JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2008; **43**: 674–679
Published online 21 January 2008 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/jms.1375

JMS

Isomerization of delta-9-THC to delta-8-THC when tested as trifluoroacetyl-, pentafluoropropionyl-, or heptafluorobutyryl- derivatives†¶

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Received 26 September 2007; Accepted 5 December 2007

For GC–MS analysis of delta-9-tetrahydrocannabinol (delta-9-THC), perfluoroacid anhydrides in combination with perfluoroalcohols are commonly used for derivatization. This reagent mixture is preferred because it allows simultaneous derivatization of delta-9-THC and its acid metabolite, 11-nor-delta-9-THC-9-carboxylic acid present in biological samples. When delta-9-THC was derivatized by trifluoroacetic anhydride/hexafluoroisopropanol (TFAA/HFIPOH) and analyzed by GC–MS using full scan mode (50–550 amu), two peaks (P1 and P2) with an identical molecular mass of 410 amu were observed. On the basis of the total ion chromatogram (TIC), P1 with a shorter retention time (RT) was the major peak (TIC 84%). To identify the peaks, delta-8-THC was also tested under the same conditions. The RT and spectra of the major peak (TIC 95%) were identical with that of P1 for delta-9-THC. A minor peak (5%) present also correlated well with the latter peak (P2) for the delta-9-THC derivative. The fragmentation pathway of P1 was primarily demethylation followed by retro Diels-Alder fragmentation (M − 15–68, base peak 100%) indicating P1 as a delta-8-THC-trifluoroacetyl compound. This indicated that delta-9-THC isomerized to delta-8-THC during derivatization with TFAA/HFIPOH. Similar results were also observed when delta-9-THC was derivatized with pentafluoropropionic anhydride/pentafluoropropanol or heptafluorobutyric anhydride/heptafluorobutanol. No isomerization was observed when chloroform was used in derivatization with TFAA. In this reaction, the peaks of delta-8-THC-TFA and delta-9-THC-TFA had retention times and mass spectra matching with P1 and P2, respectively. Because of isomerization, perfluoroacid anhydrides/perfluoroalcohols are not suitable derivatizing agents for analysis of delta-9-THC; whereas the TFAA in chloroform is suitable for the analysis. Published in 2008 by John Wiley & Sons, Ltd.

KEYWORDS: delta-9-THC to delta-8-THC isomerization; GC-MS detection; mechanism of fragmentation; fragment isotope composition

INTRODUCTION

Trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), or heptafluorobutyric anhydride (HFBA) in conjunction with a perfluoroalcohol are often used to esterify the phenolic group in (−)-delta-9-tetrahydrocannabinol (delta-9-THC). After derivatization, delta-9-THC is detected by GC-MS analysis. The alcohols used are typically pentafluoropropanol (PFPOH), hexafluoroisopropanol (HFIPOH), or heptafluorobutanol (HFBOH), respectively. The combination of the anhydride and alcohol is suitable for simultaneous derivatization and detection of delta-9-THC and its metabolite (−)-11-nor-delta-9-THC-9-carboxlic acid (delta-9-THCA) present in biological samples. The carboxylic acid in this reaction is esterified to a perfluoroalkyl ester. PFPA/PFPOH or TFAA/HFIPOH have been used as derivatizing agents for GC–MS analysis of delta-9-THC in whole blood and plasma.[1–3] TFAA alone was also used for the detection of delta-9-THC in blood, plasma, urine, and hair.[4–6]

Jurado and Gimenez[7] presented a method for the simultaneous detection of delta-9-THC and delta-9-THCA in blood using TFAA/HFIPOH, PFPA/HFIPOH, and HFBA/HFIPOH as derivatizing agents. In all cases, the chromatogram contained two peaks in GC–MS full scan analysis in addition to the peak corresponding to derivatized delta-9-THCA. Both peaks had the same molecular ions (M^+), which were m/z 410 for the TFA derivative, m/z 460 for the PFP derivative, and m/z 510 for the HFB derivative. The results were based on only one peak with no identification of the second peak mentioned. However, analysis of the

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THC metabolite, delta-9-THCA, produced only one peak. In this investigation, both peaks for derivatized delta-9-THC by GC–MS analysis are identified.

EXPERIMENTAL

Instrumental analysis

Samples were analyzed using an Agilent Technologies 6890N GC coupled to a 5973 MS (Palo Alto, CA). One-microliter split injections were made with the injector temperature held at 250 °C. The separation column was a J&W DB-5MS (20 m × 0.18-mm i.d. × 0.18 µm, Rancho Cordova, CA) with helium carrier gas maintained at a constant flow of 1.0 ml/min. An initial oven temperature of 165 °C was held for one minute before ramping to 300 °C at 10 °C/min. The transfer line temperature was set at 280 °C. The MS was operated under full scan mode (50–550 amu) with EI mode at 70 eV.

Chemicals and reagents

All solvents were HPLC grade and were purchased from Fisher Scientific (Pittsburgh, PA). Delta-8-THC, delta-9-THC, and delta-9-THC-d_3 were purchased from Cerilliant (Round Rock, TX). Chloroform, TFAA, PFPA, HFBA, HFIPOH,

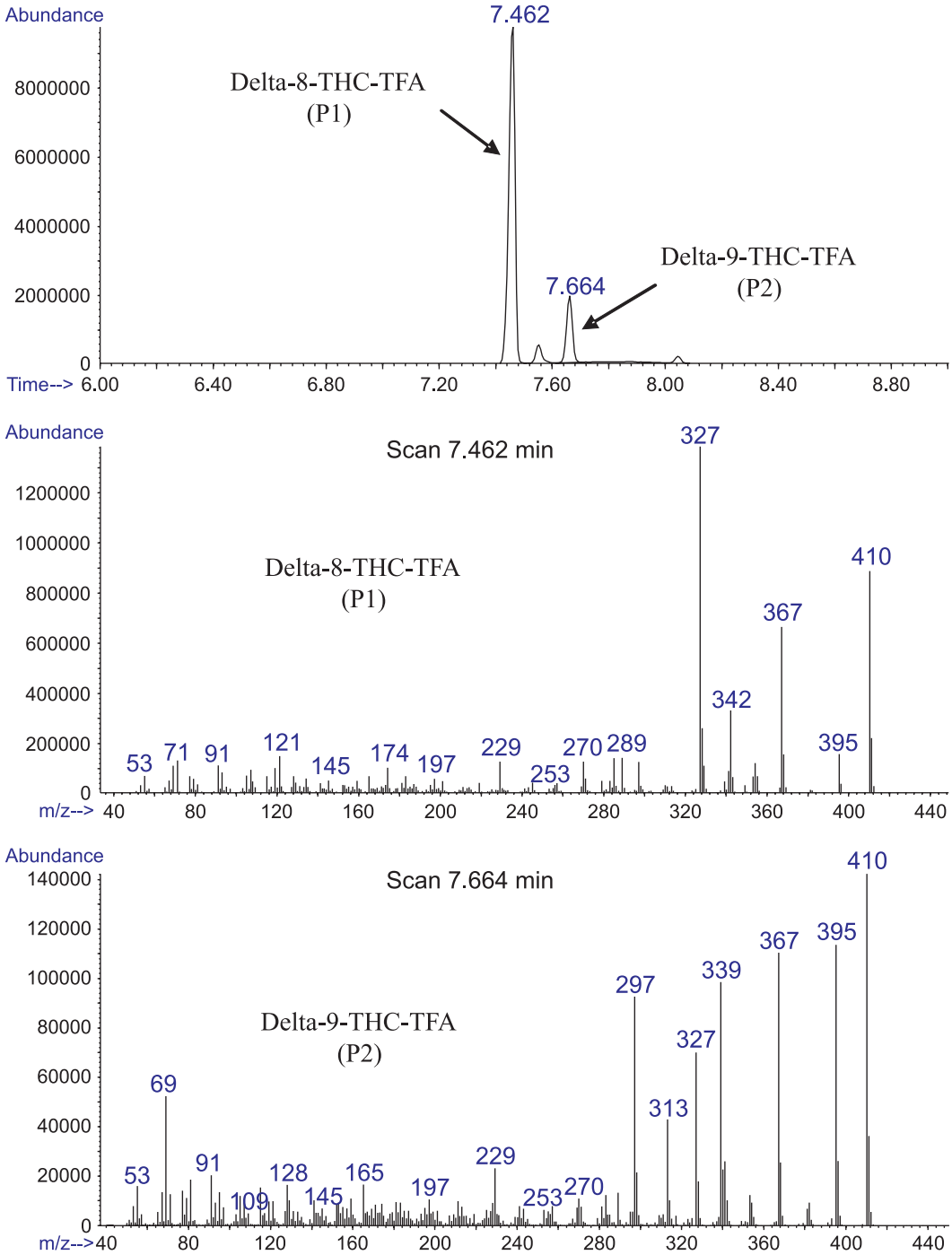

Figure 1. Mass fragmentation spectra of two peaks (P1 and P2) of delta-9-tetrahydrocannabinol (delta-9-THC) after derivatization with trifluoroacetic anhydride and hexafluoroisopropanol (TFAA/HFIPOH).

PFPOH, and HFBOH were purchased from Aldrich (Milwaukee, WI).

Sample preparation

Approximately 10 μg of delta-8-THC, delta-9-THC, and delta-9-THC-d_3 in methanol were evaporated to dryness. The compounds were either dissolved in chloroform and derivatized or derivatized directly without solvent. The reaction temperature and time were according to that reported in the literature.[1–4,6] The excess reagents were evaporated to dryness at 50 °C under nitrogen. The derivatized compounds were reconstituted in 50 μl of ethyl acetate and approximately 1 μl was injected onto the GC–MS.

RESULTS AND DISCUSSIONS

In an attempt to determine *Cannabis* compounds in blood samples, Jurado and Gimenez[7] reported two peaks when delta-9-THC was derivatized by TFAA/HFIPOH followed by GC–MS analysis. To identify the peaks, the experiments were repeated in this laboratory and similar results were found. The first peak (P1) at RT 7.462 min was the major peak (total ion chromatogram, TIC, 84%) and the other peak (P2) at RT 7.664 min was the minor peak (TIC 16%) (Fig. 1). In full scan analysis, both peaks exhibited the same molecular ion at m/z 410. To ensure that the starting material was pure, delta-9-THC was derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane and analyzed by GC–MS. The delta-9-THC as a trimethylsilyl derivative produced only one peak in the chromatogram, which was consistent with the purity (TIC 98%) claimed by the manufacturer (Cerilliant). Assuming that one of the two peaks (P1 and P2) could be delta-8-THC, delta-8-THC was derivatized with TFAA/HFIPOH and analyzed. The most abundant peak at RT 7.462 min (TIC 95%) showed fragmentation spectra identical to that from a peak of delta-9-THC-TFA at the same retention time (RT) (P1). To verify the peaks, both spectra were analyzed to determine chemical compositions (Tables 1 and 2) and fragment structures (Figs 2 and 3). The procedure for calculating the chemical composition from the areas of fragment ions and isotope ions is outlined in a previous publication.[8] The fragment composition of P1 from delta-8-THC-TFA (Table 1, example m/z 395, $C_{22}H_{26}F_3O_3$) was derived using the isotope ratio and comparing it to the theoretical value (m/z 396: 395, experimental 24.18, theoretical 24.7, match 98%). After initial demethylation ($M^+ - 15$, m/z 395), the compound undergoes a retro Diels–Alder rearrangement to produce m/z 327 ($M^+ - 15–68$, 100%) (Table 1 and Fig. 2). Retro Diels–Alder rearrangement was also observed for the

Table 1. Fragmentation and chemical composition of delta-8-THC-trifluoroacetyl derivative

Fragment[a]			Isotope/fragment Ratio	Isotope area ratio			Relative Abundance (%)
Ion	Type	Composition		Found	Theory[b]	Match (%)	
410	M^+	$C_{23}H_{29}F_3O_3$	411/410	25.00	25.85	97	65
395	M-CH_3	$C_{22}H_{26}F_3O_3$	396/395	24.18	24.7	98	12
367	M-C_2H_4-CH_3	$C_{20}H_{22}F_3O_3$	368/367	23.21	22.44	103	48
342	M-C_5H_8 (R-DL)[c]	$C_{18}H_{21}F_3O_3$	343/342	20.76	20.23	103	23
327	M-CH_3-C_5H_8 (R-DL)[c]	$C_{17}H_{18}F_3O_3$	328/327	18.77	19.08	98	100

[a] Fragmentation also supported by delta-8-THC-PFP and delta-8-THC-HFB.

[b] Calculated (Ref. 8) from the composition using $C^{13} = 1.1\%$, $H^2 = 0.015\%$ and $O^{17} = 0.037$; F has no isotope.

[c] R-DL, Retro Diels–Alder rearrangement.

Table 2. Fragmentation and chemical composition of delta-9-THC-trifluoroacetyl derivative

Fragment[a]			Isotope/fragment Ratio	Isotope area ratio			Relative Abundance (%)
Ion	Type	Composition		Found	Theory[b]	Match (%)	
410	M^+	$C_{23}H_{29}F_3O_3$	411/410	24.75	25.85	96	100
395	M-CH_3	$C_{22}H_{26}F_3O_3$	396/395	23.55	24.7	95	78
367	M-C_2H_4-CH_3	$C_{20}H_{22}F_3O_3$	368/367	23.67	22.44	106	79
339	M-C_4H_8-CH_3	$C_{18}H_{18}F_3O_3$	340/339	23.63	20.18	117[c]	70
342	nk[d]	nk	343/342	35.33	nk	nk	7
313	M-$COCF_3$	$C_{21}H_{29}O_2$	314/313	24.44	23.61	104	30
327	nk	nk	328/327	25.56	nk	nk	49
297	M-$OCOCF_3$	$C_{19}H_{21}O_3$	298/297	21.96	21.33	103	67

[a] Fragmentation also supported by d_3-delta-9-THC-TFA, delta-9-THC-PFP, d_3-delta-9-THC-PFP, delta-9-THC-HFB, and d_3-delta-9-THC-HFB.

[b] Calculated (Ref. 8) from the composition using $C^{13} = 1.1\%$, $H^2 = 0.015\%$ and $O^{17} = 0.037$; F has no isotope.

[c] Interference observed in isotope of ion m/z 339; the match is 102% for d_3-delta-9-THC-TFA.

[d] nk, not known.

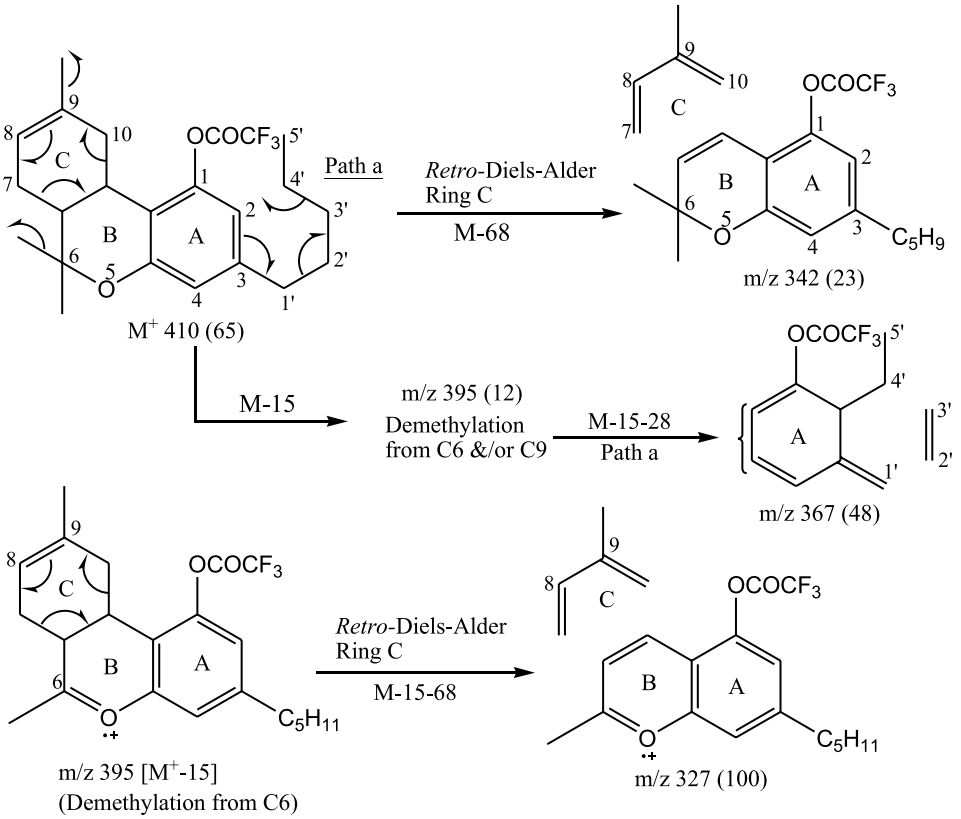

Figure 2. Fragmentation pathways and fragment structures of delta-8-THC-trifluoroacetyl derivative (M^+ 410) with percent of relative abundances in parentheses.

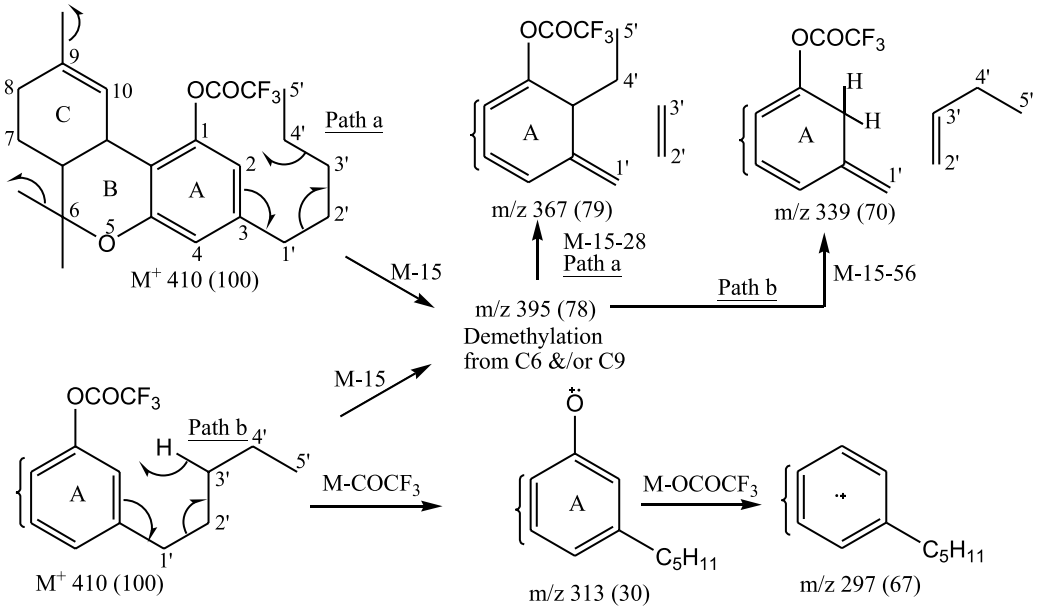

Figure 3. Fragmentation pathways and fragment structures of delta-9-THC-trifluoroacetyl derivative (M^+ 410) with percent of relative abundances in parentheses.

molecular ion to produce m/z 342 ($M^+ - 68$, 23%). This type of ring C dissociation from the rearrangement supports the position of the double bond in delta-8-THC. But in P2 from delta-9-THC-TFA, the molecular ion (m/z 410) was the base peak (Table 2 and Fig. 3, 100%). The mass fragmentation was primarily from the side chain on C3 (m/z 367 and m/z 339). Isotopic area ratio for m/z 339 (isotope/fragment 340/339) is 17% higher than the theoretical, compared to ±6% for other ions (Table 2). This small increase appeared to be from an interfering ion (m/z 340) of unknown structure overlapping with the isotope. The match is 102% for m/z 339 from d_3-delta-9-THC-TFA. Determining chemical compositions and assigning structures to ions m/z 342 and m/z 327 were difficult because

the isotopic ratios of the ions were different from that observed in delta-8-THC-TFA after C-ring dissociation from retro Diels–Alder rearrangement. The corresponding isotopic ratios for the ions m/z 342 and m/z 327 were 35.33 and 25.56 compared to theoretical values of 20.23 and 19.08, respectively for retro Diels–Alder rearrangement. As expected, ring C dissociation from retro Diels–Alder appeared unlikely in delta-9-THC-TFA. The fragmentation pattern of the delta-9-THC-TFA was also supported by an increase of 3 amu for ions m/z 395, 367, 313, and 297 and decrease of 3 amu for ion m/z 339 from deuterated-delta-9-THC-TFA with 3 deuterium atoms at C5′ position. In all cases the relative abundances of deuterated-delta-9-THC were within ±10% of the nondeuterated compound.

The fragmentation patterns of two compounds in Fig. 1 indicate that the peak P1 at RT 7.462 min is delta-8-THC-TFA and peak P2 at RT 7.664 min is delta-9-THC-TFA. It is possible that the two compounds were formed through an intermediate compound of C9 cation followed by elimination of a proton favoring the formation of delta-8-THC-TFA instead of delta-9-THC-TFA (Fig. 4). Formation of the intermediate required the presence of a proton. The increased amount of delta-8-THC-TFA (TIC 84%) from the reaction with TFAA/HFIPOH (alcoholic proton) compared to only 10% with TFAA only, supports this theory (Table 3).

Similar to TFAA in the presence of an alcohol, the reagents PFPA and HFBA also produced two major peaks for delta-9-THC. In all cases, delta-8-THC eluted first followed by delta-9-THC. The fragmentation patterns of delta-8-THC as TFA-, PFP-, and HFB- were very similar. Similar fragmentation patterns were also observed with delta-9-THC using these derivatizing agents. These similarities also support the structural assignments to the ions of delta-8-THC-TFA and delta-9-THC-TFA (Figs 2 and 3, and Tables 1 and 2). The fragmentation spectra of delta-9-THC-PFP reported by Felgate and Dinan[1] were similar to that of delta-8-THC-PFP in our study (Fig. 1). Chu and Drummer[2] also tested delta-9-THC using PFPA/PFPOH as the derivatizing agents. Three ions m/z 460 (M^+), 445 ($M^+ - 15$), and 417 ($M^+ - 43$), and two ion ratios (445/460 and 417/460) were monitored to identify the compound. The corresponding ratios reported in the literature were 18.7 and 80.2%, compared to 17.1 and 78.2% for delta-8-THC-PFP in our study. It is likely that delta-9-THC was misidentified as delta-8-THC in studies of Felgate and Dinan[1] and Chu and Drummer.[2] Foltz *et al*. and Wilkins *et al*. used only TFAA as derivatizing agent at a reaction condition of 70 °C for 10–20 min and chloroform as solvent.[4,6] In our study, no isomerization of delta-9-THC to delta-8-THC was observed under the conditions used in the Foltz and Wilkins studies (Table 3). When derivatized without chloroform, delta-9 started to isomerize to delta-8. Chloroform appeared to prevent the isomerization reaction by an unknown mechanism. Without chloroform the isomerization increased with longer reaction time (10% at 15 min to 71% at 45 min). Delta-9-THC was also derivatized under the conditions reported by Felgate and Dinan,[1] and Chu and Drummer.[2] Approximately, 90–92% of delta-9-THC was isomerized to delta-8-THC. Isomerization of delta-9-THC to delta-8-THC under different reaction conditions and derivatizing reagents are also presented in Table 3. To the author's knowledge this is the first report of acid-catalyzed isomerization of delta-9-THC to delta-8-THC as perfluoroalkyl esters. Generally, isomerization from delta-8-THC to delta-9-THC was low and within 0–9% (Table 3). No isomerization was observed when chloroform was used for TFA- and PFP-derivatives. For PFPA derivatization in the presence of chloroform the isomerization of delta-9-THC to delta-8-THC was only 2%. Therefore, both TFAA and PFPA in the presence of chloroform were suitable for derivatization of delta-8-THC and delta-9-THC.

In specimen analysis, drug controls at different concentrations are used to verify quantitative results. If a deuterated internal standard is used for quantification, the rate of isomerization of delta-9-THC and d_3-delta-9-THC (internal standard) are expected to be the same. In the experiments presented, d_3-delta-9-THC was derivatized by TFAA/HFIPOH, PFPA/PFPOH, and HFBA/HFBOH and tested by GC–MS. The percent of isomerization to d_3-delta-8-THC was comparable with that of nondeuterated delta-9-THC to delta-8-THC (within ±3%). Even though delta-9-THC was detected as delta-8-THC, the concentrations should be linear as was

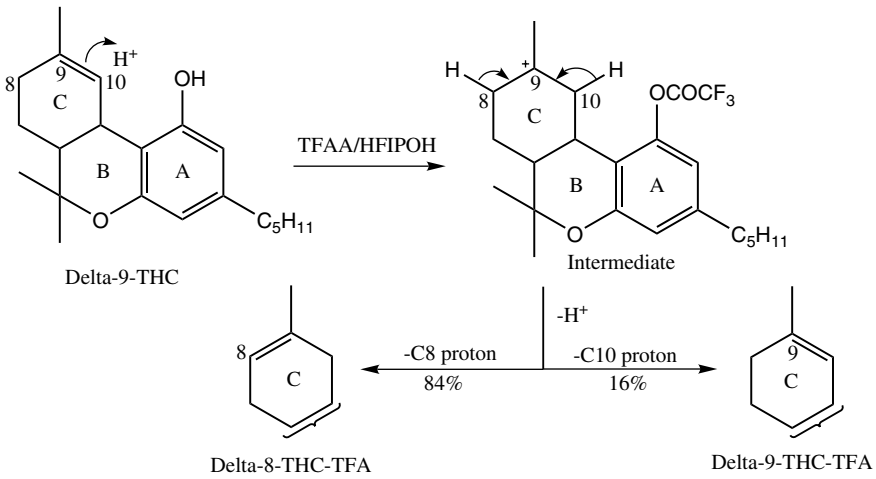

Figure 4. Mechanism of isomerization of delta-9-THC-trifluoroacetyl to delta-8-THC-trifluoroacetyl derivative.

Table 3. Isomerization between delta-9-THC and delta-8-THC under different reaction conditions and reagents

Derivatizing agent(s)[a]	Reaction Temperature (°C)	Reaction Time (min)	Reaction solvent	Isomerization[b] Delta-9 to delta-8 (%)	Isomerization[b] Delta-8 to delta-9 (%)	Reference
TFAA	70	10	Chloroform	nd[c]	nd	4
TFAA	70	30	Chloroform	nd	nd	6
TFAA	70	15	None	10	1	–
TFAA	70	30	None	63	1	–
TFAA	70	45	None	71	1	–
TFAA/HFIPOH	70	20	None	84	5	7
TFAA/HFIPOH	70	25	Chloroform	23	1	3
PFPA	70	20	None	55	7	–
PFPA	70	20	Chloroform	2	nd	–
PFPA/PFPOH	80	30	None	92	8	1
PFPA/PFPOH	70	25	None	90	9	2
HFBA	70	20	None	21	1	–
HFBA	70	20	Chloroform	19	2	–
HFBA/HFBOH	70	20	None	90	7	–
HFBA/HFBOH	70	20	Chloroform	81	6	–

[a] TFAA, Trifluoroacetic anhydride; HFIPOH, Hexafluoroisopropanol; PFPA, Pentafluoropropionic anhydride; PFPOH, Pentafluoropropanol; HFBA, Heptafluorobutyric anhydride; HFBOH, Heptafluorobutanol.

[b] We repeated the published derivatization procedures and the values are presented.

[c] nd, not detected.

found by several authors.[1–3,7] But when the specimens contain both delta-9-THC and delta-8-THC (also a natural compound), the value would represent the total THC. In that case pharmacological and pharmacokinetic interpretations should be carefully evaluated. Because of isomerization of delta-9-THC to delta-8-THC, it is recommended that perfluoroacid anhydride/perfluoroalcohol derivatization should be avoided for analysis of delta-9-THC in biological samples.

CONCLUSIONS

When perfluoroacid anhydride/perfluoroalcohol is used for simultaneous derivatization of delta-9-THC and a metabolite, delta-9-THCA, a major portion of delta-9-THC is isomerized to delta-8-THC. The amount varies with reagents and reaction conditions used and it could be as much as 84% for TFAA/HFIPOH and 90% for both PFPA/PFPOH and HFBA/HFBOH. Because of isomerization, the amount of THC in specimen analysis should represent the total THC from delta-9-THC and delta-8-THC. If the derivatization is performed only by TFAA in chloroform, no isomerization was observed. But, in this reaction delta-9-THCA is not derivatized. It is recommended that these two compounds be derivatized separately, using TFAA in chloroform to derivatize delta-9-THC and perfluoroacid anhydride/perfluoroalcohol for delta-9-THCA.

Acknowledgements

The work was supported in part by the American Registry of Pathology, Washington, D.C. 20306-6000.

REFERENCES

1. Felgate PD, Dinan AC. The determination of delta-9-tetrahydrocannabinol and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol in whole blood using solvent extraction combined with polar solid-phase extraction. *Journal of Analytical Toxicology* 2000; **24**: 127.
2. Chu MHC, Drummer OH. Determination of delta-9-THC in whole blood using gas chromatography-mass spectrometry. *Journal of Analytical Toxicology* 2002; **26**: 575.
3. Huang W, Moody DE, Andrenyak DM, Smith EK, Foltz RL. Simultaneous determination of delta-9-tetrahydrocannabinol and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol in human plasma by solid-phase extraction and gas chromatography-negative ion chemical ionization-mass spectrometry. *Journal of Analytical Toxicology* 2001; **25**: 531.
4. Foltz RL, McGinnis KM, Chinn DM. Quantitative measurement of delta 9-tetrahydrocannabinol and two major metabolites in physiological specimens using capillary column gas chromatography negative ion chemical ionization mass spectrometry. *Biomedical Mass Spectrometry* 1983; **10**: 316.
5. Johnson JR, Jennison TA, Peat MA, Foltz RL. Stability of delta-9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC in blood and plasma. *Journal of Analytical Toxicology* 1984; **8**: 202.
6. Wilkins D, Haughey H, Cone E, Huestis M, Foltz R, Rollins D. Quantitative analysis of THC, 11-OH-THC, and THCCOOH in human hair by negative ion chemical ionization mass spectrometry. *Journal of Analytical Toxicology* 1995; **19**: 483.
7. C Jurado and MP Gimenez. Determination of *Cannabis* compounds in blood samples by headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Abstract AM-o-09 Presented at 44th Annual meeting of the International Association of Forensic Toxicologists*, Ljubljana, Slovenia, 2006.
8. Paul BD, Holler JM, Lyons TP, Past MR. Stable isotopes as valid components for identification of drugs in biological specimens. *Journal of Analytical Toxicology* 2007; **31**: 447.