PRODUCTION OF IDENTICAL RETENTION TIME AND MASS SPECTRUM FOR Δ^9-TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD) FOLLOWING DERIVATISATION WITH TRIFLUORACETIC ANHYDRIDE (TFAA)

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INTRODUCTION

Methods for the analysis of cannabinoids in biological matrices are continually being developed, specifically to achieve the sensitivity required for the detection of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in hair. One of the methods used for the detection of cannabinoids that has been widely published is the use of GC-MS with negative chemical ionisation (NCI) detection. This technique is employed to achieve greater detection limits than can be achieved with electron impact (EI) ionisation.

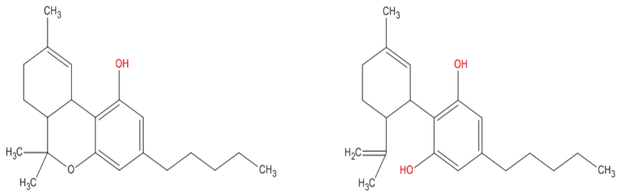
Traditionally, the most common derivatising reagent used for the analysis of cannabis under EI conditions is the silylation reagent *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (TMS). However, to make analytes suitable for NCI (i.e to make the analytes more electronegative), derivatisation using fluorinated anhydrides such as trifluoroacetic anhydride (TFAA) is employed (1). This maximises sensitivity for detection of THC-COOH. As it is not only the analysis of THC-COOH that is of interest, the same methods are often employed to analyse for the other cannabinoids, including Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD).

During our method development for the analysis of cannabinoids using two dimensional GC-MS with NCI detection, it has been observed that derivatisation with TFAA elucidates the same retention time and mass spectrum for THC-TFAA and CBD-TFAA.

STRUCTURES OF THC AND CBD

The chemical structures of THC and CBD are very similar and have the same molecular weight (314.46). The structural difference is characterized by either an open or closed pyran ring. The pyran ring is closed on the THC structure and open on the CBD structure with a hydroxyl group attached in place of the oxygen atom. This provides one possible site for derivatisation on the THC structure and two on the CBD structure.

Figure 1 – Structures of underivatised THC and CBD



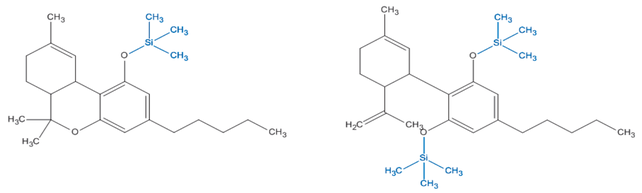
THC
Chemical Formula: $C_{21}H_{30}O_2$
Molecular Weight: 314.46

CBD
Chemical Formula: $C_{21}H_{30}O_2$
Molecular Weight: 314.46

TRIMETHYLSILYL (TMS) DERIVATISATION OF THC AND CBD

Derivatisation with TMS is most commonly used when GC-MS with EI detection is the analytical technique employed. TMS derivatives are formed by attaching to the hydroxyl groups present on the THC and CBD structures. As there are two hydroxyl groups on the CBD structure and only one hydroxyl group present on the THC structure, different retention time and mass spectrum are elucidated.

Figure 2– Structures of THC-TMS and CBD-2TMS



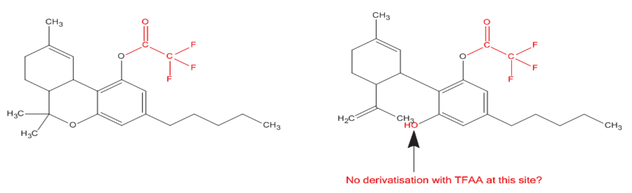
THC-TMS
Chemical Formula: $C_{24}H_{38}O_2Si$
Molecular Weight: 386.64

CBD-2TMS
Chemical Formula: $C_{27}H_{46}O_2Si_2$
Molecular Weight: 458.82

DERIVATISATION OF THC AND CBD WITH TFAA

When GC-MS with NCI detection is required, the use of the acylating reagent TFAA can be employed. Reagents containing halogen atoms (e.g fluorine) have a high electron affinity, and are used to increase a compounds detectability for NCI analysis. TFAA derivatises in the same manner as TMS by attaching to hydroxyl groups present on the THC and CBD structures. During our method development it was noted that the TFAA derivatives of THC and CBD have the same retention time and mass spectrum.

Figure 3 – Structures of THC-TFAA and CBD-TFAA



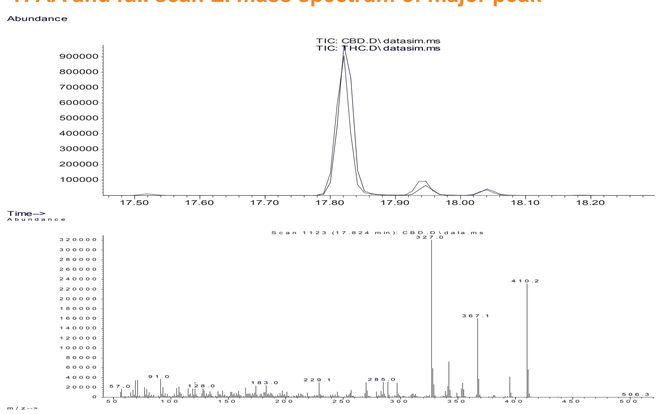
THC-TFAA
Chemical Formula: $C_{23}H_{29}F_3O_3$
Molecular Weight: 410.47

CBD-TFAA
Chemical Formula: $C_{23}H_{29}F_3O_3$
Molecular Weight: 410.47

FORMATION OF MUTLIPLE PEAKS

In addition to identical retention time and mass spectrum, it was also observed that multiple peaks are formed when derivatised with TFAA. This has been reported elsewhere, explained as the isomerisation of Δ^9-THC to Δ^8-THC with an intermediate product also being formed (2). The work here shows that the same pattern is observed with the TFAA derivative of CBD. This is more notable when hexafluoroisopropanol (HFIP) is used (required for derivatisation of the carboxyl group present on the THC-COOH molecule). The addition of chloroform during the derivatisation process can minimise this but does not eliminate the formation if HFIP is present.

Figure 4 – Identical chromatogram for THC-TFAA and CBD-TFAA and full scan EI mass spectrum of major peak



CONCLUSION

This work highlights the unsuitability of derivatisation with TFAA for the analysis of THC and CBD. The derivatisation process results in identical retention time and mass spectrum for the TFAA derivatives of THC and CBD. In addition to this, derivatisation with TFAA produces multiple peaks due to the isomerisation of THC (and CBD). These results are observed with both EI and NCI detection. Even if only analysis of THC is of interest, consideration must be given to the possibility of the presence of CBD in a sample when interpreting the results.

REFERENCES

(1) Segura J. Derivatization procedures for gas chromatographic mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents. Journal of chromatography B, Biomedical applications 1998;713(1):61-90.

(2) Holler JM, Smith ML, Paul SN, Past MR, Paul BD. Isomerization of delta-9-THC to delta-8-THC when tested as trifluoroacetyl-, pentafluoropropionyl-, or heptafluorobutyryl-derivatives. Journal of Mass Spectrometry 2008;43(5):674-9.