10a). Subsequently, the collected samples can be introduced into the GC with the usual injection method, without the need for a thermal desorption device. Wang *et al.* proposed a method for filling the needles with polydimethylsiloxane (PDMS), divinylbenzene (DVB), and Carboxen particles to collect n-alkane and benzene, toluene, xylene (BTX).\(^33\) For more information on its construction and use, see a review by Ueta.\(^34\) In Japan, it is commercialized under the name NeedlEx\(^8\) by Shinwa Chemical Industries Ltd. NeedlEx\(^8\) is a side-hole needle filled with an adsorbent that concentrates the component to be measured in the air by sucking in a headspace vial, sealed, and heated to promote volatilization of the gas from the sample and collected by a MonoTrap.\(^8\) Since the volatile components vary depending on the temperature, the collection conditions of the target substances are examined in advance to optimize them.

The MonoTrap\(^8\) is used to measure bio-volatile substances and gases emitted from food because of its compactness and ease of use. Inoue *et al.* used MonoTrap\(^8\) to investigate “ill order” and found that hexadecane and N,N-dibutyl-formamide were significantly expressed in the skin of lung-cancer patients.\(^32\) The measurement of BTX in the exhaled gas of lung cancer patients has also been attempted, with a lower limit of detection of 0.01 to 1.172 ng/L and a lower limit of quantification of 0.042 to 7.24 ng/L. It is expected to be used for clinical diagnosis and condition monitoring.\(^23\) In addition, the gas generated from cells in culture is collected by pumping the gas in the upper space of the cell flask with a syringe and measured by GC/MS for profiling analysis.\(^24\) Measurements using the disc-shaped MonoTrap\(^8\) have been used to study the early detection of cancer in the urine and skin gases (gases emitted from the skin) of cancer patients.\(^25\) The non-invasiveness, simplicity, etc. of the MonoTrap\(^8\) make it suitable for measuring odor of a baby’s head, and has been reported for common compounds with a maternal amniotic fluid.\(^26\) Other simple screening methods for toxic chemicals in health hazard management, etc., have used MonoTrap\(^8\) for 1,4-butanediol and gamma-butyrolactone (GBL)\(^27\) in water, and the development of highly sensitive and simple methods is expected. When researching the food industry, it is widely used in conjunction with the headspace method to analyze the constituents of apples,\(^28\) odor components in fish,\(^29\) and aromatic components in alcoholic beverages.\(^30\) In addition, measurements in combination with GC/MS-olfactometry (GC/MS-O) have also measured such things as the odor of Commercial fragrant rapeseed oil (CFRO)\(^11\) and fish.\(^32\) In the environmental field, it is also used for emission testing of building materials.\(^32\)
air through the needle (Fig. 10b). Four types of NeedleEx® sorbents are currently available for alcohols, organic solvents, trimethylamine, and fatty acids, which can be selected according to the measured component. The sample is collected using a pump for a detection tube.

A quantitative analysis of blood alcohol using a combination of NTD and headspace method has been reported, and the results of measurements of fatty genera and aromatic hydrocarbons in the blood of burn victims have been used to discriminate between different fuels. Also the gases generated from the cells in culture were collected by NTDs and measured by GC/MS. Significant changes of VOC compounds have been detected in tumor cells. In addition, GC/MS measurements of short-chain fatty acids associated with pathological halitosis in the oral cavity, as well as measurements of pesticide components such as diazinon and fenitrothion from intestinal contents and other sources, in combination with the headspace method, have been shown to be effective as a screening method. It is expected that the number of reports on research related to the human body will increase, since it is effective in concentrating trace amounts of gas components. In the food industry, a method combining NTD and the headspace method has been reported to determine freshness by measuring indicator substances in the gases emitted from fish. In the environmental research field, evaluating the effect of smoke separation by measuring tobacco-related combustion gases such as nicotine and toluene at nursing care sites, has been reported as a part of the qualitative analysis of odor substances in the air.

3 Summary

In recent years, the problem of odors in the living environment has become an issue, and various adsorption methods have been developed for easy use, but it is necessary to choose the method that best suits the equipment owned by each laboratory or measurement institution. Particularly in the case of TDs, the loss of SVOCs due to adsorption may occur depending on the length of the transfer line connecting the sample vial and the injection port. Portable and simplified TDs have also been developed. TD, which is characterized by its simplicity, excels in qualitative analysis and is considered to be very useful for profile comparisons between samples. The internal standard method, in which internal standards are added to headspace vials, allows for highly accurate quantitative analysis. In this case, it is thought that high precision measurement is possible by using stable isotopes of the component to be analyzed as internal standards. It is expected that adsorbents with high adsorption efficiency will be developed and used in various fields in the future.

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