Chromatography Today Help Desk

The Use of Derivatising Reagents

The GC analysis of cannabinoids is routinely performed using derivatising reagents that look to silylate the polar functional groups on the acidic form of the cannabinoid, thereby rendering the molecule more stable in the injector. This ensures both the acidic and neutral forms of the cannabis can be accurately quantified. This process also has the advantage that a non-polar column can be used in the analysis, making the chromatography more robust. Two commonly used reagents for derivatisation are N-Methyltrimethylsilyltrifluoroacetamide (MSTFA) and Bis(trimethylsilyl)trifluoroacetamide (BSTFA), however there are a vast array of derivatising agents used in both LC and GC.

The derivatising reagent can be used to alter the molecule into a form that is more amenable to the mode of chromatography being utilised, or to make the analyte more sensitive to the detector employed. The reaction undertaken by the derivatising reagent should be selective to a certain functional moiety and should also reach as close to 100% conversion to the final product as possible, with little or no side reactions occurring. It is also critical that the reagent as well as the final derivatised molecule be stable to the environment that they will be exposed to, whether that be the solvents, pressure or temperature of the resulting chromatographic system employed.

Derivatisation reactions used for gas chromatography (GC) fall into three general reaction types, namely:

- Alkylation, which is actually esterification and is the predominant mechanism employed
- Acylation
- Silylation

Each of these three processes will make the analyte either more volatile, more stable at elevated temperatures, or more detectable and hence more amenable to the analysis by GC. A classic example of this can be viewed through the derivatisation of the hydroxyl groups within the cannabinoid functionality the more thermally stable tri methyl silylated form of the molecule, thus stopping the conversion of the acid into the neutral form in the injector. The derivatisation to the tri methyl silylated form also results in a more hydrophobic molecule which delivers superior chromatography on a neutral stationary phase such as a 5% biphenyl.

In addition to choosing the correct derivatising reagent, it is also essential to consider other factors which will affect the overall assay performance. Since the derivatisation process in this example selectively targets the hydroxyl group, it is evident that if the matrix has an abundance of hydroxyl groups present then the efficiency of the derivatisation process will be reduced. This could happen for example if the cannabis being measured is in the presence of sugars, a potentially popular scenario would be a cannabis laden chocolate brownie. If GC was the only approach to the analysis of cannabinoids then this could help reduce the detection, however other orthogonal approaches such as LC-MS ensure that this is not an issue. The sample preparation approach also plays an important role here as it will determine the amount of possible interferants that are left in the sample prior to the derivatisation process.

Caution must be exercised to prevent any water from entering the samples as this will lead to hydrolysis of the derivatisation reagent and could suppress of the targeted analytes from undergoing derivatisation. Thus, when a sample pre-treatment such as SPE is utilised to clean up a a complex matrix such as blood or plasma it is essential that the final elution solvent is completely removed if water is present or an organic final elution solvent is used. In general, evaporating the final SPE eluant down to dryness before the addition of the derivatising reagent will ensure that the water content from the sample is kept to a minimum. While the use of a closed vial during the derivatisation process will keep the water level to a minimum, it is also important that the derivatising reagent is kept dry. To address this concern, the use of fresh reagents will ensure that the quality of the assay is maintained.

For the analysis of cannabis samples, the silylation reaction is driven by a good leaving group, in this case a group with a low basicity: namely chlorine. The reagents that are used to silylate will have the ability to stabilise a negative charge in the transitional state, with little or no back bonding between the leaving group and silicon atom. The mechanism involves the replacement of the active hydrogens (in the case of cannabinoids the hydrogen on the

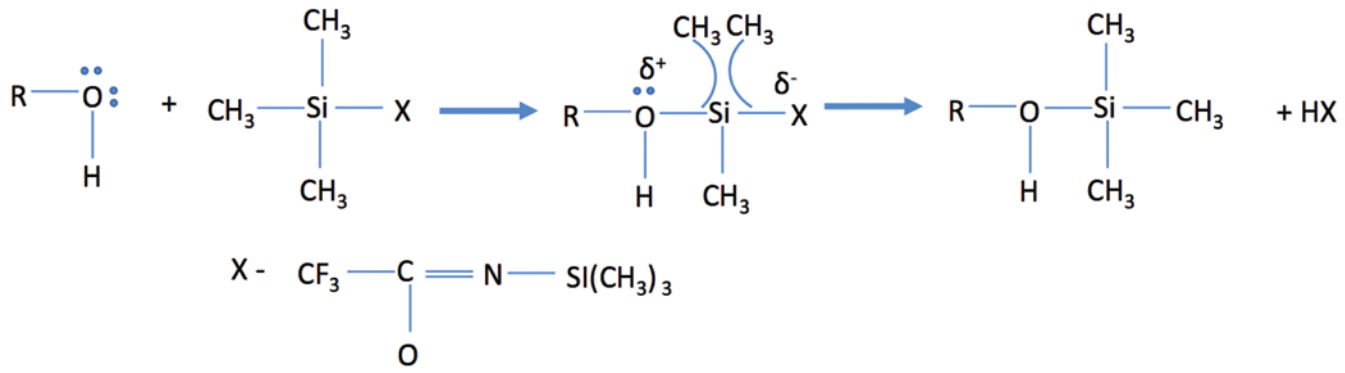

Figure 1: The general mechanism of silyation using BSTFA.

OH group) with a trimethylsilyl group. Silylation then occurs through a SN2, nucleophilic attack. The general reaction for the formation of trialkylsilyl derivatives is shown below, with the Cl atom being the leaving group, Figure 1.

Silyl reagents will react with both alcohols and acids to form trimethylsilyl ethers and trimethylsilyl esters, respectively. The derivatives formed are volatile, and for the most part, are easily separated. Silyl reagents are influenced by both the solvent system, with a common regent supplied with BSTFA and MSTFA being trimethylchlorosilane which increases the reactivity of the reagent. It is important to be aware of the effect of the solvent system, in particular if any form of sample preparation has been employed and this has resulted in the analytes being effectively transferred to another solvent.

The derivatisation of a compound is a chemical reaction that must be controlled. Ideally the process will yield only one product, with the overwhelming majority of the initial compound being converted to the final derivatised form of the molecule. However, given that in many analyses there will be a number of compounds present in the mixture prior to the derivatisation stage, this can lead to a wide range of compounds being produced. Consequently, it is often necessary to use a highly selective detector after the derivatisation process to ensure that only the compound of interest is being detected. It is also important that the reaction conditions do not cause the derivatising reagent to decompose.

An understanding of the chemistry is essential to ensure that the correct derivatised form of the molecule is produced. There are many derivatising reagents that are available and the help desk will go through some of the more common reagents that are used and also some of the challenges that can be faced when using these reagents.

There are a variety of reagents used for the silylation derivatisation process including; Hexamethyldisilzane (HMDS), Trimethylchlorosilane (TMCS), Trimethylsilylimidazole (TMSI), Bistrimethylsilylacetamide (BSA), Bistrimethylsilyltrifluoroacetamide (BSTFA), N-methyltrimethylsilyltrifluoroacetamide (MSTFA), Trimethylsilyldiethylamine (TMS-DEA), N-methyl-N-t-butyldim ethylsilyltrifluoroacetamide (MTBSTFA), and Halo-methylsilyl derivatisation reagents. The latter regents can be used with electron capture detection (ECD) to improve selective sensitivity with electron capture detectors. The most common regents for cannabinoid analysis are BSTFA and MSTFA, which due to their chemistry, react quicker than the other reagents listed with complete reactions taking less than 30 minutes.

The analysis of cannabis is seeing an increase due to increased interest in the therapeutic applications of this drug, and also in the detection of the drug when it is used in a more recreational manner. There are different approaches that can be employed to analyse the active components, but GC is still an extremely popular approach. Robust methods have been developed utilising derivatising reagents allowing for the separation and detection of the acidic and neutral forms of the cannabinoid. The sample can influence the derivatising process, as can the quality of the reagents that are used, and in the case of a silylating reagent, water contamination is critical. Other applications of derivatisation are shown in Table 1.

Table 1: Applications for chemical derivatisation

Functional Group	Reaction Type	Derivatisation Reagent
Alcohols and Phenols	Silylation	BSA, BSTFA, MTBSTFA
	Acylation	Heptafluorobutyrylimidazole, Pentafluoropropionic Anhydride, Trifluoroacetic anhydride, N-Methylbis(trifluoroacetamide)
	Alkylation	Dimethylformamide, Pentafluorobenzyl bromide
Carboxylic acids	Silylation	Bis(trimethylsilyl)–acetamide, BSTFA, Trimethylsilylimidazole, MTBSTFA
	Acylation	Pentafluoropropanol / Pentafluoropropionic anhydride
	Alkylation	Dimethylformamide, Tetrabutylammonium hydroxide
Active hydrogens	Silylation	Bis(trimethylsilyl)–acetamide, Bistrimethylsilyltrifluoroacetamide / Trimethylchlorosilane, Hydrox-Sil, N-methyl-trimethylsilyltrifluoroacetamide,
	Acylation	Pentafluoropropanol / pentafluoropropionic anhydride
Carbohydrates and Sugars	Silylation	Hexamethyldisilzane, TMSI
Amides	Silylation	BSA, N, O-bis-(trimethylsilyl)-trifluoroacetamide
	Acylation	Heptafluorobutyrylimidazole
	Alkylation	Dimethylformamide
Amines	Silylation	BSTFA, MTBSTFA
	Acylation	Trifluoroacetic anhydride, Pentafluorobenzoyl chloride, Heptafluorobutyrylimidazole
	Alkylation	Dimethylformamide (Diacetals)
Amino acids	Silylation	BSTFA, TMSI
	Acylation	Heptafluorobutyrylimidazole
	Alkylation	Dimethylformamide, Tetrabutylammonium hydroxide
Catecholamines	Silylation	TMSI
	Acylation	Pentafluoropropionic anhydride, Heptafluorobutyrylimidazole,
Inorganic anions	Silylation	BSTFA, MTBSTFA
Nitrosamine	Silylation	BSTFA
	Acylation	HFBA, Pentafluoropropionic anhydride, Trifluoroacetic anhydride
	Alkylation	Dimethylformamide, Pentafluorobenzyl bromide
Sulphonamides	Acylation	Trifluoroacetoic & Heptafluorobutyric Anhydride, Pentafluorobenzyl bromide
Sulphides	Silylation	TMSI