

An Introduction to Different Types of Gas Chromatography

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ABSTRACT

Gas Chromatography was introduced traditionally for the first time by James and Martin in 1952. As years went on, gas chromatography was further developed by combining various detectors and was used for different purposes. In this journal, 4 different types of detectors coupled with Gas Chromatography were discussed: Gas Chromatography-Mass Spectrometry (GC-MS), Gas Chromatography - Olfactometry (GC-O), Gas Chromatography - Flame Ionization (GC-FID), Gas Chromatography - Time of Flight (GC-TOF), and also a new column technology: Multidimensional Gas Chromatography (MDGC). Each type is unique and utilized differently since they differ in their end detector. These detectors may further be combined to obtain better identification of volatile compounds.

Keywords: *Gas Chromatography, Chromatogram, Column.*

HISTORY OF GAS CHROMATOGRAPHY

Gas Chromatography (GC) was introduced traditionally first by James and Martin in 1952. (Sparkman et al., 2011). However, according to Bartle and Myers (2002) and Kolomnikov et al (2018), Martin's works were just an initial pathway of the GC development. In 1941, Martin and his colleague Synge had experimented with the separation of amino acids and developed a technique to separate liquid, which was called Liquid Partition Chromatography. They also predicted that the chromatography should be possible to separate gas. However, no experimental proof of this theory was provided. In 1944, the two invented paper chromatography received a widespread use but then its use was almost

completely overshadowed by thin-layer chromatography. Later on, Martin and his new colleague James developed gas-liquid chromatography in 1952. After 1952, there has been constant development of the instrument and even combination with other instruments for further improvement and enhancement of the instrument performance. In present days, advanced instruments which are a result of various instrument like the Gas Chromatography-Mass Spectrometry (GC-MS) have been developed.

There were several researchers who claimed that they had conducted experiments on gas chromatography even before Martin and James. For example, in 1942, Gerhard Hesse introduced adsorption gas chromatography in gas-solid systems and in

1943, N.C. Turner created an instrument he claimed as the first industrial gas chromatograph in the world (Smolkova-Keulemansova, 2000; Kolomnikov et al, 2018). The instrument was used to partially fractionate natural gas. The instrument created by N.C. Turner in 1943 was nearly standing at 2-meter tall and the column was filled with charcoal, while a heated reservoir full of mercury was attached to the bottom of the column used as a displacer. Unfortunately, the instrument remained to be uncommon. In 1940s, Stig Claesson made a device that was able to analyse gases and liquid on charcoal columns with the chromatographic technique. It was claimed that the installation of the device was large enough and operated well. Despite being published, the article was completely forgotten. In the article, it was briefly mentioned that the founder of Beckman Instruments, Arnold Beckman, was interested in the device and going to produce it the idea was then abandoned as the device was deemed to be too complex and gas chromatography was a dead end (Kolomnikov et al, 2018).

HOW GC WORKS

Gas Chromatography is an instrument that works to separate, identify and quantitatively determine volatile compounds with boiling points up to 350°C or 400°C (Keulemans and Verver, 1959). GC is a partition chromatography where the liquid film is held in a solid support that acts as a stationary phase, and controlled gas flows in the surface of the liquid surface acting as the

moving phase. The temperature used in GC column is adjusted according to the mixture of components undergoing the partitioned separation (Horning et al., 1964).

Some limitations of this method include the necessity of the compounds being analyzed to be a stable volatile to some extent; and the dependency of temperature limit on the column. Horning et al. (1964) stated that even though molecular weight limitation is yet to be known, it is predicted that it will be around C_{75} for hydrocarbons (a region where carbon-carbon bond dissociation energy is equal to dispersion forces overcoming energy to provide vapor phase). Non-volatile polar substances must be derived to a less polar form before an analysis. GC also involves high temperature in the process, thus compounds decomposition cannot be avoided.

Before the process begins, there are a few things that need to be considered, such as the form of the sample, choices of column, as well as the carrier gas, oven temperature and evaporation during injections. (Sparkman et al., 2011). A sample injected to the instrument will be converted to a gaseous state and carried by a carrier gas. The separation of the components will take place at the column and will be identified by the detector. GC provides both qualitative and quantitative very important and valuable analytical data (Horning et al., 1964)

TYPES OF GC

GC is differentiated to a few types, where the difference is in its rear detector. This section will discuss a few types of GC.

Gas Chromatography-Mass Spectrometry (GC/MS). GC-MS (Gas Chromatography - Mass Spectrometry) is a combination of two different instruments, i.e Gas Chromatography and Mass Spectrometry (Sneddon et al., 2007). GC-MS could do both qualitative and quantitative analysis for volatile and semi-volatile organic compounds in various experimental samples (Sneddon et al., 2007). Scheme of GC-MS is shown in Figure 1. Compounds are injected and separated based on their volatility by GC, followed with analysis using the MS part of the instrument where the compound is shot using an electron until it breaks into ions which will come up in the detectors (Al-Bukhaiti et al., 2017). The data is transferred to the computer until the chromatogram receives it. Data obtained is in the form of chromatogram comprised peaks, recession time, retention time and a peak area which then are calculated for method validation and compound concentration (Darmapatni, 2016).

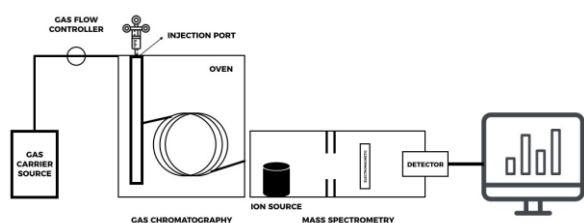


Figure 1. GC-MS Scheme (Designed with Keynote) redrawn from Shubin et al., (2012).

A column selected in an appropriate manner will produce an accurate and reliable analysis;

but if the column was selected improperly, it will generate inadequate, inaccurate, poor and unreliable separations which ultimately will lead to invalid or complex results. (Al-Bukhaiti et al., 2017). Gas Chromatography (GC) can analyse more than 10,000 compounds with more than 400 GC capillary columns (Singh et al., 2013). The choices of column and supporting instruments have a keen influence on the final result of separation optimization. The modification of the column parameters (stationary phase, length, inner diameter and film thickness) enhance chromatographers control for its column efficiency, resolution and speed of analysis (Grob and Barry, 1977).

The common carrier gases used are helium, nitrogen and hydrogen (Al-Bukhaiti et al., 2017). Inertness, absence of oxygen, dryness, safety, as well as the cost and availability of gas carriers should be considered for using (Fowles, 1995).

Application of GC-MS in food analysis involves food composition, such as food additives, flavor and aroma components, and also its contaminants, for example natural toxins, pesticides, fumigants, environmental pollutants, veterinary drugs, or packaging materials (Lehotay and Hajšlová, 2002).

Gas Chromatography-Olfactometry (GC/O)

GC/O is a technique to analyse odor activity from defined air streams which volatiles got separated using GC, by combining olfactometry or the use of human detectors (Friedrich and Acree, 1998). Scheme of GC/O is shown on Figure 2. The simplest form of GC/O, which is direct sniffing

of effluent from the gas chromatographic column has been known since 1964 (Fuller et al., 1964), but the combination of GC effluent with humid air under laminar flow started from 1970s and the quantitative dilution were developed in the mid-1980s (Friedrich and Acree, 1998).

In GC/O, the extract of food matrix is injected into a modified GC. The modification of GC is there presence of an olfactometer at the rear detector. A Sniffer is placed at the outlet of olfactometer that use to smell from humid air stream and record it (Friedrich and Acree, 1998).

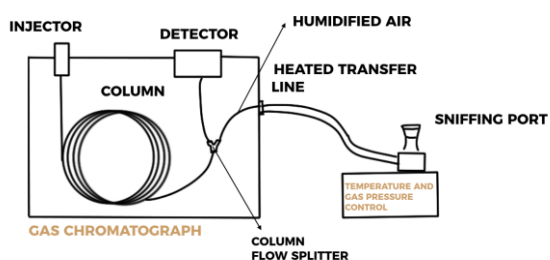


Figure 2. GC/O scheme (Designed with AutoDesk Sketchbook and Keynote) redrawn from Plutowska & Wardencki (2008)

Data produced by GC/O is a qualitative data in which the perception of the sniffer is shown. However, there has been a report of quantitative analysis using GC/O from Culleré et al., (2004) where judges gave a score between 0-3 to compounds, and the data analysis were done by ANOVA. Sniffer is trained with chemicals and standard vocabularies and it will last days-weeks (Cain, 1979). There are two ways to evaluate the data, aroma extract dilution analysis (AEDA) which and combined hedonic aroma response method (CHARM) analysis. AEDA measures

the maximum dilution of an extract that an odor is perceived in (Delahunty et al., 2006), it expresses the raw data as a flavor dilution values (Friedrich and Acree, 1998). CharmAnalysis™ records the duration of odors (Delahunty et al., 2006), expressing the raw data as Charm values (Friedrich and Acree, 1998).

Both data are comparable because it expresses a relative number of dilutions until the odor elutes from the column and become undetectable by the sniffer (Friedrich and Acree, 1998). The results of GC/O analysis could be expressed by odor activity values which can be classified as a chromatogram. The chromatogram describes the intensity and pattern of the odor-active compounds because an odor activity value is the ratio of the concentration of an odorant to its odor intensity (Friedrich and Acree, 1998). Charm values are obtained using a certain algorithm so they become proportional both to the amount of compound in the sample extract and inversely to the odor detection threshold (Delahunty et al., 2006). An easier interpretation of chromatogram is made by counting the ratio of the total amount of odor-active compounds eluting at a particular index to the threshold amount for that same mixture of compounds (Acree et al., 1984). AEDA reports the maximum dilution value, which is equivalent to the height of the Charm peak (Delahunty et al., 2006). AEDA is limited to the availability of statistical data manipulation, non-consideration of odor loss during the process of isolation and synergistic/suppressive/ distinct compounds in flavor

mixture (Zellner et al., 2008). Factor that also will affect the odor results is the dilution. This relevancy was shown by demonstrating the similarity of standard solution, a mixture of identified potent aroma compounds, to an actual food product (Gut and Gsroch, 1994; Grosch et al, 1995).

Drawbacks of GC/O analysis is directly related to the use of human detector. Abbott et al. (1993) mentioned that it is hard for panellists to detect the end of odor region. GC/O is time-intensive and typically done with 1-2 panellists. Panellists need to be carefully pre-screened for sensitivity and specific anosmia (Friedrich and Acree, 1998). It also has been shown that individual's olfactory sensitivity changes throughout the day, where ovulatory cycle occurs (Koster, 1968), which became a concern because series of dilution analysis often takes weeks to perform.

Gas Chromatography-Flame Ionization (GC/FID). GC/FID is based on the measurement of the electrical conductivity of a hydrogen flame (Horning et al., 1964). It measures the amount of carbon that is present in a sample. Horning et al. (1964) mentioned that the low electrical conductivity of burned hydrogen will increase when organic vapors are mixed in. After the sample passes through the column, it is burned in a hot, hydrogen-air flame. This combustion produced carbon ions. However, the efficiency of using this instrument debatable since only around 1 of 10^5 carbon ions are produced during combustion. But the amount of ions that is produced is proportional to the amount of

carbon that is present in the sample. Electrodes inside the instrument are used to measure the number of carbon ions present in the sample.

GC-FI is known to be a destructive instrument since all of the samples will be pyrolyzed (JoVE Science Education Database, 2019). GC-FID mostly used in quantitative analysis of hydrocarbon compounds and oil analysis. It is important to calibrate the GC-FID system using calibration standard for every analyte for quantitative analysis. The factor response method (determined based on the material standard analysis or another method before analyte measurement) also used for GC-FID calibration. FID response for hydrocarbon usually comparable with the amount of analyte carbon. Test compound quantitative analysis often done using one of the compounds in the sample as the internal calibration standard and the result is compared with the standard. With this system, only one material standard which is needed and the fee for the analysis greatly reduced compared with a conventional calibration method that need calibration standard for every analyte (Godswill et al., 2014).

The qualitative method results in a piece of information about the chemical identity from species in a sample. The qualitative analysis showed time retention ratio between chromatographic peak which contains a peak of unknown compound which is obtained for sample reference using more than one stationary phase. On the other side, the quantitative method gives numerical

information about the amount of one or more compounds in a sample. The purpose of quantitative analysis is to determine the specific amount of compound molecule in a sample. The most common of two different analytes with the same concentration will give a different response detector for chromatography. Therefore, the detector response must be measured to estimate the concentration of each compound, known as a standard curve (Godswill et al., 2014).

In GC-FID measurement, the standard material for every targeted compound is very important for a quantitative analysis. The time retention specificity for a sample and the standard methyl ester are also measured to have a calibration curve and correlation coefficient. The accuracy and precision of the method is evaluated by measurement of the standard and sample, which also decides the recovery. The average ratio for standards and sample must be close enough to 100% for the given concentration (Godswill et al., 2014).

Gas Chromatography-Time-of-Flight (GC-TOF). The technology was first developed in the 1940s by Stephens et al. Two years later, the first TOF MS was constructed by Cameron and Eggers. However, TOF MS only started gaining popularity in the 1960s due to its magnetic sector and quadrupole instruments. Currently, there are three types of GC-TOF-MS instruments differed by their basic characteristics (Cajka, 2013):

- High-resolution/accurate mass analyzer: providing moderate acquisition speed (20-50 spectra/s)
- Unit-resolution instruments that feature high acquisition speeds (500-1000 spectra/s)
- High-speed high-resolution/accurate mass analysers allowing high acquisition speeds (up to 200 spectra/s) as well as high mass resolving power (50,000 FWHM).

The new type of TOF-MS instrument combined quadrupole and TOF-MS allows analysis under conditions of high-resolution time-of-flight mass spectrometry with selections of precursor ions and monitoring the product ions throughout the entire mass range with high mass accuracy (Cajka, 2013). A GC-TOF scheme can be seen in Figure 4. Samples injected into the machine needs to be either gas or liquid. If the sample is solid, then the sample needs to be dissolved beforehand. Liquid samples are going to be vaporized first. After the sample is vaporized, gaseous ions will form from the analyte. Gaseous ions that formed by the analyte is accelerated to get constant kinetic energy and then ejected to the mass analyser, using a pulsed electric-field gradient which has a positive influence on the mass resolution of the instrument. Ions with higher mass will penetrate the reflector deeper which extends the time needed for them to reach the detector. When the ion hits the detector, the detector sends an electrical message to the computer processor. Ions with the same m/z value will reach the detector at almost the same time. In order to improve the

resolution, the ions are passed along the flight tube twice. The time taken for an ion to go through the tube and reach the detector is dependent on the mass to charge (m/z) value of the ion.

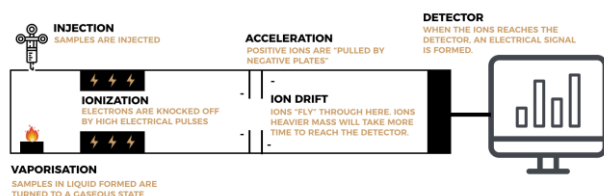


Figure 4. GC-TOF-MS scheme (Designed with Keynote.) redrawn from Boots et al., (2012)

GC-TOF-MS is advantageous for fast chromatography. One GC TOF MS instrument can measure more than 1000 mass spectra per-second. In food technology, GC TOF MS is often used for flavor components, drugs screening, petrochemical analysis or metabolomics studies. Other than that, the instrument is also used to determine pesticide residues, polybrominated diphenyl ethers (PDBEs), acrylamide and volatiles in different food matrices (Cajka and Hajslova, 2007).

Multidimensional Gas Chromatography (MDGC). MDGC is a new column of technology which can be an alternative to analyze complex samples. MDGC system consists of two or more column with individual separation to increase peak capacity by physically separating compounds from a complex (Herrero et al., 2009). There are a few types of MDGC. MDGC techniques are divided into two-dimensional gas chromatography (GC-GC), and heart-cut multidimensional gas chromatography (H/C-MDGC) (Marriott et al., 2012).

GC-GC used two columns with different compositions (Herrero et al., 2009). GC-GC Scheme is shown in Figure 5. The stationary phase in the first dimension is less polar than the second so that in the first dimension, the separation is done by differentiating boiling point properties, wherein the second used polarity (Ryan et al., 2005). GC-GC effluent transfer must be operated at high speed, so a rapid sampling which doesn't affect the second-dimension analysis is needed. To facilitate an effluent transfer, a modulator is used in between two columns, which also functions as signal amplitude increaser and the key component of GC-GC. (Herrero et al., 2009; Marriott et al., 2012). In the first dimension, the use of TOF detector that features high mass accuracy and resolution and good scanning speed which combined to a mass spectrometer is preferred, to analyze unknown compounds since it has a wide range of mass. This technique offers fast run time and increased capacity, but the drawback of this technique is that its expensive equipment and maintenance, difficult optimization method and the not so high sensitivity improvement (Herrero et al., 2009). GC-GC can be integrated with liquid chromatography (LC). Online automated LC/GC were developed by de Koning et al. (2004), which consist of two-dimensional chromatography (liquid and gas). Due to the slow GC preparation, LC was operated in sop-flow mode. Automatic transfer of sequential LC fractions to GC were done by six-port switching valve or dual side-port syringe (Marriott et al., 2012).

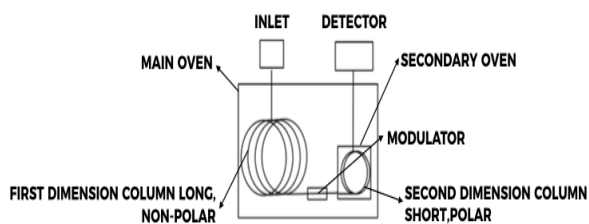


Figure 5. GC-GC Scheme (Designed with Autodesk Sketchbook and Keynote) redrawn from Murray (2012)

A H/C-MDGC instrument (shown in Figure 6) uses two approaches of effluent switching (Marriott et al., 2012). Sequential transfer of compounds from the first to second column is usually done using an on-line heart cut, only allowing the transport of some key analytes (Herrero et al., 2009). Herrero et al. (2009) highlighted that the system is based on longitudinally modulated cryogenic system (LMCS), which controls peak transfer by blocking and releasing from chromatogram between two columns. This technique broadens the peaks, but it is limited to a fast analysis, thus some types of columns could not be used and very fast detectors are needed after the second dimension (Herrero et al., 2009).

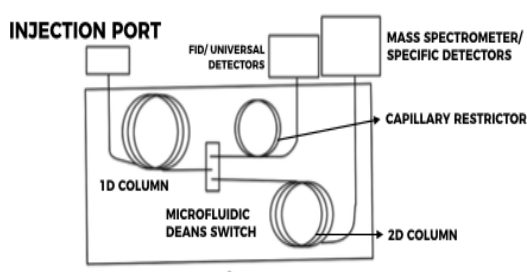


Figure 6. H/C-MDGC scheme (Designed with Autodesk Sketchbook and Keynote) redrawn from Nolvachai et al., (2017)

MDGC-Olfactometry (MDGC/O) is also popular in food sample analysis. Compounds with similar chemistry have a high probability of co-elution. Thus, there is a high probability that compounds will co-elute in single dimensional GC. Identification of a trace odor-active compound may be masked by larger odorless peak because GC-O runs under rapid GC conditions. The use of MDGC/O can resolve discrete regions of compounds which co-elute each other and separate chirals so odor intensity, potency and enantiomers can be evaluated (Delahunty et al., 2006; Begnaud & Chaintreau, 2005).

CONCLUSIONS

It is concluded that GC has a very broad utilization in food analysis. Because the types of detectors are very specific, it is usually used for different analysis, but it can also be combined to obtain better data analysis. GC/MS is used to determine wide range of samples as it has mass spectra database which can be used to interpret and compared to the sample. GC/O is used to determine volatiles and aromas present in food sample, by using human as a sniffer. GC/FID is usually used in hydrocarbons and oils analysis, because the principle is to detect conductivity of the burned sample. GC/TOF is combined with a MS detector, to identify unknown sample by calculating ion's time of flight to a detector. MDGC is combination of multiple columns (two or more) to obtain higher peak capacity, since complex compounds can be separated.

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