Chromatography:

Chromatography is an analytical technique that can be used for qualitative or quantitative analysis. It can both identify components in a mixture and determine how much of each component is present. It is a useful form of separation science that uses intermolecular forces to separate the components of a mixture according to their properties.

Stationary and mobile phases

All chromatography uses two phases to separate a mixture. These are the:

- **mobile phase**, which is a **solvent**, that moves over or through the stationary phase
- **stationary phase**, which is either a solid with a high surface area or a liquid coated onto a solid support. It always stays still.

The mobile phase moves through and carries a sample (**solute**) over the stationary phase.

Components of the sample are more attracted to either the mobile or the stationary phase, depending on their intermolecular forces. This makes the components move at different rates and separates them. Several key terms are used to describe this attraction (Figure 1).

- **Affinity**: components of a sample are attracted, by intermolecular forces, to the mobile or stationary phase. High affinity means that the component interacts strongly with the mobile or stationary phase.
- **Adsorption**: components of the sample adsorb onto the stationary phase from the mobile phase if they are attracted to, or have an affinity for, the stationary phase.
- **Desorption**: components of the sample desorb off the stationary phase into the mobile phase if they are attracted to, or have an affinity for, the mobile phase.

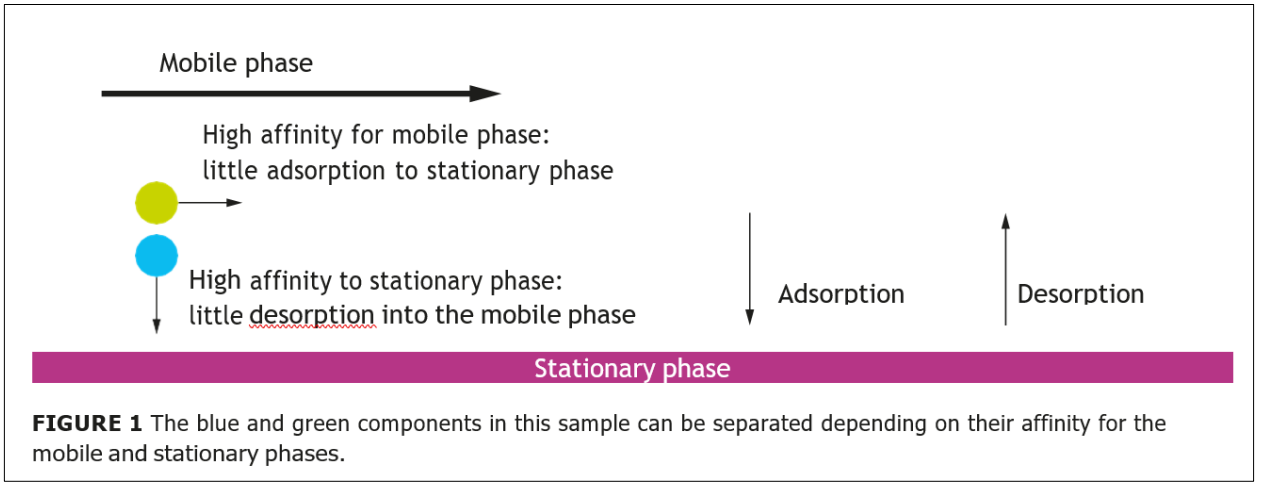


FIGURE 1 The blue and green components in this sample can be separated depending on their affinity for the mobile and stationary phases.

The 'like with like' rule

As a general rule, atoms, molecules and ions are more attracted to the mobile or stationary phase that has the same or similar properties.

Therefore, like goes with like. For example, ions contain one or more whole charges. Because of this, they are more attracted to the mobile phase if it contains charged or partially charged molecules or ions, such as water (a polar molecule).

Separation based on carbon chain length or molecular mass

Chemists often need to separate mixtures of molecules that have similar intermolecular forces such as mixtures of alcohols.

In a mixture of methanol, ethanol, propanol and butanol , all of the molecules have the same polar –OH group on the terminal, or end, carbon. Therefore, this mixture will not be separated into its components through this functional group. Rather, the mixture is separated by the size of the carbon chain – the longer the carbon chain, the greater the dispersion forces and the higher the interaction with the mobile or stationary phases.

Components are separated according to differences in the relative competition for them by the mobile phase and the stationary phase.

Paper and Thin-Layer Chromatography :

In **paper chromatography** and **thin-layer chromatography (TLC)**, a sample containing a mixture of substances is applied to the stationary phase as a spot. The spot is carried up the stationary phase by the mobile phase. The resulting **chromatogram** is usually qualitative in nature – it can only be used to identify substances, not determine how much is present.

Paper chromatography

In paper chromatography, the stationary phase is a thin strip of absorbent paper, which is cut to fit inside a container that holds the mobile phase (Figure 2). A pencil line is ruled across the bottom of the paper to mark where the sample will be placed – this is called the **origin**. Pen or ink should not be used to mark the origin because inks will separate and contaminate the sample. The sample is placed on the paper and standards of known identity can also be added to help identify the substances in the sample. In Figure 2 the standards are marked A, B and C.

The mobile phase is added to the container so that it sits *below* the origin line. The paper is then placed into the container and left to adsorb the mobile phase. The sample travels up the piece of paper, separating into its components.

Components of the sample that have a strong affinity for the stationary phase move slowly. On the resulting chromatogram, they have not moved far from the origin. Components that have a higher affinity for the mobile phase move faster. On the resulting chromatogram, they have moved further and are located closer to the **solvent front** (Figure 2).

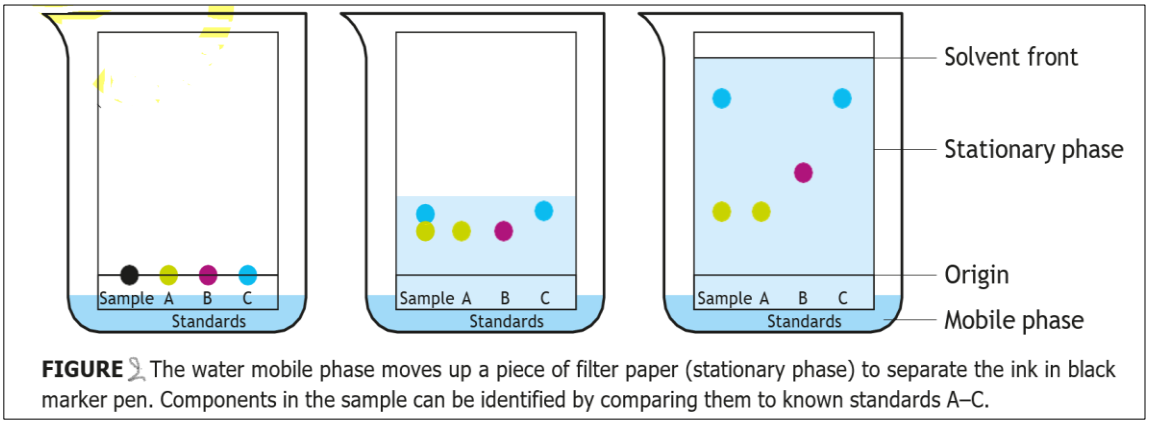


FIGURE 2 The water mobile phase moves up a piece of filter paper (stationary phase) to separate the ink in black marker pen. Components in the sample can be identified by comparing them to known standards A–C.

In Figure 2, the mobile phase is water and the stationary phase is filter paper. Paper is a derivative of cellulose, which contains many polar –OH groups. However, very few of the intermolecular forces of paper extend beyond its network of fibers (and any surface coating).

Water is also a polar molecule, but more polar than the paper. Therefore, any component of the sample that has whole or partial charges, such as ionic or polar substances, will have a higher affinity, or attraction, for the water mobile phase. These components move further from the origin. Any component of the sample that experiences dispersion forces has a higher affinity for the stationary phase and will not move as far from the origin.

FIGURE 3 Paper chromatography can be used to separate the components of photosynthetic pigment (leaf stain). The pigment is placed on the origin, and then the paper strip is placed in a container where it is in contact with the mobile phase, which is drawn up the paper by capillary action.

Retardation factor (R_f) calculations

Once the separation is completed, the resulting chromatogram is analysed to identify the substances in the sample and to determine the purity of the sample. Although we can simply look at a chromatogram to judge whether two substances are identical, it is more precise to calculate the **retardation factor (R_f)** of a substance. This is the ratio of the distance moved by a substance to the distance moved by the solvent, or mobile phase.

This can be simplified to:

$$R_f = \frac{\text{distance solute moves from origin}}{\text{distance solvent moves from origin}}$$

$$= \frac{\text{distance of sample spot}}{\text{distance of mobile phase}}$$

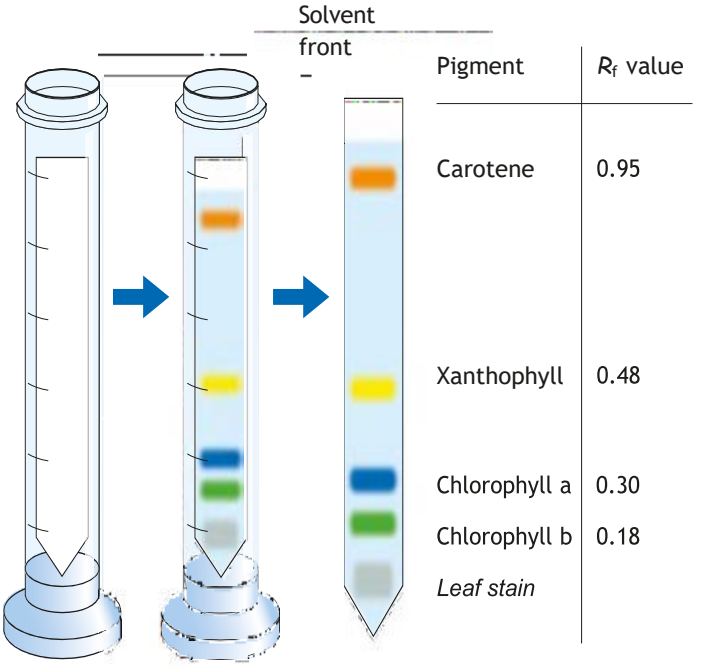


FIGURE 4 Separation of a photosynthetic pigment into its constituent pigments. The R_f value is calculated from the chromatogram.

Each atom, ion or molecule has a unique R_f

depending on the properties of the mobile and stationary phases. Increasing the polarity of the mobile phase increases its affinity to charged particles within the sample. This makes the substances move further up the paper. All R_f values must be expressed as a decimal (never a fraction) and cannot be greater than or equal to one. An R_f of 1 indicates that the substance has not separated from the mobile phase and therefore cannot be identified. The analysis must be run again under different mobile or stationary phase conditions.

Thin-layer chromatography

TLC works by the same principles as paper chromatography with one major change that makes the process more efficient (faster and more sensitive). In TLC, the support for the stationary phase is a piece of glass or plastic, which is coated in the stationary phase, consisting of silica gel, aluminium oxide or cellulose. This coating is a thin layer spread on the surface of the plastic or glass; hence, the name 'thin-layer' chromatography.

The components within the sample are separated, as with paper chromatography,

according to their affinity for the mobile or stationary phase. **FIGURE 5** TLC performed on fluorescent plates.

It is important to note that both paper chromatography and TLC are not limited to coloured compounds. Fluorescent TLC plates can be used under UV light to see the components of a sample that would not otherwise be visible. These are shown in the darker areas of the plate where the sample blocks the fluorescence of the plate (Figure 5).

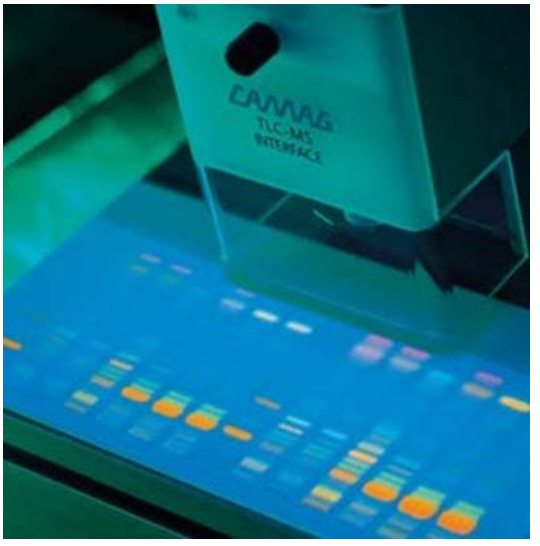


FIGURE 5 TLC performed on fluorescent plates.

Two-dimensional paper or thin-layer chromatography

Occasionally, a chromatography technique will not separate some Components sufficiently within the sample. This is because the Components interact the same amOunt with the mObile and statiOnary phases. Rather than repeating the analysis, chemists will rotate the chrOmatogram by 90°, so that the sample is on the bottom. They rule a new Origin line and run the analysis again, using a different mObile phase with different properties (Figure 6). The chrOmatogram is analysed by using Rf calculatiOns fOr analysis 1 and also for analysis 2.

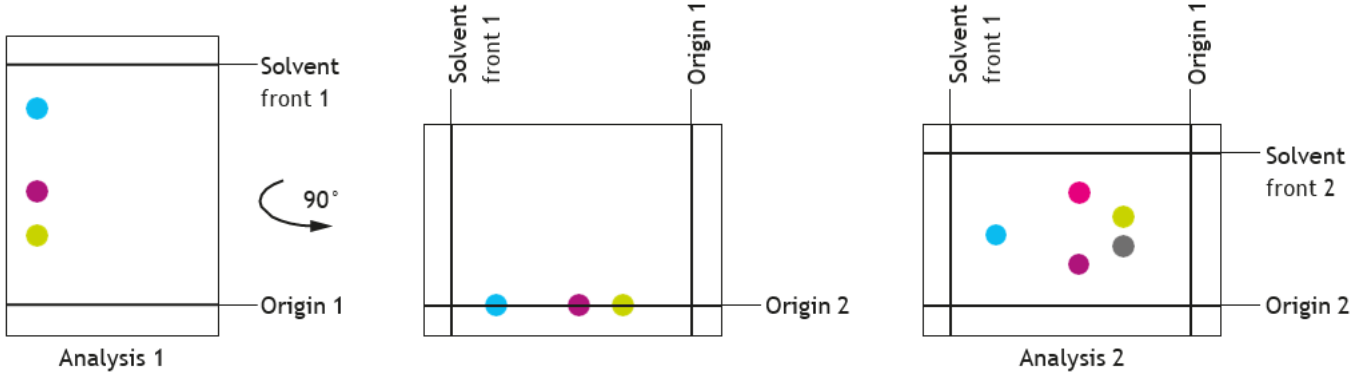


FIGURE 6 Two-dimensional paper chromatography. After the first analysis, the chromatogram is rotated 90°. This gives a new origin line. The second analysis separates the green and purple dots further into their individual components. (Origin 1 and solvent front 1 refer to the first analysis. Origin 2 and solvent front 2 refer to the second analysis.)

Column chromatography:

is a more advanced form of separation science than paper and thin-layer chromatography. It uses the same basic principles. But the stationary phase is contained within a tube, Open at both ends.

The simplest form Of column chromatography (Figure 1) uses a glass column, similar to a burette, which contains a stationary phase of aluminium Oxide or silica. The stationary phase resembles finely ground sand. The stationary phase must be packed tightly to minimize pore spaces (gaps). Otherwise, the sample will move too fast

and will not interact with the stationary phase. This means that less separation will Occur.

The mobile phase is poured into the top of the column and allowed to run slowly down through the stationary phase until it reaches the tap at the bottom. Once the column is soaked in the mobile phase, the sample is added to the top Of the column and the tap at the bottom is Opened. This allows the mobile phase to run through the column with the sample.

The sample separates into its various components depending on their affinities for the mobile or stationary phases. A higher affinity for the mobile phase means that a component spends longer interacting with it. As the mobile phase flows through the column, the component moves with it and it is collected earlier (Figure 7).

The various components Of the sample are collected in separate beakers as they elute, Or come out of, the column. Once the components are separated, further techniques are used to identify and quantify them. There are several types Of column chromatography. The two most commonly used are gas chromatography and high- performance liquid chromatography (HPLC).

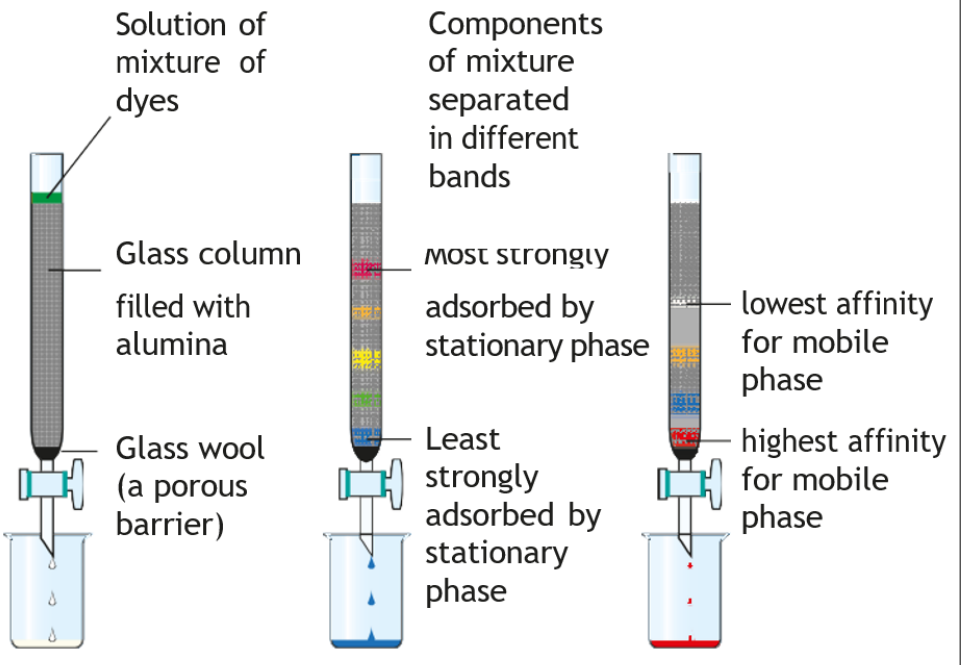
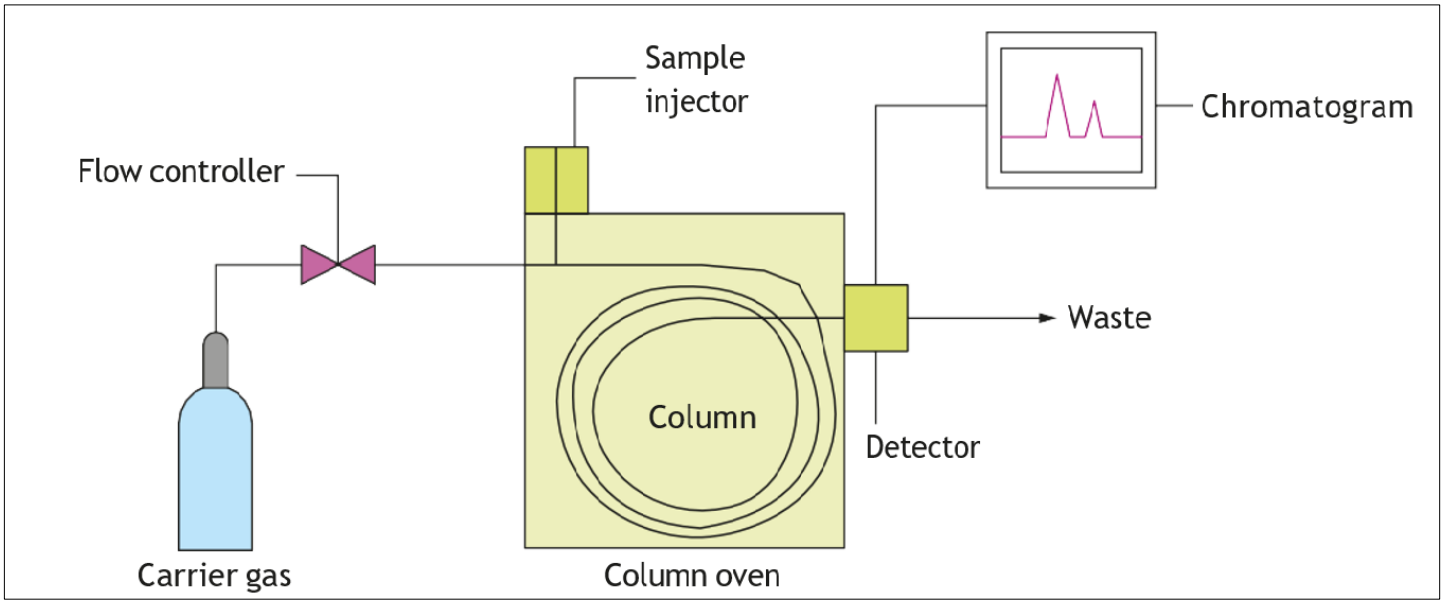


FIGURE 7 Separation of the components of a sample.

Gas chromatography:

In gas chromatography, the mobile phase is an inert gas (carrier gas), such as nitrogen (N2). The sample is vaporized and injected into the mobile phase, which carries the sample through a very small (1–2 mm diameter) and very long (up to 60 m Or longer) column containing the stationary phase. The stationary phase can be either a solid, packed into the column, Or a liquid that coats the inside of the column Both stationary phases must be able to withstand very high temperatures because the column is contained within an oven to ensure that the sample remains vaporized throughout the separation. The sample separates as each component interacts with the stationary phase. There is no attraction to the mobile phase because it is an inert gas; its purpose is to move the sample through the column. The components with a low affinity for thestationary phase elute from the column

first – they are not retained On the column for long. The components with a high affinity for the stationary phase are retained On the column for a longer period Of time.
The resulting chromatogram is Obtained when the detector recognizes the components as they elute from the column. Each component forms a peak in the chromatogram as it elutes .



Interpreting a gas chromatogram:

Figure 8 depicts a gas chromatogram that has been obtained from the separation of a mixture of alcohols. The detector is started at time zero, when the mixture is injected into the column. At 39 seconds, methanol has eluted from the column. Therefore, methanol must have the lowest affinity for the stationary phase. The chromatogram ends with hexanol, which elutes from the column at 162 seconds. Hexanol has the highest affinity for the stationary phase, interacting with it more and eluting last.

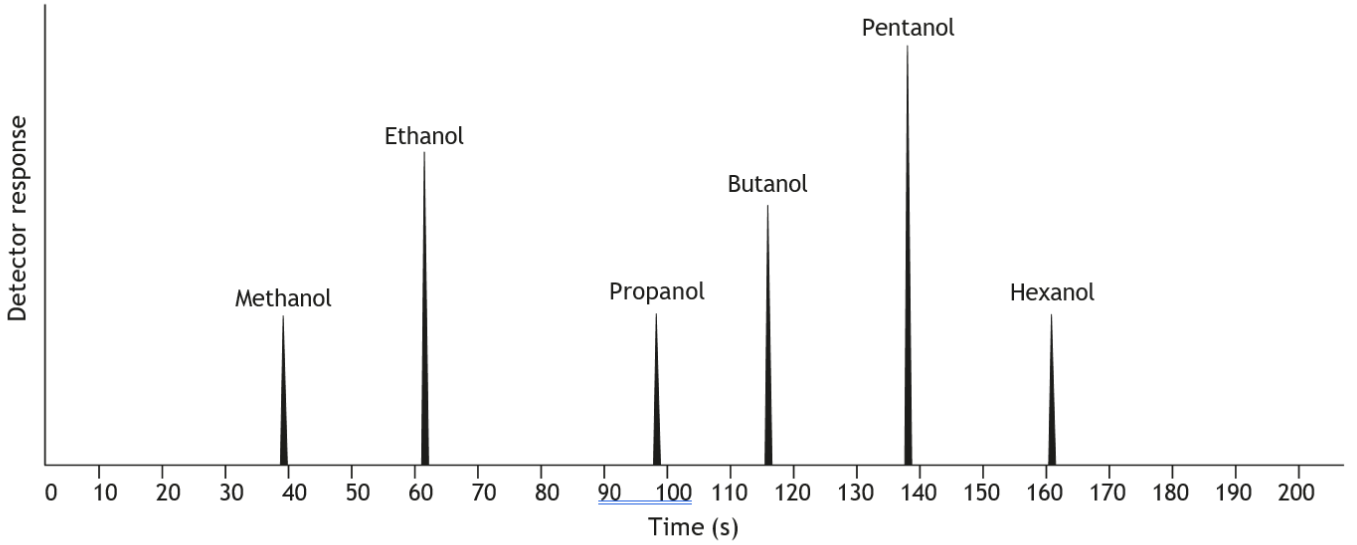


FIGURE 8 A gas chromatogram obtained from the separation of a mixture of alcohols.

Retention time:

The time that it takes a sample to move through the column, from injection to detection, is called the retention time (Rt). It is the time that each component is retained by the column. Therefore, it indicates the amount of interaction that has Occurred between each component Of the sample and the stationary phase.

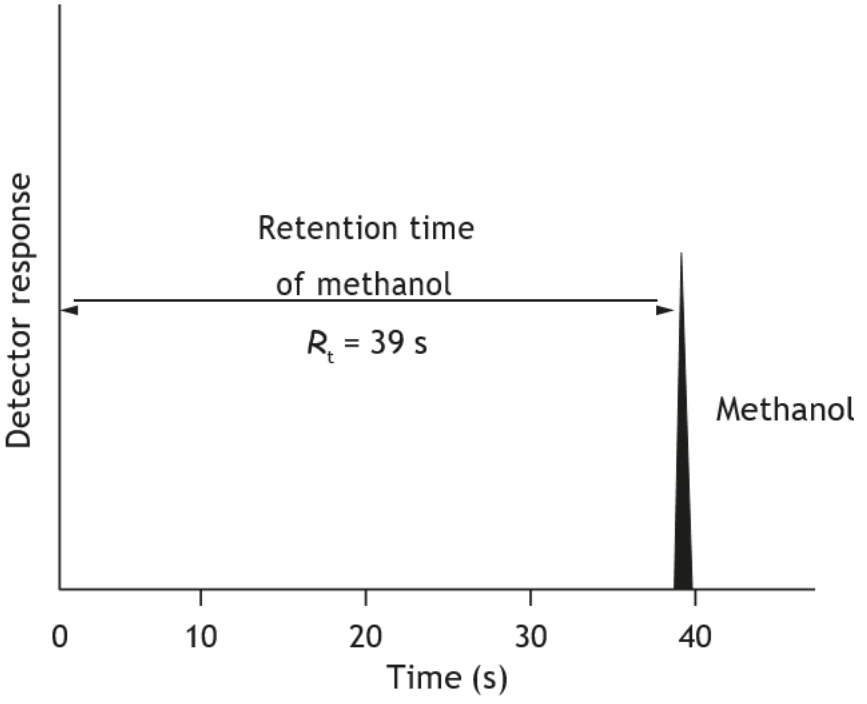


FIGURE 9 The retention time is the length of time that a component is retained by the column.

Identifying components

As with paper chromatography and TLC, standards are used in gas chromatography to identify the components within a mixture qualitatively. Figure 10 shows a chromatogram of a propanol standard run under the same conditions as the chromatogram in Figure 5. Propanol has an Rt of 98 seconds. If the standards are run under the same conditions as the sample, they will have identical retention times to the components Of the sample. Therefore, standards that are run under the same conditions can be used to identify components of a sample.

Component of the sample	R_t (s)
Methanol	39
Ethanol	63
Propanol	98
Butanol	115
Pentanol	138
Hexanol	162

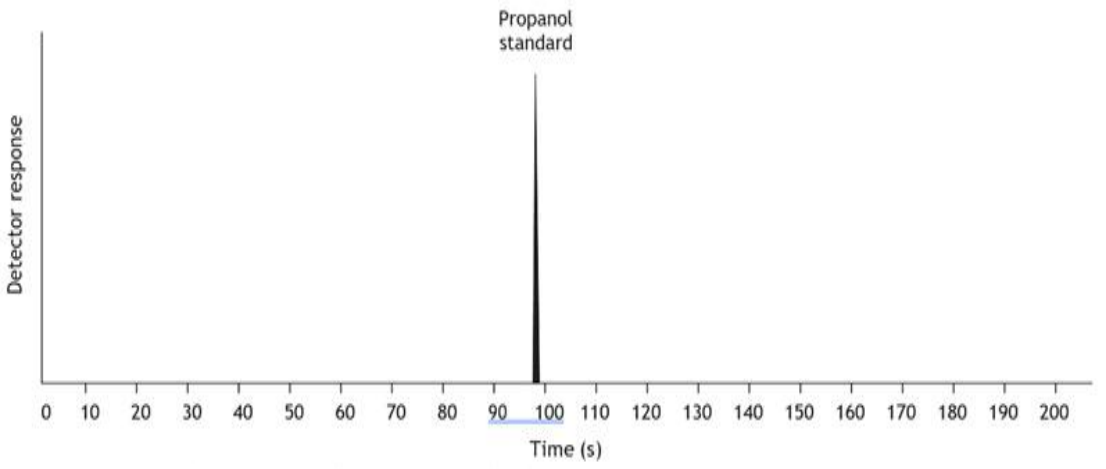


FIGURE 10 A gas chromatogram of a propanol standard.

Concentration of components

Column chromatography has the advantage of measuring the components Of a sample quantitatively. This is done by measuring the height of the peak Or the area of the peak. Fortunately, most chromatographs are computerized and programmed to complete this calculation. A computerized gas chromatography system usually measures peak area rather than height. The y-axis Of the chromatogram measures peak area in cm2. The greater peak height is due to more of that compound eluting over the same very short interval. Figure 11 demonstrates how this technique is performed. In this example, five pure samples Of ethanol were run on a gas chromatograph under identical conditions. Each sample had an accurately known concentration. A sample of ethanol Of unknown concentration was also run. As well as the resultant chromatograms, a table of peak area data was generated .

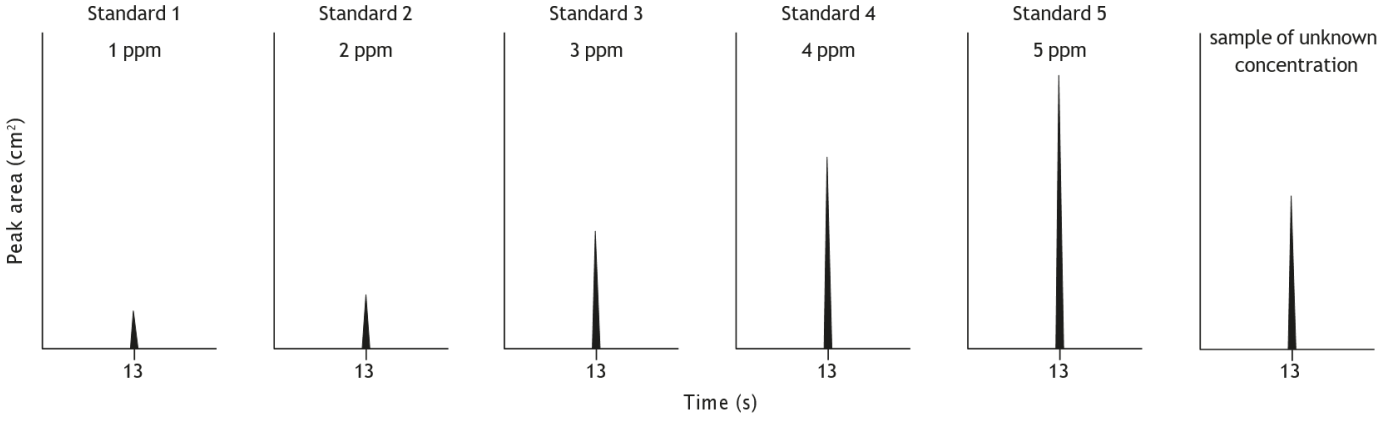


FIGURE 11 The chromatograms of five standards of ethanol and an ethanol sample of unknown concentration.

Sample	Concentration (ppm)	Peak area (cm²)	
Standard 1	1	5	
Standard 2	2	10	
Standard 3	3	15	
Standard 4	4	20	
Standard 5	5	25	
Unknown sample	–	19	

A graph can be constructed from the data in Table above, using only the standards (Figure 12). This plots peak area against concentration. The graph demonstrates a relationship between peak area and the concentration of the standards. This is called a calibration curve. If the peak area of the unknown sample is within the range of the standards (Table above), the calibration curve can be used to determine its concentration. Figure 12 shows that a peak area of 19 cm2 corresponds to a concentration Of 3.8 ppm. This can be determined by ruling lines to the linear calibration line (shown as purple dashed lines On the graph).

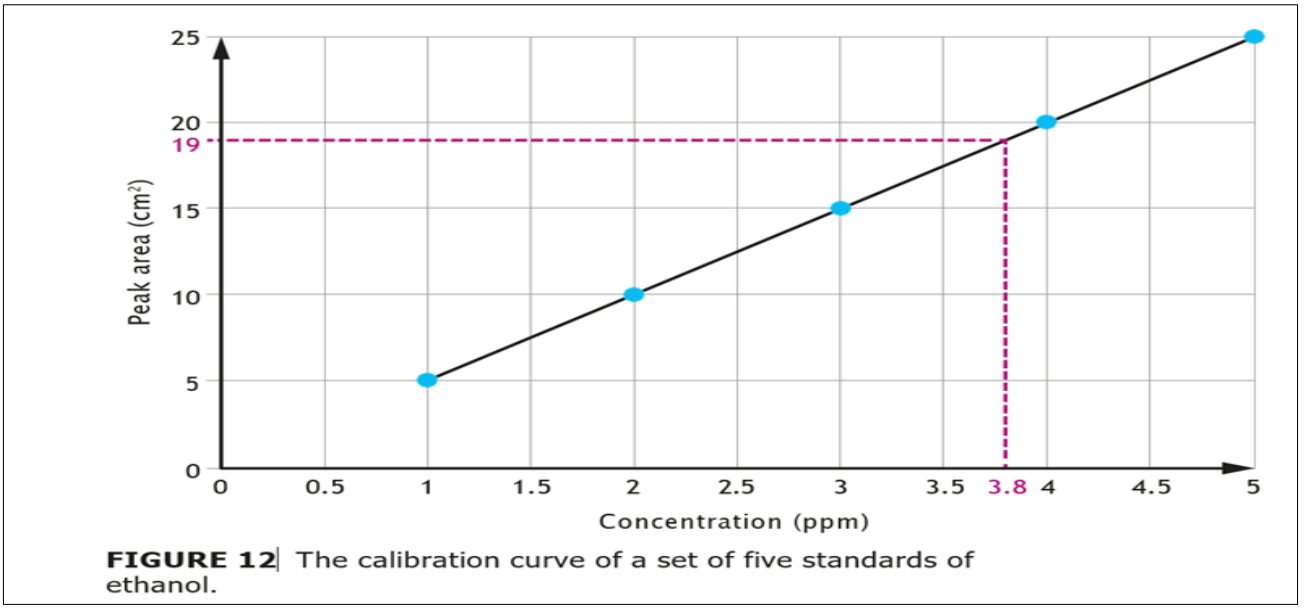


FIGURE 12 The calibration curve of a set of five standards of ethanol.

Problems with chromatograms

Sometimes, when a sample is run through a chromatograph, the first chromatogram includes peaks that overlap. This may make it impossible to measure retention time. Figure 13 shows a chromatogram with two overlapping peaks. The tops Of the peaks can be easily observed, so retention time could be measured, even though the most accurate results are gained when there is complete separation of the peaks. However, the peak area cannot be measured because not all Of the peak can be observed. TO avoid overlapping peaks in a chromatogram, a chemist can change the material in the stationary phase, the flow rate of the mobile phase or the temperature of the column.

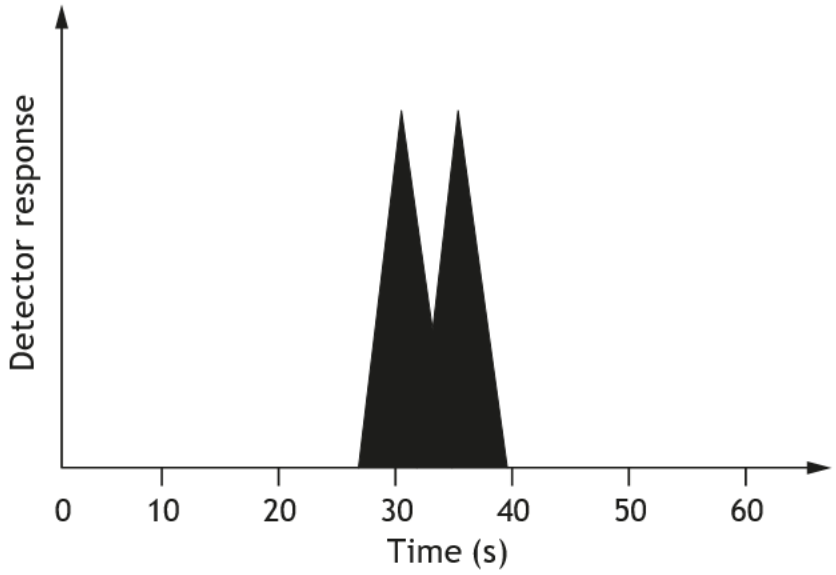


FIGURE 13 Overlapping peaks on a chromatogram can make it impossible to measure retention time and peak area.

High-performance liquid chromatography

HPLC Operates On the same principles as Other chromatographic techniques. HPLC uses a liquid Or aqueous mobile phase, which is pumped through an instrument. The sample is injected, and the mobile phase moves the sample through the column at a specified flow rate. This flow rate can be altered to pump the mobile phase with the sample through faster or slower. The column in a high performance liquid chromatography is a solid with a high surface area, packed at very high pressure into a plastic piece of tubing. It is very similar to the column in chromatography, but particles are much smaller and more tightly packed. As the sample moves through the column, it interacts with other mobile and stationary phases to separate into its various components. The detector recognizes the components as they elute from the column and generates a chromatogram similar to that generated by gas chromatography. The temperature of the column can be altered, but not as a much as with a gas chromatograph, because the column is made of plastic, which melts at high temperatures.

Interpreting a high-performance liquid chromatogram

High-perfromance liquid chromatograms are analyzed in the same way as gas chromatograms are. The peaks are identified by running standards under identical conditions and analyzing retention times. Concentration is determined by using a set of standards Of accurately known concentration, plotting a calibration curve and measuring an unknown sample on the calibration curve.