Analysis of Hexahydrocannabinols: Eliminating Uncertainty in its Identification

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## MAIN FINDINGS AND CONCLUSIONS

- Hexahydrocannabinol (HHC) is one of the newest substances to be marketed in the cannabis business sector.

- "Natural" HHC can be prepared by hydrogenating Δ^8-THC or Δ^9-THC with hydrogen and a metal catalyst such as palladium on charcoal or Adams' catalyst.

- The HHC produced by hydrogenation of Δ^8-THC or Δ^9-THC retains the stereochemistry at C-6a and C-10a but possesses a new stereogenic center at C-9.

- The resulting diastereomers differ in psychoactivity with the (6a*R*,9*R*,10a*R*)-HHC diastereomer being about as active as Δ^8-THC and ten to twenty times more active than (6a*R*,9*S*,10a*R*)-HHC.

- Analytical methods that are fit-for-purpose for determining the individual diastereomers of natural HHC and differentiating them from other related substances have been developed and are being used to test raw materials and finished consumer products.

- The use of the abbreviation HHC to include substances other than hexahydrocannabinol has created uncertainty about the identities of raw materials and components of finished consumer products.

- Consequently, methods have been developed to differentiate the diastereomers of HHC as well as diastereomers of 9-hydroxyhexahydrocannabinol and various cannabinoids.

- Results of analysis of samples labelled HHC have been shown to contain mixtures of cannabinoids with no evidence for any substances identified as HHC.

INTRODUCTION

The cannabis business sector has recently witnessed an influx of numerous products containing (-)-hexahydrocannabinol ((-)-HHC) and related substances in a wide variety of formulations. Although (-)-HHC has been reported from cannabis, the amounts are too low for commercial production so the (-)-HHC in these products is obtained by catalytic hydrogenation of (-)-Δ^9-*trans*-tetrahydrocannabinol (*i.e.*, Δ^9-THC) or (-)-Δ^8-*trans*-THC)

(see Figure 1). The configurations at C-6a and C-10a are retained in the product of hydrogenation but a new stereogenic center is created at carbon atom at C-9. The resulting product is a mixture of the diastereomers (6a*R*,9*R*,10a*R*)-HHC and (6a*R*,9*S*,10a*R*)-HHC as illustrated in Figure 1. The product mix obtained from hydrogenation of (-)-*trans*-Δ^9-THC or (-)-*trans*-Δ^8-THC is sometimes characterized as natural HHC because it is obtained from the natural cannabinoid (-)-*trans*-Δ^9-THC without change of configuration at C-6a and C-10a. It is also abbreviated as (-)-HHC because both diastereomers are levorotatory [1]. The relative abundances of the diastereomers in the mixture depend on the starting materials and the reaction conditions. Since the 9*R*-diastereomer is approximately as active as (-)-*trans*-Δ^8-THC and ten to twenty-fold more active than the 9*S*-diastereomer of (-)-HHC in tests of psychoactivity [2], quantification of individual epimers is required for comparisons of commercial products. Since these epimers of (-)-HHC (*i.e.*, (6a*R*,9*R*,10a*R*)-HHC and (6a*R*,9*S*,10a*R*)-HHC) are diastereomers and not enantiomers, they are amenable to separation by achiral chromatographic methods (Sams, unpublished) as will be illustrated later in this review.

Figure 1. Acid-catalyzed intramolecular ring closure of cannabidiol (CBD) to Δ^9-THC and Δ^8-THC followed by hydrogenation of both THC isomers to (6aR,9R,10aR)-HHC and (6aR,9S,10aR)-HHC
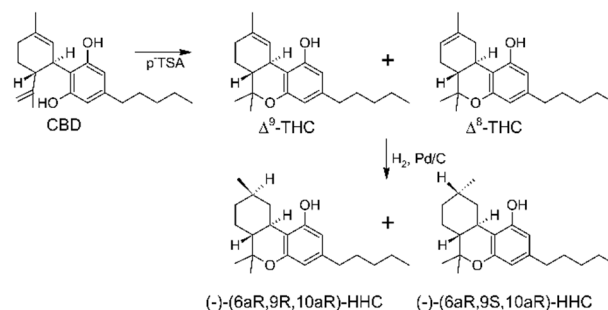
In addition to the diastereomers of (-)-HHC, certain substituted hexahydrocannabinols such as 9b-hydroxyhexahydrocannabinol may have been substituted for (-)-HHC intentionally or due to confusion regarding the use of the abbreviation HHC for hexahydrocannabinol as well as substituted analogues. Therefore, analytical methods need to be able to identify and differentiate these substances. The purpose of this review is to describe those analytical methods that have been demonstrated to be fit for purpose for determining the diastereomers of (-)-HHC and that have been used to differentiate these substances from other substances that have been marketed as HHC.

ORIGINS OF HHC

The first published report of the synthesis of (-)-hexahydrocannabinol came from the Noyes Chemical Laboratory at the University of Illinois in 1940 during a series of studies to isolate and identify the active principles of *Cannabis sativa* L [3]. One of the studies involved the hydrogenation of (-)-*trans*-Δ^9-THC and (-)-*trans*-Δ^8-THC using platinum oxide in acetic acid (Adam's catalyst) in efforts to locate the position of the double bond in the psychoactive substances that they had obtained by acid-catalyzed intramolecular ring closure of cannabidiol [3]. This study revealed that hydrogenation of either substance resulted in the same product thereby demonstrating that they differ only in the position of the double bond in the cyclohexenyl ring [3]. The correct assignments of the locations of the double bonds required the application of nuclear magnetic resonance (NMR) spectroscopy by Mechoulam's group in Israel nearly 25 years later [4].

The hydrogenation of (-)-*trans*-Δ^9-THC and (-)-*trans*-Δ^8-THC was investigated using instrumental methods (*e.g.*, NMR) that enabled investigators to locate the positions of the double bonds in the cyclohexenyl rings and to characterize the diastereomers resulting from the hydrogenation reactions [1]. The results of these investigations revealed that (6a*R*,9*R*,10a*R*)-HHC and (6a*R*,9*S*,10a*R*)-HHC were found in a relative abundance of about 3:1 in the crude reduction product of (-)-*trans*-Δ^8-THC, while under the same reaction conditions, these isomers were formed from (-)-*trans*-Δ^9-THC in a ratio of 1:2 [1]. The results of this study indicate that the more psychoactive isomer is produced in greater abundance when the starting material is (-)-*trans*-Δ^8-THC instead of (-)-*trans*-Δ^9-THC.

The (-)-hexahydrocannabinol that is being produced commercially is usually obtained by catalytic hydrogenation of (-)-*trans*-Δ^8-THC that has been obtained by acid-catalyzed intramolecular ring closure of cannabidiol. The hydrogenation is typically catalyzed using Raney nickel or palladium on charcoal. The Δ^8-THC may have been purified before hydrogenation or the reaction mixture from the conversion of CBD may have been used without purification. Residual Δ^9-THC in the reaction mixture is converted to the mixture of isomers but s with a different

distribution of their relative concentrations [1]. Since (6a*R*,9*R*,10a*R*)-HHC is substantially more active than (6a*R*,9*S*,10a*R*)-HHC [2], stereoselective and stereospecific syntheses of the active isomer have been reported.

A synthetic procedure to produce enantiomerically pure (6a*R*,9*R*,10a*R*)-HHC starting with condensation of (*R*)-(+)-citronellal with 5-n-pentyl-1,3- cyclohexanedione at 100 °C in dimethylformamide followed by aromatization and warming to produce (6a*R*,9*R*,10a*R*)-HHC [5]. Substitution of (*S*)-(-)-citronellal in this reaction sequence results in the production of (6a*S*,9*S*,10a*S*)-HHC [5]. Other approaches to the synthesis of these enantiomers using (*R*)-(+)-citronellal and (*S*)-(-)-citronellal have been reported [6; 7]. Although these synthetic routes to the stereospecific synthesis of (-)- and (+)-hexahydrocannabinol have been known for a decade or more, commercially available

ANALYSIS OF HHC AND RELATED SUBSTANCES

Analysis of the diastereomers of (-)-hexahydrocannabinol by gas chromatography-mass spectrometry under isothermal conditions on an intermediate polarity column without derivatization affords baseline resolution of these diastereomers from each other and from other substances including cannabinol (CBN), cannabidiol (CBD), (-)-*trans*-Δ^8-THC, (-)-*trans*-Δ^9-THC, and other related substances. The separations were achieved on a 30 m fused silica capillary gas chromatography column using s 35% diphenyl / 65% dimethylpolysiloxane bonded-phase system such as Phenomenex ZB-35 (part# 7HG-G003-11) [1]

The recent availability of reference standards for both diastereomers of (-)-HHC allows them to be differentiated and quantified separately by GC-MS (Sams, unpublished observations). Note that the diastereomers of (-)-HHC are completely resolved and that the more psychoactive isomer (*i.e.*, (6aR,9R,10aR)-HHC) elutes after (6aR,9S,10aR)-HHC under these GC-MS conditions (see Figure 2) (Sams, unpublished observations). Mass spectral data for the two diastereomers under electron-impact ionization conditions at 70 eV are shown in Figure 3 and Figure 4 (Sams, unpublished observations).

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[1] https://www.phenomenex.com/Products/Part/7HG-G003-11

Figure 2. Total ion chromatogram of reference standards of (6aR,9S,10aR)-HHC, (6aR,9R,10aR)-HHC, 9a-hydroxy-HHC, and 9b-hydroxy-HHC analyzed by isothermal gas chromatography on a DB35ms column at 240 °C.
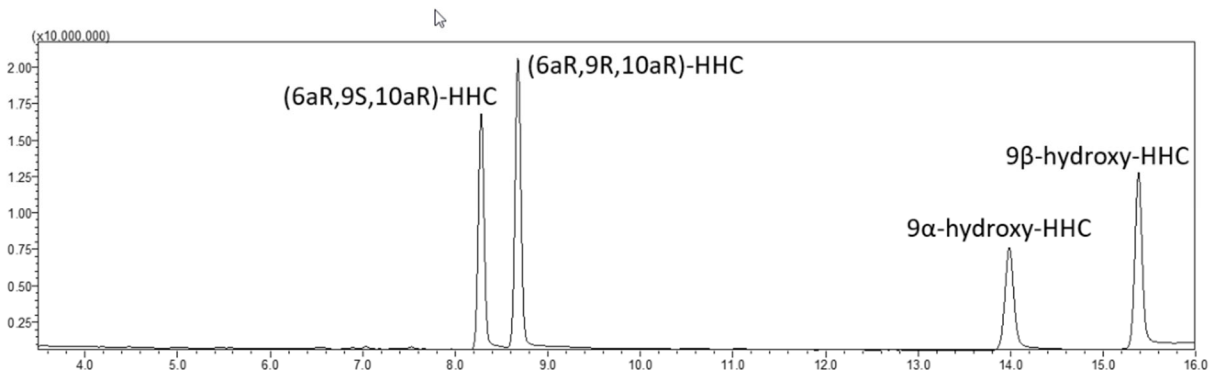
Electron-impact (-70 eV) ionization mass spectra of the diastereomers of natural HHC (*i.e.*, (6a*R*,9*S*,10a*R*)-HHC and (6a*R*,9*R*,10a*R*)-HHC) in Figure 3 and Figure 4 indicated only small differences in their mass spectra as would be expected but the chromatographic resolution provides unambiguous identification of these substances.

Figure 3. Electron-impact (-70 eV) mass spectrum of a reference standard of (6aR,9S,10aR)-HHC analyzed by isothermal gas chromatography on a DB35ms column at 240 °C.
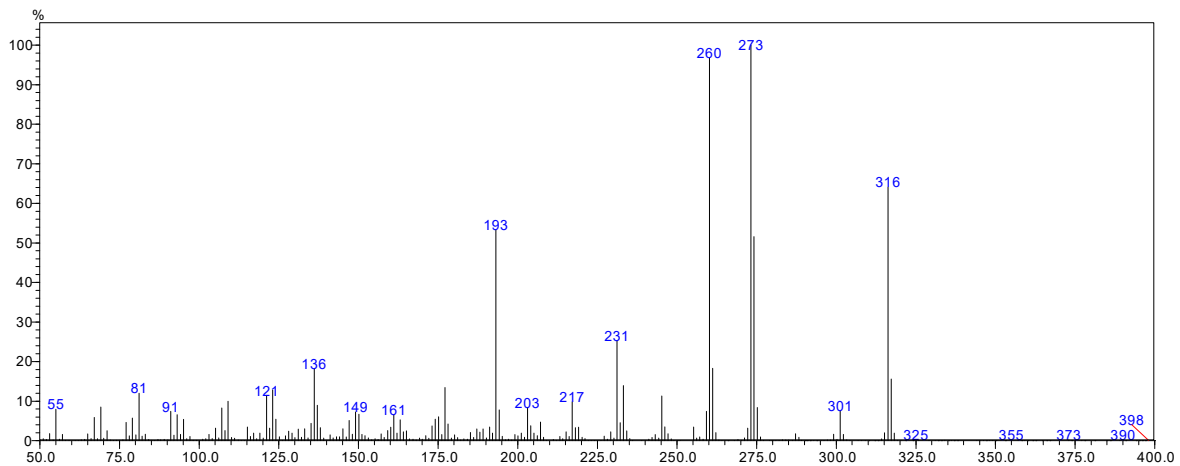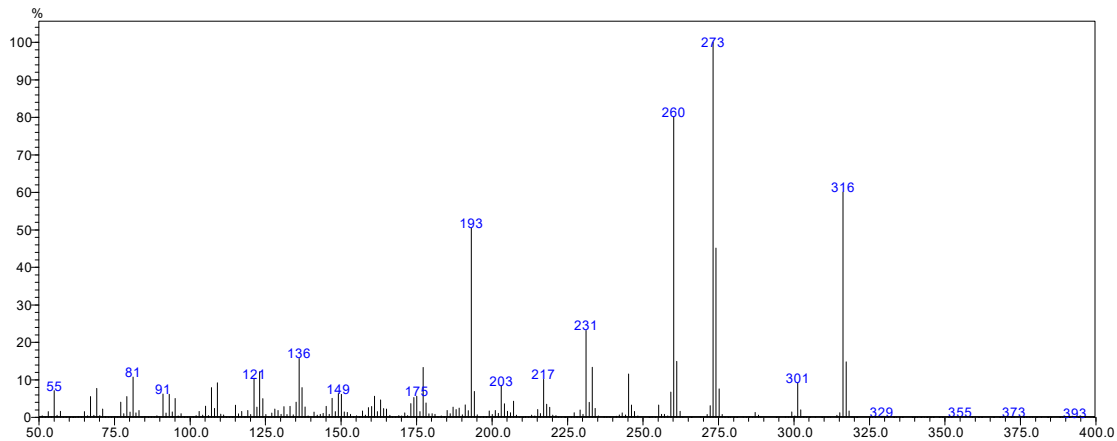
Figure 4. Electron-impact (-70 eV) mass spectrum of a reference standard of (6aR,9S,10aR)-HHC analyzed by isothermal gas chromatography on a DB35ms column at 240 °C.

Analysis of the diastereomers of natural HHC by reversed-phase chromatography on C18 bonded-phases indicated complete separation (see Figure 5) and with a reversed elution order compared to analysis by GC-MS (Sams, unpublished observations). The HPLC analyses were performed using a 150 mm x 4.6 mm i.d., containing 2.7 mm particles (Raptor ARC-18, Cat # 9314A65)[2] using a mobile consisting of 5 mM ammonium formate and 0.1 % formic acid (25 %) and acetonitrile containing 0.1 % formic acid (75 %).

Figure 5. Reversed-phase HPLC separation of the diastereomers of natural HHC (i.e., (6aR,9R,10aR)-HHC and (6aR,9S,10aR)-HHC) under isocratic conditions.
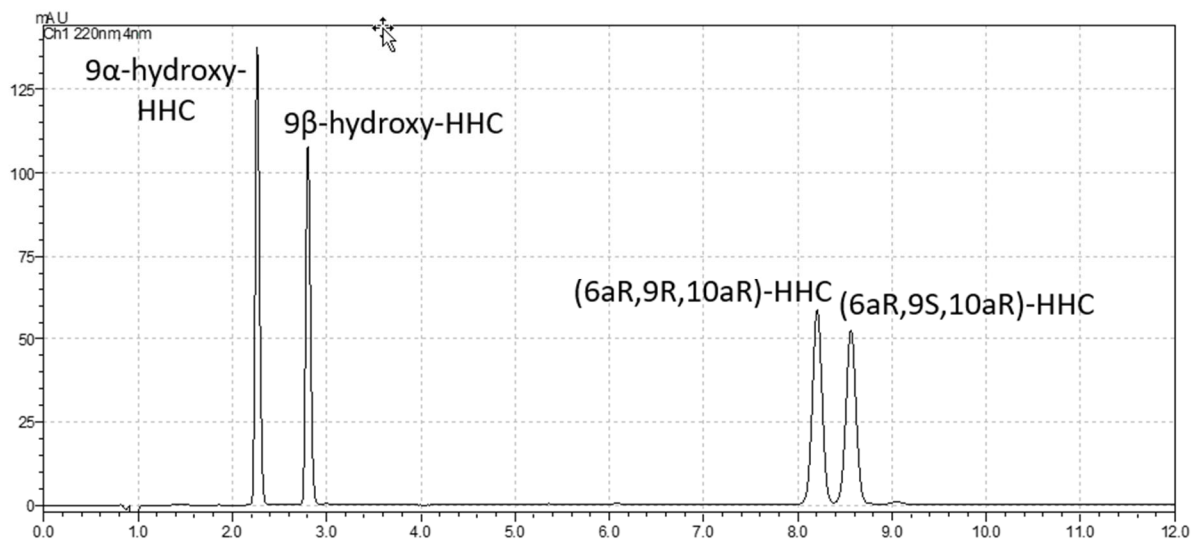

Some samples labelled as "HHC" have been submitted to KCA for analysis but, when they were analyzed by RP-HPLC-PDA or GC-MS, neither diastereomer of (-)-HHC was detected and no substances characterized by a molecular mass of 316 were detected

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[2] https://www.restek.com/en/products/columns/HPLC--UHPLC-Columns/Analytical-Columns/41315/

by mass spectral screening (Sams, unpublished observations). However, the results of GC-MS analyses of these samples indicated the presence of (-)-*trans*-Δ^8-THC, (-)-*trans*-Δ^9-THC, and $\Delta^{6a,10a}$-THC (see, for example, Figure 6).

Figure 6. Results of GC-MS analysis of a sample submitted to the laboratory for analysis as an "HHC" sample
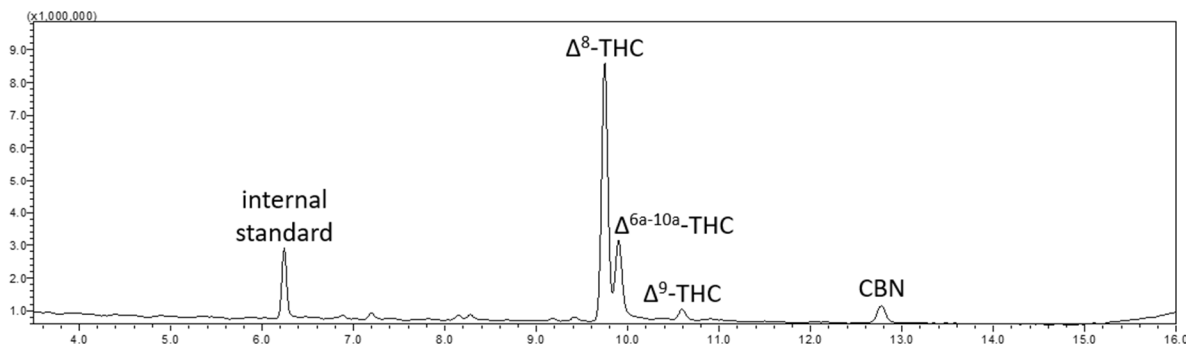
Aware that HHC is also being used as an abbreviation for 9b-hydroxyhexahydrocannabinol[3] (9b-OH-HHC), we sought to determine whether the results for these test samples could be attributed to loss of water from 9b-OH-HHC during GC-MS analysis thereby producing a mixture of tetrahydrocannabinoids such as those shown in Figure 6 [8]. However, analysis of reference standards for both (±)-9a-hydroxyhexahydrocannabinol (Item No. 36129)[4] and (±)-9b-hydroxyhexahydrocannabinol (Item No. 35266)[5] indicated that both substances are amenable to nondestructive analysis by HPLC, LC-MS, and GC-MS analysis under the conditions that we use to analyze (-)-HHC and that the analysis proceeds without producing a mixture of tetrahydrocannabinols such as was observed in Figure 6 (Sams, unpublished observations). In fact, no evidence for loss of water from either substance during GC-MS analysis was detected.

Both hydroxy-HHC analogues were clearly separated by reversed-phase HPLC at early retention times indicative of their higher polarity relative to the diastereomers of (-)-HHC (see Figure 5) (Sams, unpublished observations).

Furthermore, the results of GC-MS analysis of both hydroxy-HHC analogues indicated long retention times relative to those of the diastereomers of (-)-HHC consistent with their predicted higher boiling points under GC analysis conditions (see Figure 2). The electron-impact ionization mass spectra of these two substances confirmed their molecular masses (*i.e.*, 332 amu). The mass spectra were both characterized by loss of 18 amu indicating loss of water in the source (see Figure 7 and Figure 8) but not during GC analysis. Since the mass spectra confirmed the molecular mass and the retention times of the hydroxy-HHC analogues were different from those of Δ^8-THC, Δ^9-THC, and $\Delta^{6a,10a}$-THC, we concluded that loss of water during sample preparation for analysis and then HPLC or GC-MS analysis was not responsible for the anomalous findings in the samples labelled as HHC. Therefore, we have concluded that the analyses of natural (-)-HHC and 9a-OH-HHC and 9b-OH-HHC under both HPLC and GC-MS conditions, as described above, proceed unambiguously and that findings for various tetrahydrocannabinoids are not artifacts produced by loss of water from the OH-HHC isomers during analysis by either method.

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[3] https://dailycbd.com/en/hhc/ accessed February 22, 2022

[4] https://www.caymanchem.com/product/36129/helping-make-research-possible

[5] https://www.caymanchem.com/product/35266/(%C2%B1)-9%CE%B2-hydroxy-hexahydrocannabinol

Figure 7. Electron-impact (70 eV) ionization mass spectrum of a standard of (±)-9a-hydroxyhexahydrocannabinol analyzed by isothermal gas chromatography on a DB35ms column at 240 °C.
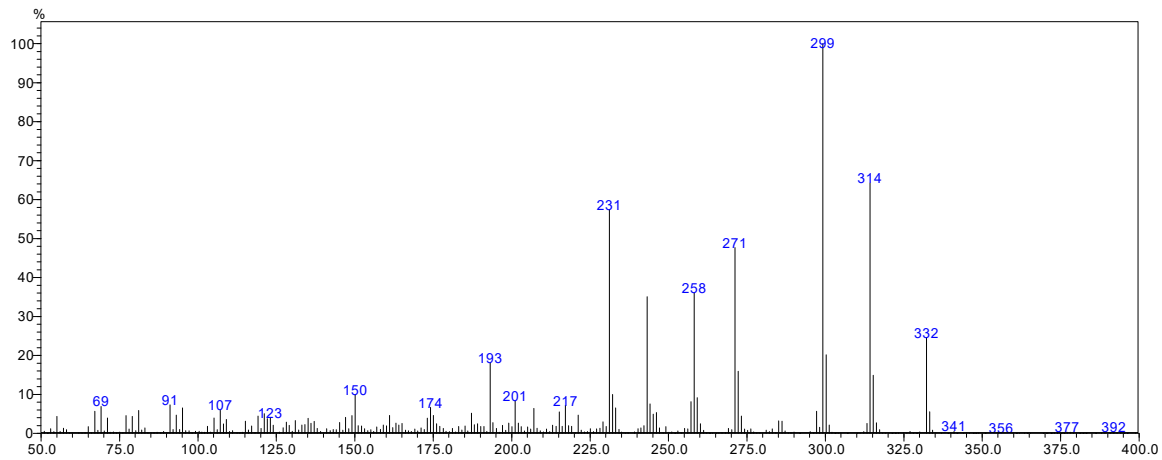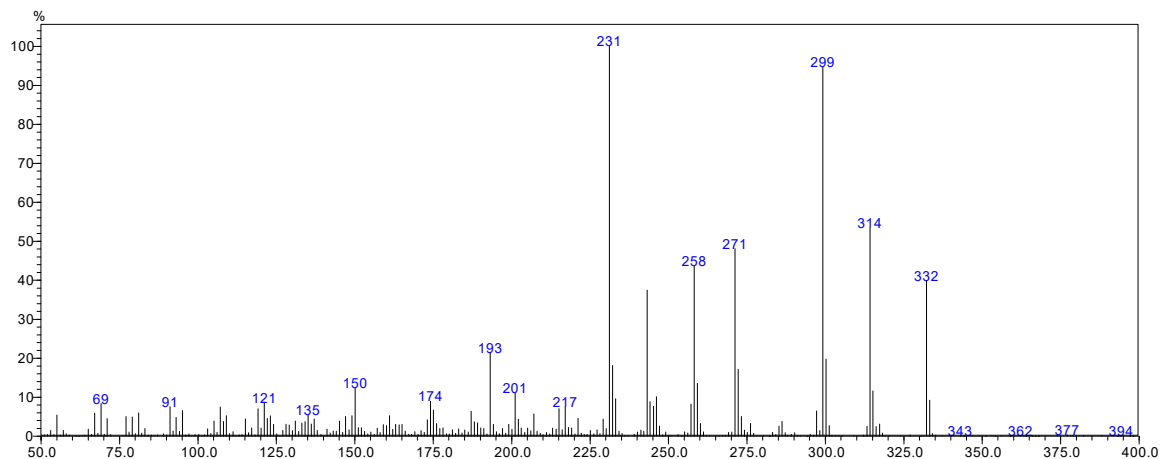

Figure 8. Electron-impact (70 eV) ionization mass spectrum of a standard of (±)-9b-hydroxyhexahydrocannabinol analyzed by isothermal gas chromatography on a DB35ms column at 240 °C.

CONCLUSION

We have demonstrated that the diastereomers of natural HHC can be separated and identified by HPLC, LC-MS, and GC-MS methods with complete resolution of the two diastereomers from each other and from other substances tested. Furthermore, we have conclusively determined that some samples labelled to contain "HHC" do not contain any evidence for the presence of (-)-HHC or OH-HHC but instead contain primarily (-)-*trans*-Δ^8-THC with smaller quantities of (-)-*trans*-Δ^9-THC and $\Delta^{6a,10a}$-THC.

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