Production of Identical Retention Times and Mass Spectra for Δ⁹-Tetrahydrocannabinol and Cannabidiol Following Derivatization with Trifluoracetic Anhydride with 1,1,1,3,3,3-Hexafluoroisopropanol*

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The use of perfluorinated anhydrides coupled with perfluoroalcohols for the derivatization of cannabinoids has been well documented. Derivatization is used in the detection of cannabinoids using gas chromatography–mass spectrometry (GC–MS) with both electron impact ionization (EI) and negative chemical ionization (NCI). During method development for the analysis of cannabinoids in biological samples using GC–MS in EI and NCI mode, it was observed that when Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD) were derivatized with trifluoracetic anhydride (TFAA), the resultant derivatives produced the same retention times and mass spectra. This was not observed with the trimethylsilyl (TMS) derivatives of THC and CBD. This complication is due to the conversion of CBD to THC under acid conditions. The work here highlights the unsuitability of the derivatizing reagent TFAA for the detection of THC and CBD. For the analysis of case samples, even if only THC is of interest, the presence of CBD cannot be excluded, and other derivatization techniques should be used.

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-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in hair. To date, there have been several methods regarding instrumental methods used for the analysis of cannabis published. The methods commonly include the use of gas chromatography–mass spectrometry (GC–MS) operated in electron impact (EI) mode or negative chemical ionization (NCI) mode. The latter technique is employed to achieve lower detection limits than can be achieved in EI mode.

In NCI mode, acylation of hydroxyl groups with perfluorinated anhydrides such as trifluoroacetic anhydride (TFAA) and esterification of carboxylic acids with perfluoroalcohols such as 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) is often employed. This combination of derivatizing reagents is used to maximize the sensitivity and selectivity for the analysis of THC-COOH in hair using NCI detection (1). The same methods are often employed to analyze for the other cannabinoids, including Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD). The use of perfluorinated anhydrides and perfluoroalcohols for the analysis of cannabinoids in addition to THC-COOH has been reported frequently in the literature (2–15).

During method development for the analysis of cannabinoids using two-dimensional GC–MS (2D GC–MS) with NCI detection, it was observed that derivatization with TFAA-HFIP elucidates the same retention time and mass spectrum for the trifluoracetyl derivatives of THC and CBD. This has been observed in both EI and NCI mode using 2D GC–MS. This observation has not been well documented previously, with only two authors acknowledging this complication of PFPA-PFPOH use (8, 9). The purpose of this article is to highlight the problem of using this reagent for the detection of THC and CBD.

Experimental
To illustrate our observation that THC and CBD produce the same retention time and mass spectrum after derivatization with TFAA-HFIP, a simple experiment was conducted to compare the characteristics of the trimethylsilyl (TMS) and trifluoroacyl derivatives of THC and CBD. Although this phenomenon was initially observed during development of a 2D GC–MS method with NCI detection, the work was carried out on a standard GC–MS setup with EI detection as this was the most effective way to illustrate this.

Apparatus
The instrument used was an Agilent 5973B MSD coupled with a 6890N GC fitted with a split/splitless injector and a 7683B automatic liquid sampler (Agilent, Edinburgh, U.K.). The analytical column was a Restek RTX-5MS (30 m × 0.25-mm i.d. × 0.25-mm d.f.) fitted with a retention gap of uncoated deactivated silica (1 m × 0.25-mm i.d.). The GC temperature conditions were programmed as follows: an initial temperature of 80°C held for 1 min increased to 300°C at 10°C/min, held for 6 min (total run time of 29 min). The carrier gas (helium) was operated in constant flow mode at 1 mL/min. The injector was maintained at 280°C. The GC–MS was programmed to perform a 1-µL splitless injection. The MSD was operated in EI using simultaneous full scan mode (50–750 amu) and selected ion monitoring (SIM/SCAN). The software used for data acquisition and manipulation was Enhanced MSD Chemstation version D.03.00.611.

Standards and reagents
The drug standards for THC (1 mg/mL) and CBD (1 mg/mL) were purchased from LGC Standards (Tedddington, U.K.). The derivatizing reagents MSTFA, TFAA, and HFIP were purchased from Sigma-Aldrich (Poole, U.K.). Methanol and toluene were of HPLC grade and purchased from VWR (Lutterworth, U.K.).
Preparation of standard solutions

Separate stock solutions of THC and CBD were prepared in methanol at a concentration of 100 μg/mL and stored at –20°C. For analysis, separate aliquots of approximately 500 ng of THC and CBD were evaporated to dryness. Both compounds were derivatized separately with TMS and TFAA/HFIP according to current laboratory procedure. The TMS derivatives were formed by the addition of 50 μL of TMS to the standards. The trifluoroacetyl derivatives were formed by the addition of 50 μL of TFAA/HFIP (50:30). Both sets of derivatives were heated at 70°C for 30 min. The excess TFAA-HFIP was subsequently evaporated to dryness at 50°C under nitrogen and the derivatized compounds reconstituted in 50 μL of toluene. For each derivatized compound, 1 μL was injected onto the GC–MS under the conditions described.

Results and Discussion

The total ion chromatograms (TIC) and mass spectrum that are produced from the derivatization with MSTFA and TFAA/HFIP are shown in Figures 1–3. For the TMS derivatives, only one peak for each compound was observed in the TIC (Figures 1 and 2). For the trifluoroacetyl derivatives, three peaks were observed in the TIC for both THC and CBD. The three peaks had the same retention times and the same mass spectrum for each compound (Figure 3). THC and CBD have the same molecular weight (314.46) and differ by an open or closed pyran ring (Figure 4). 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.

TMS derivatives are formed by a silyl group attaching to the hydroxyl groups present on the THC and CBD molecules. 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 to give THC-TMS and CBD-2TMS as shown in Figure 5.

As TFAA derivatizes in the same manner by attaching to hydroxyl groups, it would be expected that THC-TFAA and CBD-2TFAA would be produced. However, this is not the case: the same chromatographic pattern (three peaks with identical retention times) and mass spectrum are formed for both THC and CBD following derivatization. The derivatization is shown in Figure 6.

Since the isolation of CBD in the 1940s (16) and the discovery of the correct structure in the 1960s (17), it has been well...
Figure 3. Total ion chromatogram (A) and mass spectra (B,C,D) when THC or CBD is derivatized with trifluoroacetic anhydride (TFAA) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP). The mass spectra shown are for Δ⁸-THC-TFAA (B), an intermediate peak (C), and Δ⁹-THC-TFAA (D).

Figure 4. Underivatized structures of Δ⁹-THC (A) and CBD (B).

Figure 5. Trimethylsilyl derivatives of Δ⁹-THC (A) and CBD (B).
reported that CBD cyclizes to $\Delta^8$-THC and $\Delta^9$-THC under acidic conditions (18–25) by means of a Lewis-acid-catalyzed process (18). The amounts of $\Delta^8$-THC and $\Delta^9$-THC that are produced are dependent on the reaction conditions (i.e., strength of acid, reaction time, and temperature) (20–22). The perfluorinated anhydrides are highly acidic (they must be removed prior to analysis to prevent damage to the GC column). TFAA provides an acidic medium for the closure of the open ring present on the CBD structure to a closed pyran ring thus producing THC.

In addition to identical retention time and mass spectrum, it was also observed that multiple peaks are formed following derivatization with TFAA-HFIP. This has been reported elsewhere, explained as the isomerization of $\Delta^9$-THC to $\Delta^8$-THC with an intermediate product also being formed to give three peaks on the chromatogram. This conversion occurs more readily with the combined derivatization mixture of TFAA-HFIP (84%) than with TFAA alone (10%) (26). This pattern has been reported with the PFPA-PFPOH derivatives (7). The addition of chloroform during the derivatization process can minimize this conversion but does not eliminate it if a perfluoroalcohol is present (26). The work here shows that the same pattern is observed when CBD is derivatized with TFAA-HFIP due to the conversion of CBD to THC.

There have been numerous publications detailing the analysis of cannabinoids using perfluorinated anhydrides alone or coupled with perfluoroalcohols (2–15). To the authors' knowledge, there are only two publications that report the production of identical retention time and mass spectrum for CBD and THC when derivatized with PFPA-PFPOH (8, 9) and none that detail the same reaction with TFAA-HFIP.

Conclusions
The purpose of this article is to demonstrate the unsuitability of derivatization with TFAA-HFIP for the analysis of THC and CBD. The same analytical difficulty has been reported previously with PFPA-PFPOH derivatives of THC and CBD. The derivatization process results in identical retention times and mass spectra when TFAA-HFIP is used to derivatize THC and CBD. This is further complicated by the production of multiple peaks due to the isomerization of THC in acidic conditions. Even if analysis of THC only is of interest, consideration must be given to the possibility of the presence of CBD in a sample when choosing the method of derivatization and interpretation of the results.

References


