White Paper

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Understanding Solvent Focusing Gas Chromatography and How it can be Optimized for Splitless Injections

Introduction

Discussions of the basic mechanism of gas chromatography (GC), samples can start as a liquid, solid, or a gas. These samples, when not in gas phase, are converted into a vapor made up of solvent and analytes that are then separated using a GC column. The type of film or stationary phase used in the column helps to separate the compounds of a sample mixture. Meanwhile, the role of the gaseous mobile phase is to move compounds through the column without chemically interacting with them.[1] With splitless injections, almost the entire sample is transferred onto the column head, making it ideal for trace level analysis, but more susceptible to solvent interactions due to the large volume of solvent also introduced to the column. It is important to understand all the variables and how to optimize them to successfully perform splitless injections, including the role of the sample solvent and related solvent effects.

Solvent focusing, peak shape, and phase chemistry

Once vaporized, the sample enters the head of the GC column and, if the oven is cool enough, recondenses upon entry into the oven. The amount of reconcentration will be determined by the difference in temperature of the GC Inlet and the GC oven and is determined by Equation 1 and demonstrated in Figure 1.[2] During the reconcentration process the liquid will form a thin film on the head of the column, called the flooded zone. The reconcentrated solvent helps to trap analytes in this zone and prevents them from moving through the analytical column, by forming a barrier, as seen in Figure 2, also known as a retention hill. When the flooded zone is optimally concentrated, this would be deemed as being optimally focused. In general terms, the greater the reconcentration, the greater potential for sharper analyte bands.

As the condensed solvent evaporates, the portion closest to the hot inlet will evaporate first, the recondensed solvent will be replaced by solvent vapor, and the thin layer of solvent phase will evaporate. This evaporation leaves the solute condensed on the column head, as seen in Figure 3. The rate of solvent evaporation depends on the volatility of solvent and volume of solvent present. If analytes have a similar volatility to the solvent or a greater affinity to the solvent, this can lead to peak distortion.

$$\text{Reconcentration Factor} = e^{0.0462\ (\text{temperature difference})}$$

Equation 1. Calculation of reconcentration factors based as a function of the temperature difference between the inlet temperature and the starting temperature of the oven.

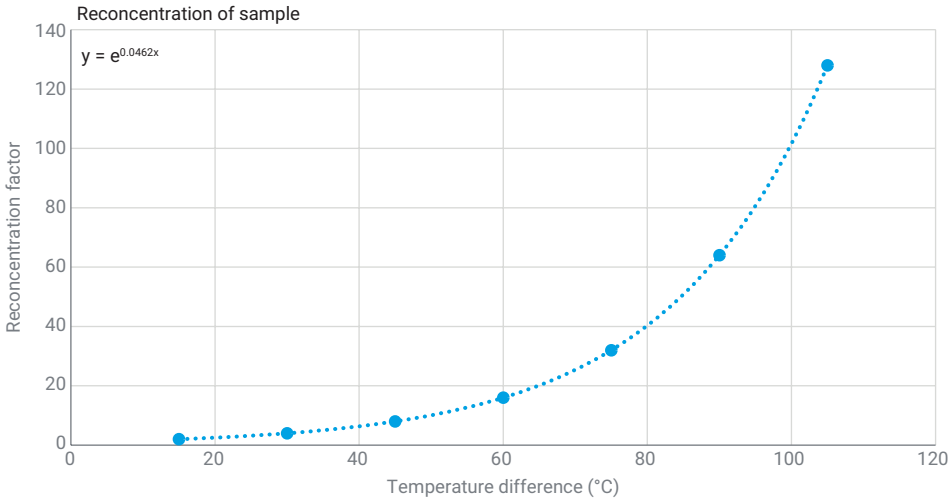
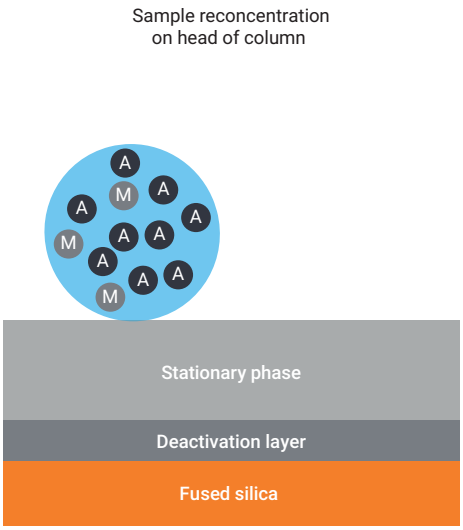


Figure 1. Demonstration of sample reconcentration on column as a function of temperature difference between inlet temperature and oven temperature.



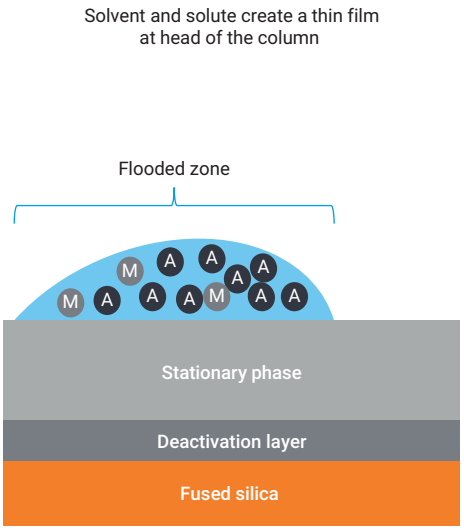


Figure 2. Example of how a sample reconcentrates on the head of the gas chromatography column and spreads to form a thin film.

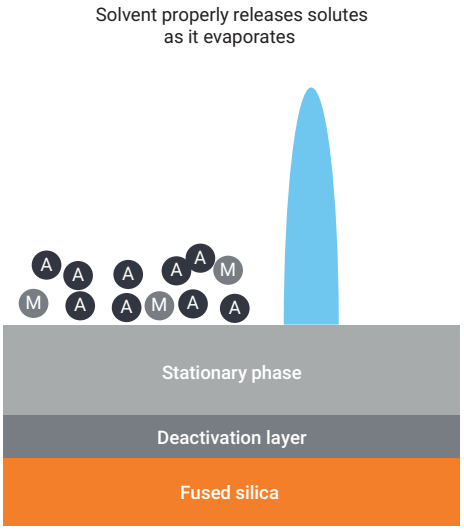


Figure 3. Example of how a solvent evaporates and leaves analytes at the head of the gas chromatography column.

In addition to the recondensation and solvent focusing, there will also be interactions with the column phase that can lead to proper or improper wettability. If the polarity of the solvent and the polarity of the column phase are not properly matched, this can lead to an increase in size of the flooded zone, demonstrated in Figure 4 as the sample will bead up similarly to water on a freshly waxed surface.[3] The solutes will then recondense unevenly. As the size of the flooded zone is increased, the retention hill will be decreased, allowing some solutes to escape the solvent, leading to split peaks as well as increasing peak widths.[2] This effect can be referred to as a reverse solvent effect.

Additionally, as the analytes are spread out along a larger flood zone, there is also potential for increasing interactions between the solvent phase and the column phase. If the analyte has a greater affinity for the solvent than the column phase, it can get partially trapped in the solvent and decrease the retention on the head of the column, causing a decrease in analyte response. Increasing column length can help to decrease this effect, such as increasing the column length from 30 meters to 60 meters. But conversely, this will mean that shorter columns, such as 20 meter high efficiency columns, are increasing the impact solvent choice has on the potential sensitivity of the analytical method.

To determine which solvents should be used with which column phases, and which solvents should be avoided, a general rule is to match the polarities of a column phase with the polarity of a solvent and is shown in Table 1. If a column with low polarity, like an Agilent J&W DB-5 column is used, it is recommended to use a solvent that also has a low polarity, such as hexane. Occasionally it can be possible to use a nonpolar column with a midpolar solvent, such as using dichloromethane (DCM) with a DB-5 type column, but it is generally not recommended to use a polar solvent, such as acetone or acetonitrile.

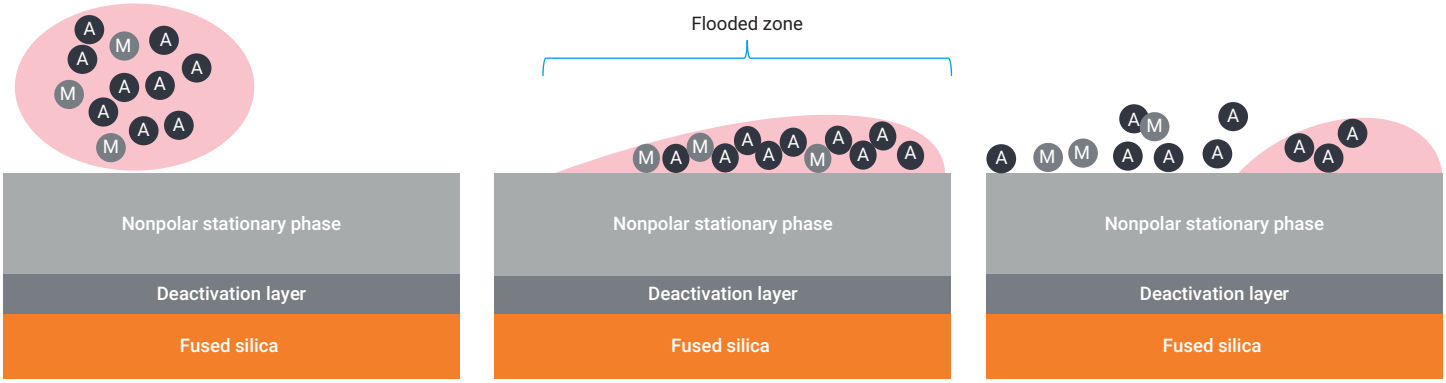


Figure 4. Example of how the flooded zone increases and solvent evaporation can be affected by solvent polarity and column phase polarity mismatch.

Table 1. Polarity index of various solvents used in gas chromatography and general column phase compatibility.

Solvent	Polarity Index	Phase Polarity	Sample Phase Types
Hexane	0.1	Low	DB-1, DB-5
Carbon Tetrachloride	1.6		
Toluene	2.4		
Tert-Butyl Methyl Ether	2.4		
Chloroform	2.7	Middle	DB-35, DB-17
Dichloromethane	3.1		
Isopropanol	3.9		
Tetrahydrofuran	4.0		
Ethyl Acetate	4.4		
Acetone	5.1	High	DB-624, DB-Wax
Methanol	5.1		
Acetonitrile	5.8		
Water	9.0		

Use of an uncoated column before the analytical column, such as a retention gap or guard column, can be used to help refocus the solvent and solute at the head of the analytical column and improves peak shape. The terms "retention gap" and "guard column" both refer to a piece of uncoated (usually deactivated) capillary column that is prior to the analytical column, but they are not always the same thing. The role of a retention gap is to function as an extension of the liner and help to focus the analytes on the head of the analytical column. A retention gap can be the same diameter or wider than the analytical column. It is never recommended that the retention gap or guard column be of a smaller diameter than the analytical column. The role of a guard column is to protect the analytical column and is usually the same diameter as the analytical column, and while it can have some sample focusing power, the purpose is to protect the analytical column from heavy matrices.

When a sample is initially introduced onto the head of an uncoated column the solvent and solutes will still create a flood zone as they are reconcentrated. But, as the guard column contains no phase, there will be a low retention factor, allowing the solvent and solutes to move faster through the guard column, and will be slowed down and recondenses a second time upon interacting with the analytical column phase, as demonstrated in Figure 5. This will provide the solvent with a second chance to form a clear retention hill and can reconcentrate a solute band by a factor of 100, decreasing the solute band size, and sharpening peak shape as demonstrated in Figure 6.[4,5]

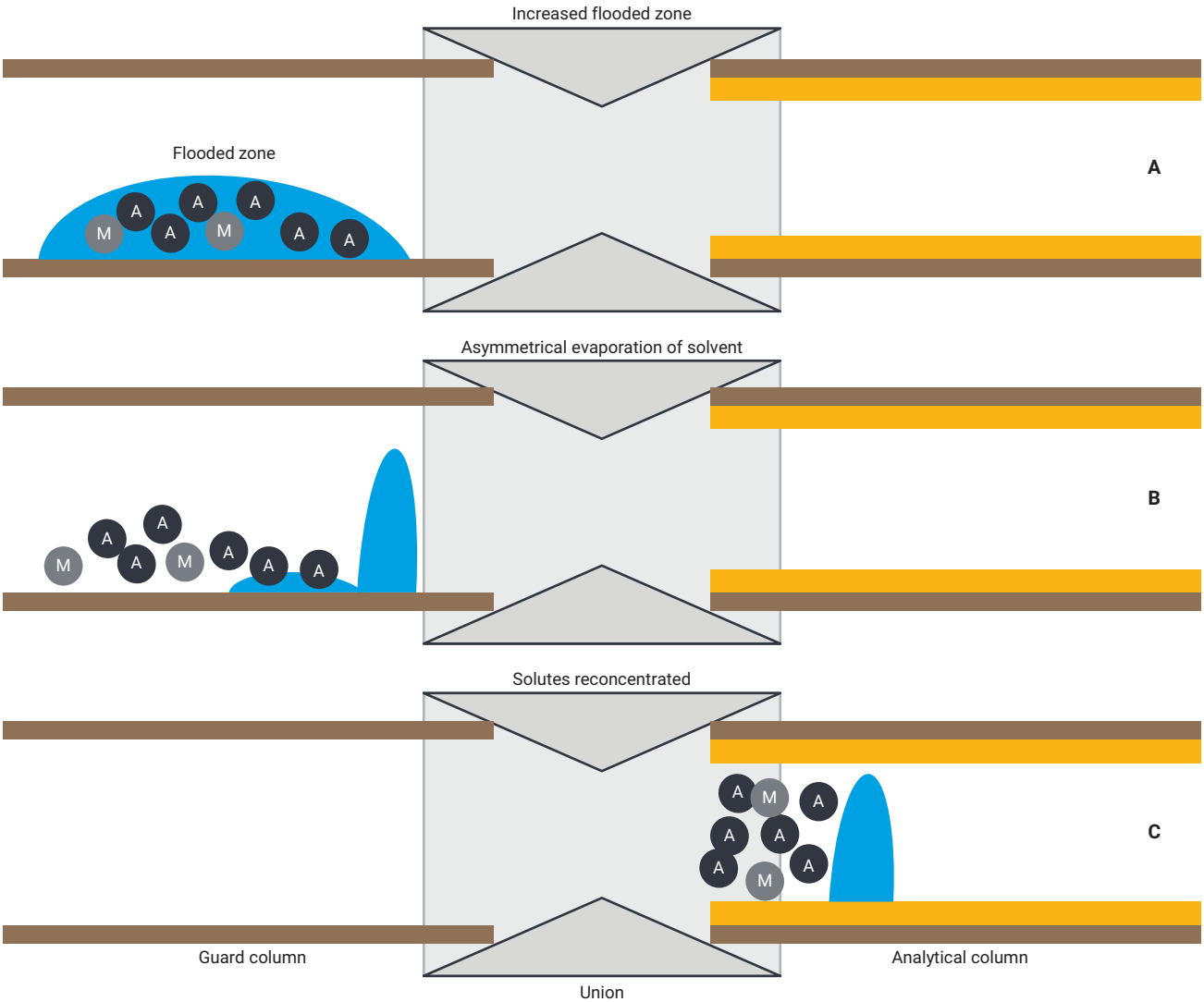


Figure 5. Example of how solvents and solute band are refocused using a retention gap.

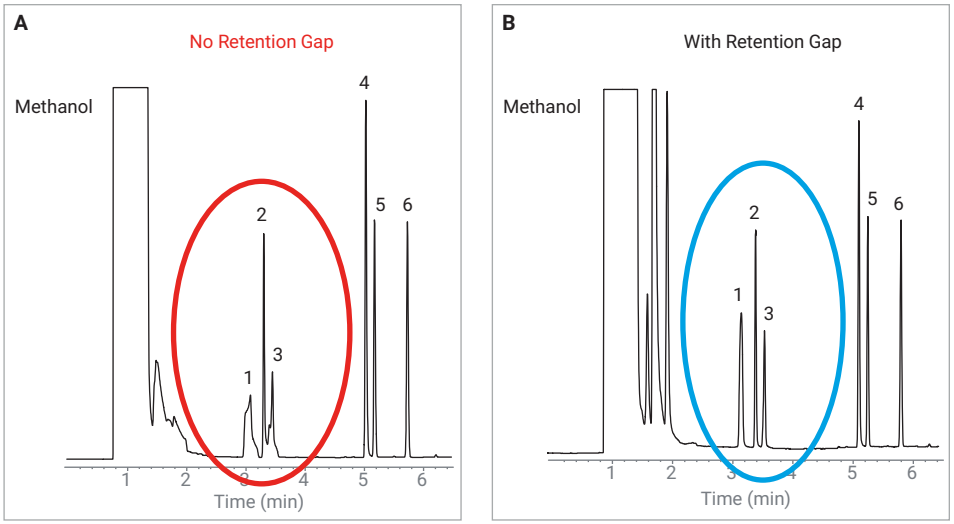


Figure 6. (A) Standard analyzed on an Agilent J&W DB-1 (15 m × 0.25 mm, 0.25 µm) without a retention gap. (B) Has the addition of a retention gap of 1 m × 0.32 mm . Peak identification: (1) 1,3-DCP; (2) 3-hexanol; (3) butyl acetate; (4) 1-heptanol; (5) 3-octanone; (6) 1,2-dicholorbenzene.

Solvent focusing and cold trapping

An additional factor to consider when selecting the optimum solvent for an analytical method is the relationship between the starting oven temperature and the boiling point of the solvent. As previously discussed, the relationship between the inlet temperature and the starting oven temperature will result in a reconcentration factor, but this reconcentration will also be impacted by the boiling point of the solvent. Ideally, after the solvent and analytes create the short-lived film at the head of the column, the solvent evaporates leaving the analytes at the head of the column. This film can only occur once the oven temperature is greater than the boiling point of the solvent. As many gas chromatography methods begin with an oven temperature of 40 °C or greater, a solvent such as dichloromethane that has a boiling point at 39.6 °C proves to be an optimum solvent for evaporation and leaving analytes at the head of the analytical column. If a solvent has a higher boiling point, such as acetonitrile, which has a boiling point of 82 °C, is used in an oven program that begins below its boiling point not only will the sample be reconcentrated, but the solvent will not evaporate until the oven temperature reaches its boiling point. This is often referred to as cold trapping.[4] As the evaporation of solvent is delayed, the flooded zone increases, increasing the potential for solvent effects and peak distortion, as is seen in Figure 6A in solutes 1, 2, and 3.

Sample preparation and solvent selection

Prior to efficient sample preparation methods for the analysis of pesticides, a guard column was used to help protect the analytical column from heavy matrix or nonvolatile analytes. The primary method of sample preparation used was solvent dilution in hexane or dichloromethane, which potentially passed through a filter, and then injected directly into the inlet and analyzed on a 5% phenyl phase type column. This can be referred to as the dilute and shoot method. As dichloromethane and hexane are both compatible solvents with a 5% phenyl phase, the guard column was only needed to protect the analytical column from matrix, and not from adverse wettability.

With the adoption of more involved sample preparation techniques, traditionally used in liquid chromatography, samples typically finish their preparation in the polar solvent acetonitrile. For GC analysis solvent exchange is employed, which is the process of drying down a sample in one solvent under nitrogen gas and reconstituting it in another solvent such as hexane. This procedure is performed to ensure proper interactions between solvent and column phase. Since these cleaned up samples contained less matrix, the need for a guard column was decreased.[6] Gradually the requirement for solvent exchanges was decreased to decrease the overall sample preparation time, but this left the prepared samples in a final solvent of acetonitrile.

As acetonitrile is a more polar solvent, and the analysis of pesticides is performed on a nonpolar 5% phenyl column phase, this creates a potential for improper wettability and an effect to peak shape and causes a decrease in response that is seen in Figure 7.

Thermal stability and solvent focusing

One attribute of the column dimensions that can have an impact on the size of the flooded zone is the film thickness of the column phase. As the thickness of the column phase is increased, the area of the flooded zone is decreased, which can lead to smaller bands and sharper peak shape. This will also allow for the ability to analyze more concentrated samples and increase the overall capacity of the column without causing overloaded peaks. Table 2 provides examples of the range of capacity of a column based on the film thickness. While thicker film columns can offer an increase in capacity, the increase in column phase will also provide a greater potential for the presence of column bleed.

Table 2. Example of column capacity based on various film thickness of gas chromatography columns.

Film Thickness (mm)	Capacity (ng)
0.10	50 to 100
0.25	125 to 250
0.5	250 to 300
1.0	500 to 1,000
3.0	15,000 to 3,000
5.0	2,500 to 5,000

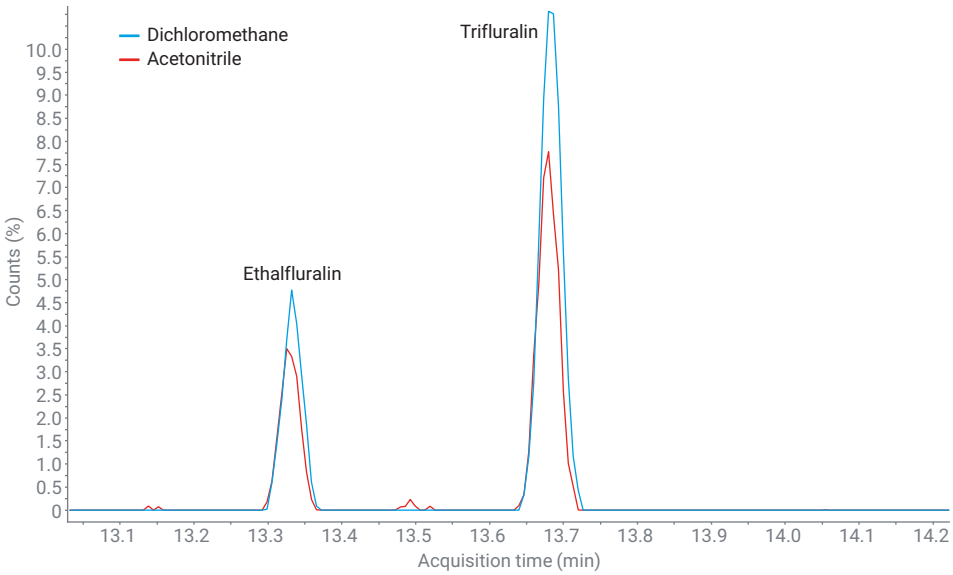


Figure 7. Example of a sample of pesticides prepared at 20 ppb and analyzed on an Agilent J&W DB-5ms UI column prepared in dichloromethane (DCM) as well as acetonitrile, using the analytical method in described in Agilent publication 5994-0916EN.

Column bleed occurs when heat is applied and the terminal end of the stationary phase polymer starts to bend back and attacks itself, which is called backbiting. Ring structures, which are thermodynamically stable, preferentially become liberated and increase the noise and raise the baseline signal, as seen in Figure 9. The backbiting process is then perpetuated. An increase in the rise of a baseline can be problematic for low signal-to-noise analytes, as peak integration can negatively impact data quality. Stable, flat baselines optimize the accuracy and repeatability of peak integration. When working with sensitive detectors, such as mass spectrometers (MS), an increase in the sensitivity of the detector also increases the background noise and bleed detection, causing excess column bleed and reducing the sensitivity of trace-level compounds. This is one reason why columns with phase thicknesses of 0.25 µm and less are typically used in GC/MS applications.[7]

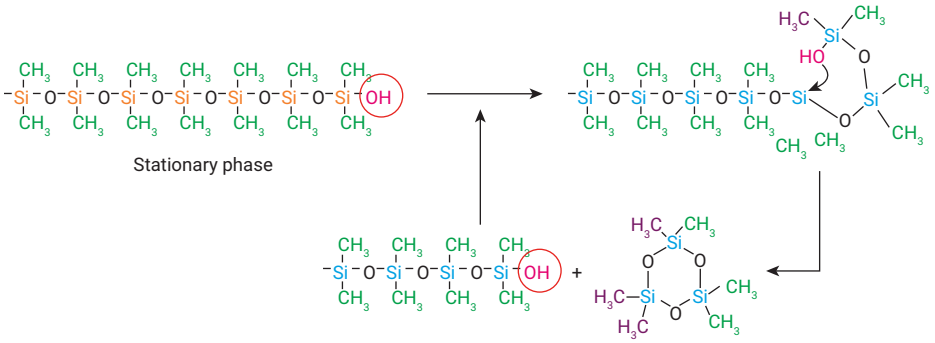


Figure 8. Mechanism of column stationary phase back biting and releasing cyclical structures causing an increase in background signal which is also known as column bleed.

A factor in determination of thermal stability of a GC column, is also due in part to the deactivation process of the fused silica and the natural presence of silanols on the surface of the fused silica. Depending on how the fused silica is produced, there can be differences in the concentration of silanols present, fully hydrated silica, and can range from 6 to 10 silanols per square nanometer on the capillary surface. As analytes and solvent diffuse into and out of the analytical column phase, they will also interact with free silanols. This interaction can lead to activity that can influence peak shape, which is why it is important to perform a deactivation to decrease the silanol content as much as possible, a process that be done in a variety of ways.[8] Polar solvents will have a greater affinity for silanols, and when the solvent is recondensed at the head of the column, the interaction between the solvent and silanols can cause a decreased flooded zone. Conversely, and dependent on the fused silica surface chemistry when less free silanols are present, certain solvents can have less affinity (even repulsion) for the column surface-phase chemistry which will cause an increase in the flooded zone.

In recent advancements in deactivation technologies, it has been possible to create deactivation that further decreases the silanol content on the fused silica surface, creating ultra thermally stable and ultra inert columns. But as their silanol content is further decreased, mismatch in solvent polarity with the column phase can lead to an increase of the flooded zone. Use of a guard column allows for the refocusing of the solvent and analytes and corrects the effects due to solvent mismatch and column phase interactions.

For example, the Agilent J&W 5Q series GC columns undergo a different deactivation process for the fused silica and purification of the phase polymer, which allows it to be the most thermally stable GC column.[7] Through this deactivation process there is a significant decrease of silanols at the polymer-fused silica surface creating an ultra-thermally stable analytical column. For these columns with such low free silanols, if the solvent is not properly matched with the phase, it can lead to an increase of the flooded zone and cause peak distortion such as in Figure 9.
A standard of a pesticide mix was prepared separately in two solvents with different polarities, dichloromethane, and acetonitrile. This standard was prepared at a concentration of 20 ppb and analyzed on an Agilent 8890 GC system coupled with an Agilent 7010D triple quadrupole GC/MS in dynamic multiple reaction mode (dMRM). The dramatic decrease of peak height and distortion of peaks when prepared in acetonitrile is due to the polar solvent interacting with the nonpolar column phase/surface, causing an increased flooded zone and defocused solute band. To refocus the solvent and analytes on to the analytical column, in this case, a retention gap was an effective solution. By adding one meter of uncoated and deactivated fused silica before the analytical column, it was possible to refocus the analytes and improve the peak shape, as is demonstrated in Figure 10.

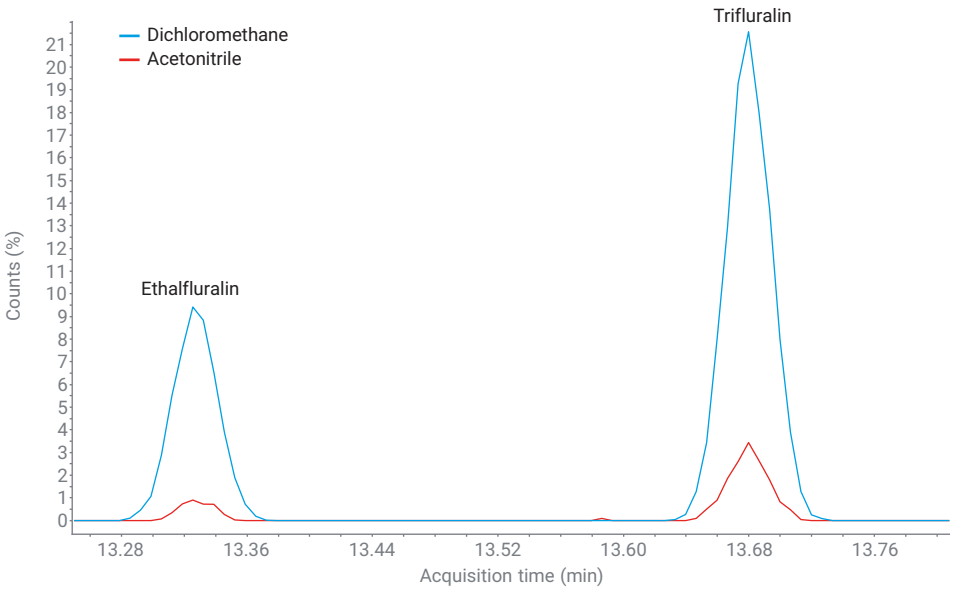


Figure 9. A sample of pesticides prepared at 20 ppb and analyzed on an Agilent J&W DB-5Q column prepared in dichloromethane (DCM) as well as acetonitrile, using the analytical method, without a guard column, as described in Agilent publication 5994-0916EN.

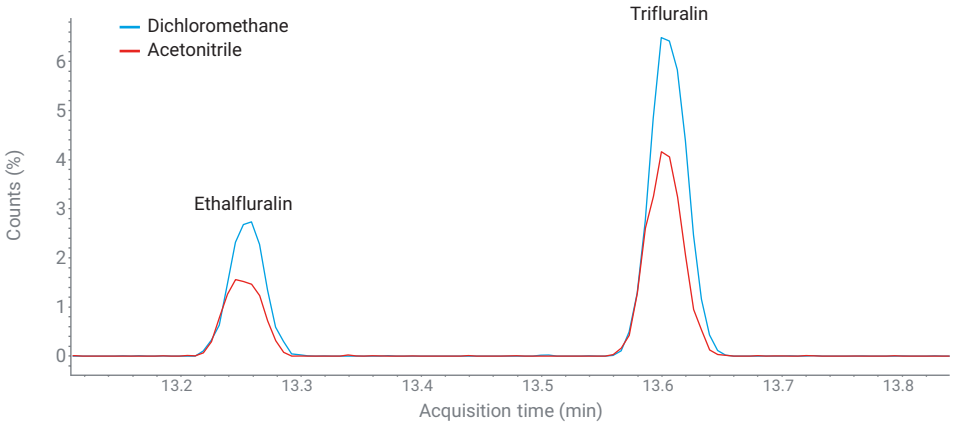


Figure 10. Example of a sample of pesticides prepared at 20 ppb and analyzed on an Agilent J&W DB-5Q column prepared in dichloromethane (DCM) as well as acetonitrile, using the analytical method in described in Agilent publication 5994-0916EN with a 1-meter guard column with an identical internal diameter as the analytical column.

Conclusion

For a gas chromatography analysis to be successful there are many factors to be considered. These factors include injection speed, solvent choice, installation of the column, and the column phase selection. Solvent selection is important in more decisions than determining which solvent will dissolve analytes. Solvent selection can impact vaporization volume in the liner, as well as impact reconcentration at the head of the GC column.

Splitless injections will be more affected by solvent selection, as there will be a greater amount of solvent introduced to the column and greater interactions between the solvent and column phase. If the solvent and column phase are mismatched, there will be an increase in the flooded zone, and a subsequent impact to the peak shape of analytes. To mitigate the problem of using a polar solvent with a nonpolar column, a guard column can be used to help refocus the solvent and analytes at the head of the analytical column and improve analyte peak shape.

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