CHAPTER II
LITERATURE REVIEWS

2.1 Insecticide

Insecticide is a group of pesticide used for control of insects. This group includes ovicides and larvicides which are used for killing eggs and larvae of insects respectively. Insecticides are used mostly in agriculture and the household. The use of insecticides is believed to be one of major factors behind the increase of agricultural productivity in the 20th century. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; and others are contaminated in the food chain.Other good qualification of pesticide are repellent, attractants, chemosterilants and growth regulators in which fipronil is certainly included [11].

2.2 Fipronil

Fipronil is a phenylpyrazole insecticide discovered by Rhône-Poulenc Ag Company (now Bayer CropScience) in 1987, introduced in 1993, and registered in the U.S. in 1996 [12],[13] .

This insecticide can control a broad spectrum of insects such as aphids, beetles, worms, grasshoppers, ticks, and fleas in both larval and adult stages [14]. Fipronil is effective, at low field application rates, against insects that are resistant to pyrethroids, organophosphates, and carbamate insecticides [15]. It can be formulated as solid, liquid spray, or as a granular product. Fipronil is the active ingredient in Frontline®, Ascent®, Termidor® and Top Spot® [16].

The chemical structure of fipronil is shown in figure 2.1 fipronil can be degraded by oxidation and decomposition of chemical reactions with light in the form of fipronil-sulfide, fipronil-sulfone, fipronil-.desulfinyl and fipronil-amide, respectively, which normally occurs during the use in crop cultivation [17]. The structure of fipronil after degrading is shown in figure 2.2.

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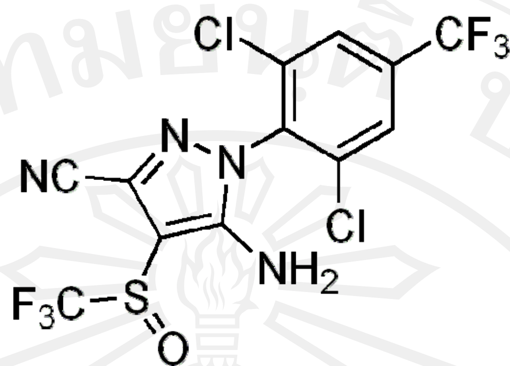

Figure 2.1 The structure of fipronil. (molecular weight 437.2 g/mol).

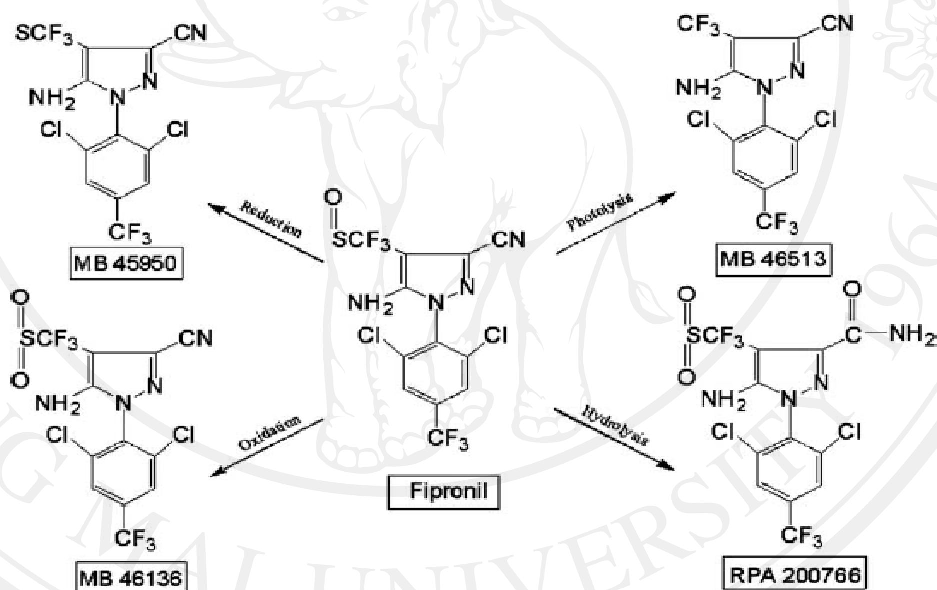

Figure 2.2 Derivatives of fipronil.

2.3 Physical-chemical properties of fipronil

Fipronil is low to moderately soluble in water, prefers lipophilic (organic) matrices such as lipids, oils, lignin, proteins, and organic solvents, and is stable at room temperatures (Table 2.1).

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Table 2.1 Physical-chemical properties of fipronil. All parameters are at 25°C unless specified.

Property	Condition	Value
Chemical Abstract Service registry number (CAS #)[1]		120068-37-3
Molecular weight (g/mol)[1]		473.2
Solubility[1]	Water (mg/L; pH = 5)	1.90
	Water (mg/L; pH = 9)	2.40
	Hexane (mg/L)	28.0
	Toluene (mg/L)	3000
Melting point (°C)[1]		200-201
Density (g/mL 20°C)[1]		1.48-1.63
Vapor pressure (mPa; calculated)[6]		3.7×10^{-4}
Henry's constant (m^3·atm/mol; experimental)[2]		6.60×10^{-6}
Henry's constant (m^3·atm/mol; calculated)[6]		8.50×10^{-10}
Octanol-water partition coefficient (Log K_{ow})[6]		3.50
Organic carbon normalized partition coefficient (averaged K_{oc})[3]		825
Aqueous photolysis (days; pH = 5)[6]		0.33
Hydrolysis half-life (days)[4]	pH = 5.5	>100
	pH = 7.0	>100
	pH = 9.0	32.08
	pH = 10	4.75
	pH = 11	0.45 (11 hours)
	pH = 12	0.1 (2.4 hours)
Aerobic soil half-life (days)[5]		188

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Table 2.1 Physical-chemical properties of fipronil. All parameters are at 25°C unless specified.(cont.)

Anaerobic soil half-life (days) [2]	Dry flowable formulation	19.3-22.2
	Granular formation	18.3
Anaerobic water half-life (days) [1]	Dry flowable formulation	0.92-2.83
	Granular formation	5.20

[1] The Pesticide Manual, 2000; [2] Ngim and Crosby, 2001; [3] Ying and Kookana, 2001; [4] Bobe et al., 1998b; [5] Ying and Kookana, 2002; [6] Rhône-Poulenc Ag Company, 1998.

2.4 Mode of Action

Fipronil is a "new generation" insecticide as its mode of action does not follow the common biochemical pathways of pyrethroids (sodium channel blockers), organophosphates, and carbamate (cholinesterase inhibitors) which are classical insecticides to which some insects have developed resistance found that fipronil interferes with the γ-aminobutyric acid (GABA)-gated channels; fipronil disrupts normal nerve influx transmission (e.g., passage of chloride ions) by targeting the GABA-gated chloride channel and at sufficient doses, causes excessive neural excitation, severe paralysis, and insect death(18),(19). Fipronil demonstrates a selective toxicity toward insects (LC_{50} can be low as 24.8 nM or ~11.7 μg/L) by having a tighter binding affinity toward the GABA-regulated chloride channels of insects than the mammalian GABA receptors (20).

2.5 Toxicity of fipronil

WHO (World Health Organization) is an organization, is responsible for grouping the toxication of a chemical insecticide. This organization has appointed fipronil as a moderately hazardous chemical insecticide, testing the oral toxicity to rat at 97 mg/kg (Acute oral LD_{50}) and the skin toxicity to rat at 2000 mg/kg (Acute dermal LD_{50}). Besides, the LD_{50} toxicity to bird is 11.3 ug/kg, to rainbow trout (fish) is 31 ug/kg and to bee is 4 ng/1bee) (USEPA, 1996). Anyhow, there is no report about toxicity to human. But there is a study report on fipronil and rat, finding that giving the high volume of fipronil continuously for a long time can cause thyroid tumors in

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rats. In addition, fipronil also effects ecosystem of the Organisms in soil and water and the environment [21].

There was a study to find the amount of fipronil residue continuously in the past because the world realizes of the danger of fipronil remained in the product and of the impact towards human and environment. The results of the study on the amount of fipronil residue are as follows:

In 2001, Vilchez *et al.,* [5] analyzed fipronil residues in soil, water and human urine by gas chromatography using the MS (mass spectrometry) measurement tool. The sample was extracted by acetonitrile and cleaned up by SPME; 85 um polyacrylate. The % recovery was found to be in the range of 94-102%, with the % RSD in the range 8.5 to 10.5% and the LOD was 0.08 milligram/kilogram. The LOQ was 0.1 milligram/kilogram.

In 2004, Pei *et al.*,[6] determination fipronil residues in pakchoi by gas chromatography with the ECD detection. The sample was extracted with dichloromethane and cleaned up by SPE; alumina and the cartridge was rinsed by benzene: acetonitrile 70: 30, v / v, the % recovery was found to be in the range of 83-86%, % RSD in the range of 4.5 to 5.1% and the LOD was 0.02 milligram/kilogram and the LOQ was 0.03 milligram/kilogram.

In 2006, Reza *et al.*, [7] analyzed fipronil in water and soil from the rice fields by using HPLC with UV detection. The sample was extracted with dichloromethane: acetone with C-18 column and rinsed a acetonitrile the % recovery in the range of 87-102%, % RSD in the range of 2.8 to 4.9% and the LOD was 0.01 milligram/kilogram and the LOQ was 0.1 milligram/kilogram.

In 2008, Bichon *et al.*, [8] analyzed fipronil in ovine plasma by GC/MS/MS. The sample was extracted with acetonitrile: isooctane and clean up by SPE; ATOOL XC 96 well plate and PS-DVB and the cartridge was rinsed by methanol, the % recovery in the range of 122-129%, % RSD in the range of 2.8 to 4.9% and the LOD was 0.1 milligram/kilogram and the LOQ was 0.2 milligram/kilogram.

In 2008, Jim'enez *et al.,* [9] analyzed fipronil in honey by Gas chromatography with the MS (mass spectrometry) measurement tool. The sample was extracted with hexane and clean up by SPE; florisil. The cartridge was rinsed by hexane:

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dichloromethane, the % recovery in the range of 68-82%, %. RSD in the range 4.9 to 8.0% and the LOD was 0.05 milligram/kilogram and the LOQ was 1.0 milligram/kilogram.

In 2008, Liu *et al*., [10] analyzed fipronil in Chinese cabbage by gas chromatography with the MS (mass spectrometry) measurement tool. The sample was extracted with acetonitrile and clean up by SPE; alumina and the cartridge was rinsed by petroleum ether: ethyl acetate ratio 7 / 3, v, v. The % recovery in the range of 78-87%, % RSD in the range. 2.5 to 4.9% and the LOD was 0.05 milligram/kilogram and the LOQ was 0.1 milligram/kilogram.

From the research above, the finding was the LOQ (Limit of quantitation) of the residue in each country was likely high in the comparison of Codex MRLs in vegetable was at 0.02 milligram/kilogram and EU MRLs was appointed in sweet green pepper at 0.005 milligram/kilogram. Thus, the LOQ in research was unsafe to the consumers. The objectives of this research are to improve the analytics method to find the amount of the fipronil residue which is safer to consumers. Another reason to do this research is that there has not been any report about the analysis on finding the amount of the fipronil residue in crop and agricultural produce in Thailand before.

2.6 The sample preparation for GC analysis. [22]

The SPE (Solid Phase Extraction) is a good technique for sample preparation. It has been brought to replace the LLE (Liquid-Liquid Extraction) because of having low cost and less solvent use. In sample preparation, the technique mootly used for extraction is LLE, but it has disadventages because of emulsion may occur. More over, it takes a long times, using more glassware, which increasing contaminances to interfere with analysis. Organic solvent is expensive and must be used at a high quantity. This brings the problem on toxic waste to the environment afterward. To reduce the amount of the solvent used, more appropriate technique is requided. Solid Phase Extraction (SPE) is a good technique for sample preparation which can replace the LLE. This technique has a low cost and using less organic solvent. SPE has a similar principle to LLE in partition process but different from LLE in the way that the partition does not occur between liquid and liquid but it occurs between solid

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(absorbent in SPE cartridge) and the liquid added. The good things for using SPE can be concluded as follows:

1. Reducetion of organic solvents used.
2. Short-time consuming.
3. Reducetion of sample preparation; extraction, increasing concentration and clean up are in the same step.
4. It is easy to choose appropriate SPE due to varieties of SPE.

It can be seen that SPE is technique used for reduction of complicated sample preparation process and can delimiting the interference of analysis. It can help improving efficacy of analysis and extending long-life of the column use.

To choose the adsorbent solid phase extraction technique, should be done with the polarity to absorb the target substance to be separated. If it is a non-polar compounds. (hydrophobic), the adsorbent should have the same non-polar, such as carbon -18 (C18) in the form of bonded silica, The absorbing carbon -18 can be used to analyze small amounts of organic matter in water samples. The separation of such a non-polar solvent with water as solvent can be used to absorb in Table 2.2

Table 2.2 Selection of adsorbents in SPE cartridge [23].

Mode	**Sorbent type**	**Property Of the adsorbent.**	**Analysis**
Reversed phase	C18, C8, C4, C2, Cyclohexyl, Phenyl	Hydrophobic bonded silica with Non-polar or with medium polar	Separate group of non-polar or hydrophobic compounds from solution, aqueous solution.
Normal phase	Silica, Florisil, Amino, Cyano, Diol, Alumina	Hydrophilic with polar	Separate polar compounds from non-polar solution.
Anion exchange	SAX	Anionic compound	The separation of negatively charged ions.
Cation exchange	SCX	Cationic compound	The separation of positively charged ions.

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The type of SPE can be selected based on solubility of the target substance as shown in 2.3

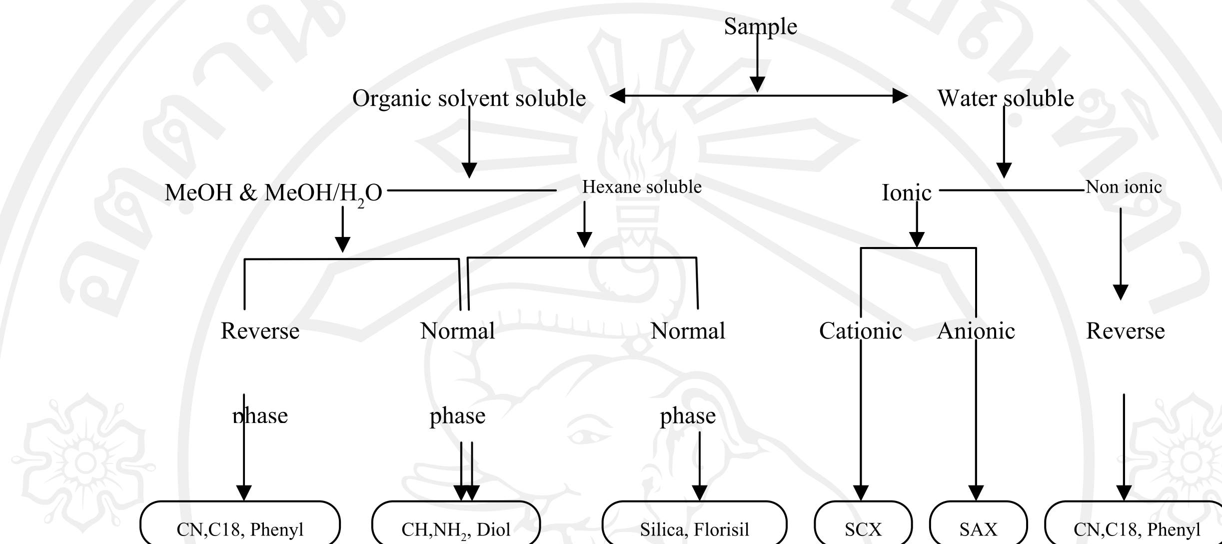

Figure 2.3 The selection of SPE, considering from the solubility of the sample substance.

At present, the suitable way to analyze fipronil is to use LC-MS or LC-MS-MS as a tool, but, the tools are very expensive and are not available in the laboratory. It is necessary to analyze the fipronil residue in sweet green pepper with the use of gas chromatography - mass spectrometry. The fipronil residue is extracted by the suitable solvent and cleaned up by the technique of Solid Phase Extraction. Then, it is analyzed by gas chromatography and is measured by MS detector, using the statistical parameter to validate methods.

2.7 The validity for analytical method.

1. Accuracy is a measure of the difference between the true value and a set of experimentally determined data. The Accuracy of the test solution is measured by percentage recovery (% Recovery) and is analyzed by adding the standard substance in the sample for the concentrations level at low, medium and high for at least 10 times repeatedly, then, calculated from the formula.

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$$\%Recovery = (\bar{x}_1 - \bar{x}_2 / C) \times 100$$

When $\bar{x}_1$ = volume analyzed

$\bar{x}_2$ = existing sample volume

C = volume added to the sample

The accepted criteria will depend on the concentration level of standard added in the samples, as shown in Table 2.3.

Table2.3 Criteria for analysis of pesticide residues [24].

Concentration	Repeatability %CV	Reproducibility %CV	Range of Mean %Recovery
≤ 1 μg/kg	35	53	50-120
> 1 μg/kg ≤ 0.01 mg/kg	30	45	60-120
> 0.01 mg/kg ≤ 0.1 mg/kg	20	32	70-120
> 0.1mg/kg ≤ 1 mg/kg	15	23	70-110
> 1 mg/kg	10	16	70-110

Sources; GUIDELINES ON GOOD LABORATORY PRACTICE IN RESIDUE ANALYSIS., CAC/GL 40-1993, Rev.1-2003. page 25.

2. The precision is the closeness of agreement between a series of measurements obtained from multiple sampling of same homogeneous sample. The precision of the test solution is measured by percentage of the coefficient of variation (% CV) and is analyzed by adding the standard substance in the sample for at least 10 times repeatedly. Results will be calculated from the formula indicated below:

$$\%CV = (SD / \bar{x}) \times 100$$

When SD = Standard deviation

$\bar{x}$ = The average volume of residue in the sample

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The accepted criteria will depend on the concentration level of the standard added in the sample. The value must not exceeded the value, listed in Table 2.3 which will be divided into two types.

2.1 The precision of repeatability is calculated from analyzing the samples in the same set, on the same day, by the same person, or with the same analytical tools.

2.2 The precision of the reproducibility is calculated from analyzing the samples in different set on different day, by different person and with different analytical tools.

3. The Limit of detection (LOD) is a parameter that indicates the ability of the test method to detect residues in the sample, informing the concentration level (mg / kg) at the lowest quantity in the sample detected. It is measured from standard deviation (SD) to analyze the sample added at low concentration of standard substance for 10 times repeatedly, then, calculate the average LOD value.

$$LOD_{approx} = 3 \bullet SD$$

When LOD_{approx} = The average LOD value

SD = Standard deviation

To make a confirmation by preparing the samples with concentrations equal to the LOD approx. This must be repeated. Signal of the peak from GC must not less than 3 times of the noise.

4. The Limit of quantitation (LOQ) is a parameter that indicates the ability of the test method to detect residues in the sample, being able to measure lowest concentration level (mg / kg) in the sample and can tell the quantity in figure. It is measured from standard deviation to analyze the sample added the low standard substance for 10 times repeatedly, then, calculate the average LOQ value.

$$LOQ_{approx} = 10 \bullet SD$$

When LOD_{approx} = The average LOQ value

SD = Standard deviation

Confirmation by the preparing the sample with concentrations equal to the LOQ approx. Percentage of recovery was brought to compare with accepted criteria as shown in Table 2.3

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2.8 Gas chromatography [25]

Gas chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical use of GC includes testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.

In gas chromatography, the mobile phase (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside there is a piece of glass or a metal tube called a column (a homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences. Firstly, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas in column chromatography the stationary phase is a solid and the mobile phase is a liquid Hence the full name of the procedure is "Gas-liquid chromatography", referring to the mobile and stationary phases, respectively. Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences. However, fractional distillation is typically used to

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separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale (i.e. microscale).

Gas chromatography is also sometimes known as vapor-phase chromatography (VPC), or gas-liquid partition chromatography (GLPC). These alternative names, as well as their respective abbreviations, are frequently found in scientific literature. Strictly speaking, GLPC is the most correct terminology, and is thus preferred by many authors.

Gas analysis

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature.

In a GC analysis, a known volume of gaseous or liquid analyze is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyze molecules through the column, this motion is inhibited by the adsorption of the analyze molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyze mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by

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the order in which they emerge (elute) from the column and by the retention time of the analyze in the column.

Physical components

Autosamplers

The autosampler provides the means to introduce a sample automatically into the inlets. Manual insertion of the sample is possible but is no longer common. Automatic insertion provides better reproducibility and time-optimization.

Different kinds of autosamplers exist. Autosamplers can be classified in relation to sample capacity (auto-injectors vs. autosamplers, where auto-injectors can work a small number of samples), to robotic technologies (XYZ robot vs. rotating robot – the most common), or to analysis:

- Liquid
- Static head-space by syringe technology
- Dynamic head-space by transfer-line technology
- Solid phase microextraction (SPME)

Traditionally autosampler manufacturers are different from GC manufacturers and currently no GC manufacturer offers a complete range of autosamplers. Historically, the countries most active in autosampler technology development are the United States, Italy, Switzerland, and the United Kingdom.

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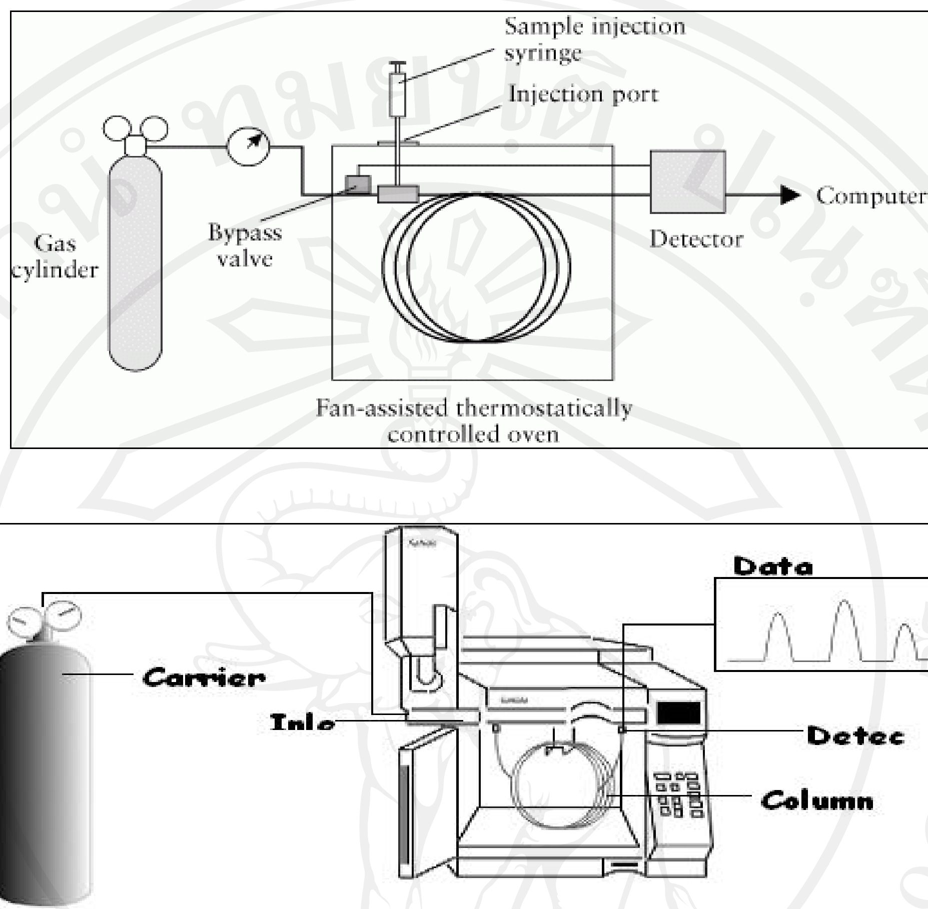

Figure 2.4 Diagram of a Gas Chromatograph

Inlets

The column inlet (or injector) provides the means to introduce a sample into a continuous flow of carrier gas. The inlet is a piece of hardware attached to the column head.

Common inlet types are:

- S/SL (Split/Splitless) injector; a sample is introduced into a heated small chamber via a syringe through a septum - the heat facilitates volatilization of the sample and sample matrix. The carrier gas then either sweeps the entirety (splitless mode) or a portion (split mode) of the sample into the column. In split mode, a part of the sample/carrier gas mixture in the injection chamber is exhausted through the split vent.

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Split injection is preferred when working with samples with high analyte concentrations (>0.1%) whereas splitless injection is best suited for trace analysis with low amounts of analytes (<0.01%). In splitless mode the split valve opens after a pre-set amount of time to purge heavier elements that would otherwise contaminate the system. This pre-set time can equal the total runtime to effectively keep the purge closed.

- On-column inlet; the sample is here introduced directly into the column in its entirety without heat.
- PTV injector; Temperature-programmed sample introduction was first described by Vogt in 1979. Originally Vogt developed the technique as a method for the introduction of large sample volumes (up to 250 μL) in capillary GC. Vogt introduced the sample into the liner at a controlled injection rate. The temperature of the liner was chosen slightly below the boiling point of the solvent. The low-boiling solvent was continuously evaporated and vented through the split line. Based on this technique, Poy developed the Programmed Temperature Vaporizing injector; PTV. By introducing the sample at a low initial liner temperature many of the disadvantages of the classic hot injection techniques could be circumvented.
- Gas source inlet or gas switching valve; gaseous samples in collection bottles are connected to what is most commonly a six-port *switching valve*. The carrier gas flow is not interrupted while a sample can be expanded into a previously evacuated *sample loop*. Upon switching, the contents of the sample loop are inserted into the carrier gas stream.
- P/T (Purge-and-Trap) system; An inert gas is bubbled through an aqueous sample causing insoluble volatile chemicals to be purged from the matrix. The volatiles are 'trapped' on an absorbent column (known as a trap or concentrator) at ambient temperature. The trap is then heated and the volatiles are directed into the carrier gas stream. Samples requiring preconcentration or purification can be introduced via such a system, usually hooked up to the S/SL port.

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The choice of carrier gas (mobile phase) is important, with hydrogen being the most efficient and providing the best separation. However, helium has a larger range of flowrates that are comparable to hydrogen in efficiency, with the added advantage that helium is non-flammable, and works with a greater number of detectors. Therefore, helium is the most common carrier gas used.

Detectors

A number of detectors are used in gas chromatography. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before an FID (destructive), thus providing complementary detection of the same analytes.

Other detectors are sensitive only to specific types of substances, or work well only in narrower ranges of concentrations. They include:

- catalytic combustion detector (CCD), which measures combustible hydrocarbons and hydrogen.
- discharge ionization detector (DID), which uses a high-voltage electric discharge to produce ions.
- dry electrolytic conductivity detector (DELCD), which uses an air phase and high temperature (v. Coulsen) to measure chlorinated compounds.
- electron capture detector (ECD), which uses a radioactive Beta particle (electron) source to measure the degree of electron capture.
- flame photometric detector (FPD)
- flame ionization detector (FID)
- Hall electrolytic conductivity detector (ElCD)
- helium ionization detector (HID)
- Nitrogen Phosphorus Detector (NPD)

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- Infrared Detector (IRD)
- mass selective detector (MSD)
- photo-ionization detector (PID)
- pulsed discharge ionization detector (PDD)
- thermal energy(conductivity) analyzer/detector (TEA/TCD)
- thermionic ionization detector (TID)

Some gas chromatographs are connected to a mass spectrometer which acts as the detector. The combination is known as GC-MS. Some GC-MS are connected to an NMR spectrometer which acts as a backup detector. This combination is known as GC-MS-NMR. Some GC-MS-NMR are connected to an infrared spectrophotometer which acts as a backup detector. This combination is known as GC-MS-NMR-IR. It must, however, be stressed this is very rare as most analyses needed can be concluded via purely GC-MS.

Method

The method is the collection of conditions in which the GC operates for a given analysis. Method development is the process of determining what conditions are adequate and/or ideal for the analysis required.

Conditions which can be varied to accommodate a required analysis include inlet temperature, detector temperature, column temperature and temperature program, carrier gas and carrier gas flow rates, the column's stationary phase, diameter and length, inlet type and flow rates, sample size and injection technique. Depending on the detector(s) (see below) installed on the GC, there may be a number of detector conditions that can also be varied. Some GCs also include valves which can change the route of sample and carrier flow. The timing of the opening and closing of these valves can be important to method development.

Carrier gas selection and flow rates

Typical carrier gases include helium, nitrogen, argon, hydrogen and air. Which gas to use is usually determined by the detector being used, for example, a DID requires helium as the carrier gas. When analyzing gas samples, however, the carrier is sometimes selected based on the sample's matrix, for example, when

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analyzing a mixture in argon, an argon carrier is preferred, because the argon in the sample does not show up on the chromatogram. Safety and availability can also influence carrier selection, for example, hydrogen is flammable, and high-purity helium can be difficult to obtain in some areas of the world. (See: Helium--occurrence and production.) As a result of helium becoming more scarce, hydrogen is often being substituted for helium as a carrier gas in several applications.

The purity of the carrier gas is also frequently determined by the detector, though the level of sensitivity needed can also play a significant role.Typically, purities of 99.995% or higher are used. The most common purity grades required by modern instruments for the majority of sensitivities are 5.0 grades, or 99.999% pure meaning that there is a total of 10ppm of impurities in the carrier gas that could affect the results. The highest purity grades in common use are 6.0 grades, but the need for detection at very low levels in some forensic and environmental applications has driven the need for carrier gases at 7.0 grade purity and these are now commercially available. Trade names for typical purities include "Zero Grade," "Ultra-High Purity (UHP) Grade," "4.5 Grade" and "5.0 Grade."

The carrier gas linear velocity affects the analysis in the same way that temperature does (see above).The higher the linear velocity the faster the analysis, but the lower the separation between analytes. Selecting the linear velocity is therefore the same compromise between the level of separation and length of analysis as selecting the column temperature. The linear velocity will be implemented by means of the carrier gas flow rate, with regards to the inner diameter of the column.

With GCs made before the 1990s, carrier flow rate was controlled indirectly by controlling the carrier inlet pressure, or "column head pressure." The actual flow rate was measured at the outlet of the column or the detector with an electronic flow meter, or a bubble flow meter, and could be an involved, time consuming, and frustrating process. The pressure setting was not able to be varied during the run, and thus the flow was essentially constant during the analysis. The relation between flow rate and inlet pressure is calculated with Poiseuille's equation for compressible fluids. Many modern GC, however, electronically measure the flow rate, and electronically control the carrier gas pressure to set the flow rate. Consequently, carrier pressures

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and flow rates can be adjusted during the run, creating pressure/flow programs similar to temperature programs.

Stationary compound selection

The polarity of the solute is crucial for the choice of stationary compound, which in an optimal case would have a similar polarity than the solute. Common stationary phases in open tubular columns are cyanopropylphenyl dimethyl polysiloxane, carbowax polyethyleneglycol, biscyanopropyl cyanopropylphenyl polysiloxane and diphenyl dimethyl polysiloxane. For packed columns, there are more options available [26].

Inlet types and flow rates

The choice of inlet type and injection technique depends on if the sample is in liquid, gas, adsorbed, or solid form, and on whether a solvent matrix is present that has to be vaporized. Dissolved samples can be introduced directly onto the column via a COC injector, if the conditions are well known; if a solvent matrix has to be vaporized and partially removed, a S/SL injector is used (most common injection technique); gaseous samples (e.g., air cylinders) are usually injected using a gas switching valve system; adsorbed samples (e.g., on adsorbent tubes) are introduced using either an external (on-line or off-line) desorption apparatus such as a purge-and-trap system, or are desorbed in the S/SL injector (SPME applications).

Sample size and injection technique

Sample injection

The real chromatographic analysis starts with the introduction of the sample onto the column. The development of capillary gas chromatography resulted in many practical problems with the injection technique. The technique of on-column injection, often used with packed columns, is usually not possible with capillary columns. The injection system in the capillary gas chromatograph should fulfil the following two requirements:

1. The amount injected should not overload the column.

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2. The width of the injected plug should be small compared to the spreading due to the chromatographic process. Failure to comply with this requirement will reduce the separation capability of the column. As a general rule, the volume injected, V_{inj}, and the volume of the detector cell, V_{det}, should be about 1/10 of the volume occupied by the portion of sample containing the molecules of interest (analytes) when they exit the column.

Some general requirements which a good injection technique should fulfill are:

- It should be possible to obtain the column's optimum separation efficiency.
- It should allow accurate and reproducible injections of small amounts of representative samples.
- It should induce no change in sample composition. It should not exhibit discrimination based on differences in boiling point, polarity, concentration or thermal/catalytic stability.
- It should be applicable for trace analysis as well as for undiluted samples.

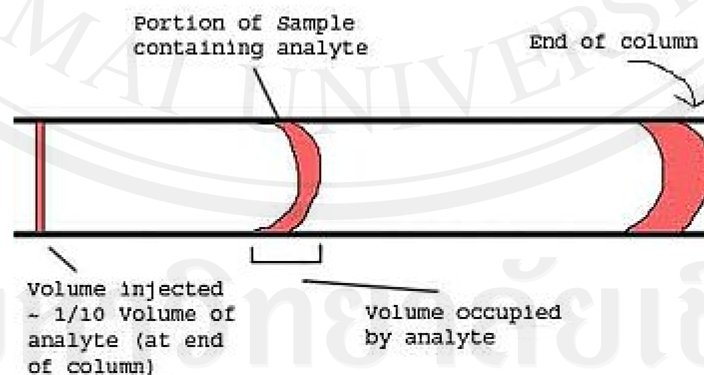

Figure2.5 The rule of ten in gas chromatography

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Column selection

The choice of column depends on the sample and the active measured. The main chemical attribute regarded when choosing a column is the polarity of the mixture, but functional groups can play a large part in column selection. The polarity of the sample must closely match the polarity of the column stationary phase to increase resolution and separation while reducing run time. The separation and run time also depends on the film thickness (of the stationary phase), the column diameter and the column length.

Column temperature and temperature program

The column(s) in a GC are contained in an oven, the temperature of which is precisely controlled electronically. (When discussing the "temperature of the column," an analyst is technically referring to the temperature of the column oven. The distinction, however, is not important and will not subsequently be made in this article.)

The rate at which a sample passes through the column is directly proportional to the temperature of the column. The higher the column temperature, the faster the sample moves through the column. However, the faster a sample moves through the column, the less it interacts with the stationary phase, and the less the analytes are separated.

In general, the column temperature is selected to compromise between the length of the analysis and the level of separation.

A method which holds the column at the same temperature for the entire analysis is called "isothermal." Most methods, however, increase the column temperature during the analysis, the initial temperature, rate of temperature increase (the temperature "ramp") and final temperature is called the "**temperature program**."

A temperature program allows analytes that elute early in the analysis to separate adequately, while shortening the time it takes for late-eluting analytes to pass through the column.

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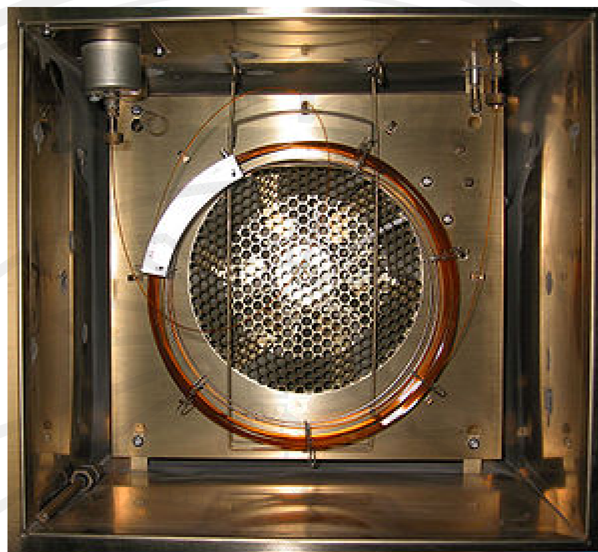

Figure 2.6 A Gas Chromatography oven, open to show a capillary column

Data reduction and analysis

Qualitative analysis:

Generally chromatographic data is presented as a graph of detector response (y-axis) against retention time (x-axis), which is called a chromatogram. This provides a spectrum of peaks for a sample representing the analytes present in a sample eluting from the column at different times. Retention time can be used to identify analytes if the method conditions are constant. Also, the pattern of peaks will be constant for a sample under constant conditions and can identify complex mixtures of analytes. In most modern applications however the GC is connected to a mass spectrometer or similar detector that is capable of identifying the analytes represented by the peaks.

Quantitative analysis:

The area under a peak is proportional to the amount of analyte present in the chromatogram. By calculating the area of the peak using the mathematical function of integration, the concentration of an analyte in the original sample can be determined. Concentration can be calculated using a calibration curve created by finding the response for a series of concentrations of analyte, or by determining the relative response factor of an analyte. The relative response factor is the expected ratio of an analyte to an internal standard (or external standard) and is calculated by finding the

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response of a known amount of analyte and a constant amount of internal standard (a chemical added to the sample at a constant concentration, with a distinct retention time to the analyte).

In most modern GC-MS systems, computer software is used to draw and integrate peaks, and match MS spectra to library spectra.

Application

In general, substances that vaporize below ca. 300 °C (and therefore are stable up to that temperature) can be measured quantitatively. The samples are also required to be salt-free; they should not contain ions. Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.

Various temperature programs can be used to make the readings more meaningful; for example to differentiate between substances that behave similarly during the GC process.

Professionals working with GC analyze the content of a chemical product, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water. GC is very accurate if used properly and can measure picomoles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples.

In practical courses at colleges, students sometimes get acquainted to the GC by studying the contents of Lavender oil or measuring the ethylene that is secreted by *Nicotiana benthamiana* plants after artificially injuring their leaves. These GC analyses hydrocarbons (C2-C40+). In a typical experiment, a packed column is used to separate the light gases, which are then detected with a TCD. The hydrocarbons are separated using a capillary column and detected with an FID. A complication with light gas analyses that include H_2 is that He, which is the most common and most sensitive inert carrier (sensitivity is proportional to molecular mass) has an almost identical thermal conductivity to hydrogen (it is the difference in thermal conductivity between two separate filaments in a Wheatstone Bridge type arrangement that shows when a component has been eluted). For this reason, dual TCD instruments are used

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with a separate channel for hydrogen that uses nitrogen as a carrier are common. Argon is often used when analysing gas phase chemistry reactions such as F-T synthesis so that a single carrier gas can be used rather than 2 separate ones. The sensitivity is less but this is a tradeoff for simplicity in the gas supply.

2.9 Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification.

Instrumentation

The GC-MS is composed of two major building blocks; the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules take different length of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio.

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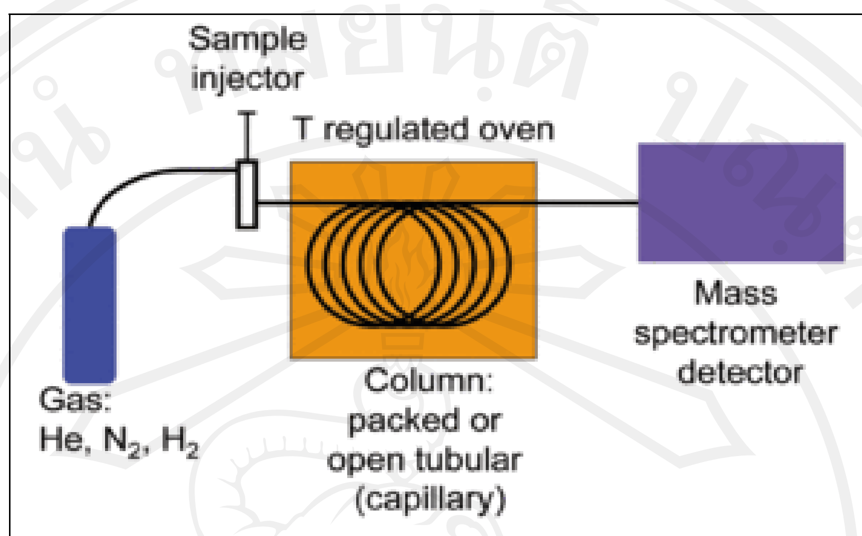

Figure 2.7 GC-MS schematic

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is impossible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detects multiple molecules that happen to take the same length of time to travel through the column (i.e. have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in similarly in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically tends to increase certainty that the analyte of interest is in the sample.

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Purge and trap GC-MS

For the analysis of volatile compounds a Purge and Trap (P&T) concentrator system may be used to introduce samples. The target analytes are extracted and mixed

with water and introduced into an airtight chamber. An inert gas such as Nitrogen (N_2) is bubbled through the water; this is known as purging. The volatile compounds move into the headspace above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber. The volatile compounds are drawn along a heated line onto a 'trap'. The trap is a column of adsorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then heated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system. P&T GC-MS is particularly suited to volatile organic compounds (VOCs) and BTEX compounds (aromatic compounds associated with petroleum) [27].

Types of mass spectrometer detectors

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, sometimes referred to by the Hewlett-Packard (now Agilent) trade name "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally one may find a magnetic sector mass spectrometer; however, these particular instruments are expensive and bulky and not typically found in high-throughout service laboratories. Other detectors may be encountered such as time of flight (TOF), tandem quadrupoles (MS-MS) (see below), or in the case of an ion trap MS^n where n indicates the number mass spectrometry stages.

Analysis

A mass spectrometer is typically utilized in one of two ways: Full Scan or Selective Ion Monitoring (SIM). The typical GC-MS instrument is capable of performing both functions either individually or concomitantly, depending on the set up of the particular instrument.

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Full scan MS

When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be m/z 50 to m/z 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set too low to look for mass fragments or else one may detect air (found as m/z 28 due to nitrogen), carbon dioxide (m/z 44) or other possible interferences. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

Full scan is useful for determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

Selected ion monitoring

In selected ion monitoring (SIM), certain ion fragments are entered into the instrument method and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place at each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard.

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Types of ionization

After the molecules travel along the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods

with typically only one method being used at any given time. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected. The ionization technique chosen is independent of using Full Scan or SIM.

Electron ionization

The most common and perhaps standard form of ionization is electron ionization (EI). The molecules enter the MS (the source is a quadrupole or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not much unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. Hard ionization is considered by mass spectroscopists as the employ of molecular electron bombardment, whereas "soft ionization" is charged by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with library spectra using manufacturer-supplied software or software developed by the National Institute of Standards (NIST-USA). Spectral library searches employ matching algorithms such as Probability Based Matching [28] and Dot-product [29] matching that are used with methods of analysis written by many method standardization agencies.

Chemical ionization

In chemical ionization; a reagent gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and the analyte which cause a 'soft' ionization of the molecule of interest. A softer ionization fragments the molecule to a lower degree than the hard ionization of EI. One of the main benefits of using chemical ionization is that a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced.

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Positive Chemical Ionization

In Positive Chemical Ionization (PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts.

Negative Chemical Ionization

In Negative Chemical Ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply.

The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data.

Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC-MS. Typically, this identification done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample.

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A “full spectrum” analysis considers all the “peaks” within a spectrum. Conversely, selective ion monitoring (SIM) only monitors selected peaks associated with a specific substance. This is done on the assumption that at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if the analyst has previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity of the analysis increases. So, SIM analysis allows for a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced.

GC-tandem MS

When a second phase of mass fragmentation is added, for example using a second quadrupole in a quadrupole instrument, it is called tandem MS (MS/MS). MS/MS can sometimes be used to quantitate low levels of target compounds in the presence of a high sample matrix background.

The first quadrupole (Q1) is connected with a collision cell (q2) and another quadrupole (Q3). Both quadrupoles can be used in scanning or static mode, depending on the type of MS/MS analysis being performed. Types of analysis include product ion scan, precursor ion scan, Selected Reaction Monitoring (SRM) (sometimes referred to as Multiple Reaction Monitoring (MRM)) and Neutral Loss Scan. For example: When Q1 is in static mode (looking at one mass only as in SIM), and Q3 is in scanning mode, one obtains a so-called product ion spectrum (also called "daughter spectrum"). From this spectrum, one can select a prominent product ion which can be the product ion for the chosen precursor ion. The pair is called a "transition" and forms the basis for SRM. SRM is highly specific and virtually eliminates matrix background.

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Applications

Environmental monitoring and clean up

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies. There are some compounds for which GC-MS is not sufficiently sensitive, including certain pesticides and herbicides, but for most organic analysis of environmental samples, including many major classes of pesticides, it is very sensitive and effective.

Criminal forensics

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and the results, used in court, need to be highly accurate.

Law enforcement

GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs.[1] It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.

Security

A post–September 11 development, explosive detection systems have become a part of all US airports. These systems run on a host of technologies, many of them based on GC-MS. There are only three manufacturers certified by the FAA to provide these systems, one of which is Thermo Detection (formerly Thermedics), which produces the EGIS, a GC-MS-based line of explosives detectors. The other two manufacturers are Barringer Technologies, now owned by Smith's Detection Systems, and Ion Track Instruments, part of General Electric Infrastructure Security Systems.

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Food, beverage and perfume Analysis

Foods and beverages contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful and which is often controlled by governmental agencies, for example pesticides.

Medicine

Dozens of congenital metabolic diseases also known as Inborn Error of Metabolism (IEM) are now detectable by newborn screening tests, especially the testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders. This is an increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better outcome. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS.

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the labeling and the measurement of ^{13}C-^{12}C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few select ions and return values as ratios.

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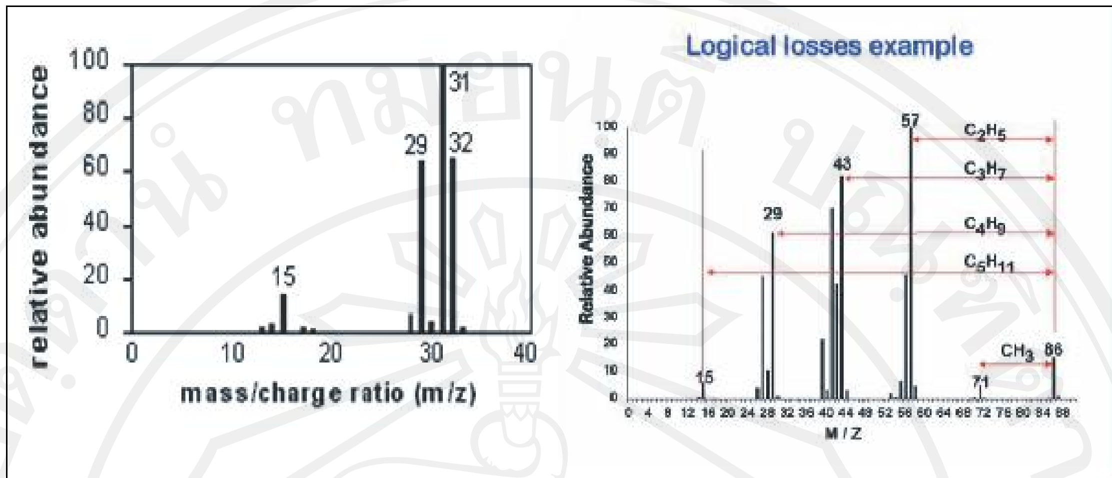

Figure 2.8 Interpreting spectra

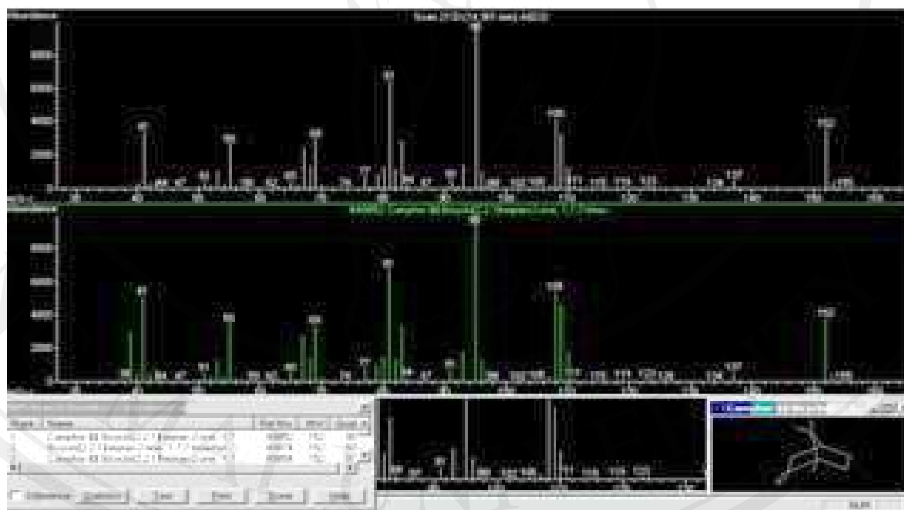

Figure 2.9 Library search results

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