

SAW/GC

Technical Application Note
Revised May 2003



Title:

Identification of the Physiologically Active and Inactive Components of Marijuana using the 4200 SAW/GC from a Variety of Cannabis Samples

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Introduction

There is a sociological need for the rapid identification of illicit drugs. Among those substances classified as ‘drugs of abuse’ is marijuana or cannabis derived from the female Indian Hemp plant Cannabis Sativa. Hemp grows easily in temperate and tropical locations to a height sometimes of over six feet high. The plant often differs in its production of secondary metabolites due to growth patterns and climatic location. Secondary metabolite production may be due to sub-species variants, tropical or subtropical location and many other less specific reasons. Different sections of the plant produce different varieties of biologically active secondary metabolites some of which are also physiologically active. The leaves and shoots of the plant contain a wide variety of volatile, active substances which give the plant its characteristic odor. The seeds however contain no psychologically active compounds. The aroma of the plant, which is a function of its volatile compounds, is particularly noticeable when it has been air dried and smoked.

Four major volatile chemical components (see Table 1) derived from hemp are released during smoking. These compounds are related to the Cannabinol family and can be present in varying amounts. The physiologically and hallucinogenic active component of the plant which distinguish it from other dried herb is, tetrahydrocannabinol (THC). THC exists as two positional isomers Δ^9 & Δ^8 tetrahydrocannabinol which are responsible for the euphoric effect of cannabis when smoked or consumed.

The 4100 SAW/GC produces a signature chromatogram of cannabis in under ten

Table 1- Standard Cannabis Volatile Components

	RT	Compound	Comments
1	3.14s	Δ^9 Tetrahydrocannabinol	Physiologically active Δ^9 isomer
2	3.14s	Δ^8 Tetrahydrocannabinol	Physiologically active Δ^8 isomer
3	3.70s	Cannabinol	Physiologically inactive
4	3.66s	Cannabinadiol	Physiologically inactive

seconds often containing up to eight major peaks from the available volatile compounds in the plant. Single component standard compounds are commercially available and can be used to characterize the important volatile components of cannabis in terms of their chromatographic retention time in the instrument. Using these standards the THC peak and the other significant volatile components as shown in Table 1 can be distinguished.

Results

Sampling Methods

The normal method of collecting ultra-rapid chromatograms is to prepare cannabis samples either in a 40mL VOA vial or in a temperature controlled glass tube with constant air flow through the tube. The VOA vial allows room temperature sampling in an easy and convenient manner which is also easily adaptable for field work. The sample is contained in the vial and secured with a plug of glass wool to prevent particles from entering the inlet of the SAW/GC analyzer during sample acquisition.

The air flow tube method of sampling allows temperature control of the sample which may be stabilized at any given temperature above room temperature (RT). The sample is packed into a 100mm x 5mm diameter glass tube and secured between two glass wool plugs. The tube is inserted into the thermostat and the temperature allowed to equilibrate. A constant flow of air is delivered through the tube which allows vapors to be acquired, carried through the chromatograph, and then delivered to the detector.

GC Method

The chromatographic method used with the SAW/GC is shown in Figure 1. The column was temperature programmed at a constant 18°C per minute rate from 40°C to 175°C. The complete method is shown sequentially in the lower portion of Figure 1. Vapors were pumped through the system preconcentrator for 5 seconds. Then the valve was switched into the inject position and the preconcentrator (focus) activated so as to release vapor samples collected. These released vapors pass through the valve and onto the column where they are temporarily immobilized. After allowing approximately 5 seconds for this to occur, the valve is switched back to the sample position and the column temperature ramp sequence started. Simultaneous with starting the ramp, data acquisition from the SAW detector is carried out for 10 seconds. After the data has been collected the detector temperature is raised so that all detected vapor samples are desorbed so that the SAW detector is ready for the next machine cycle.

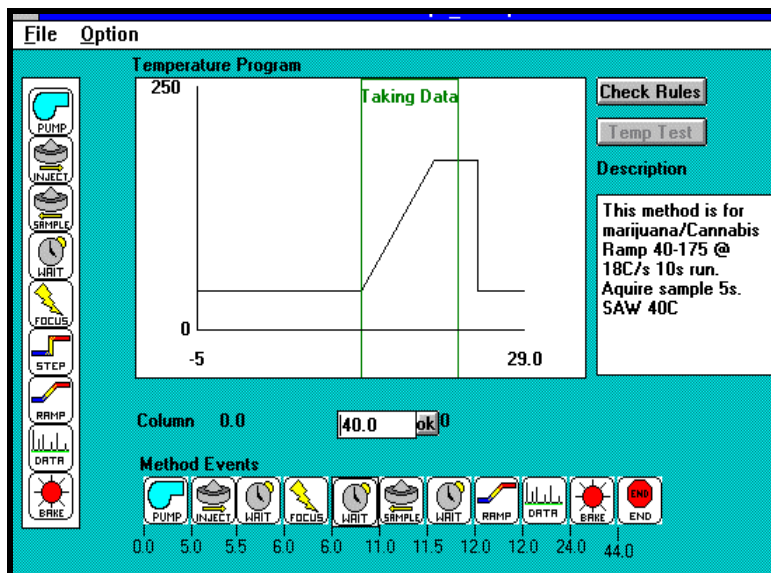


Figure 1- Chromatographic method screen taken from the instrument used for the analysis of cannabis volatiles

Sample Chromatograms

Sample chromatograms were obtained from different samples of marijuana grown under different conditions and obtained from geographically different locations, e.g. New York, Jamaica, California, Mexico. Four chromatograms, labeled HG-NY, AP, M1, and M2, are displayed in Figure 2 through Figure 5 and are representative of these samples.

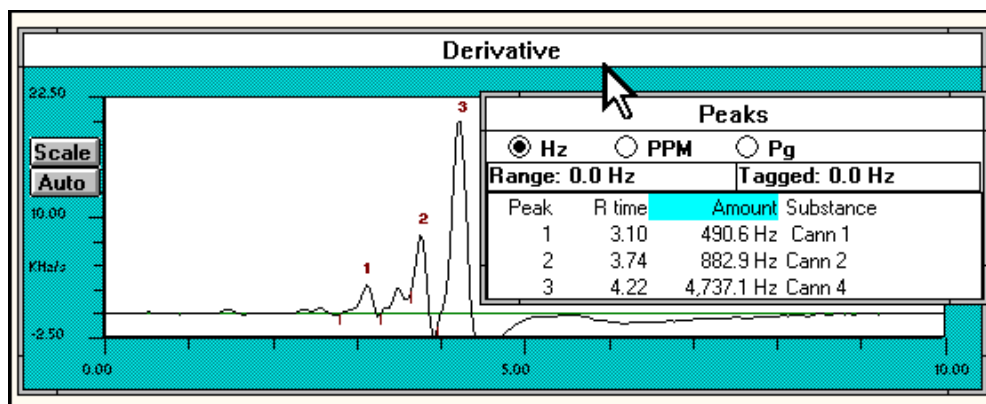


Figure 2- Cannabis Sample Labeled 'AP'.

The chromatograms shown in Figure 2 (AP) and Figure 3 (HG-NY) are nearly identical and the amount of each compound is shown in the accompanying peak table. Amounts are shown in native detector units, Hz. Each peak has been labeled simply as Cann 1, Cann 2, etc..

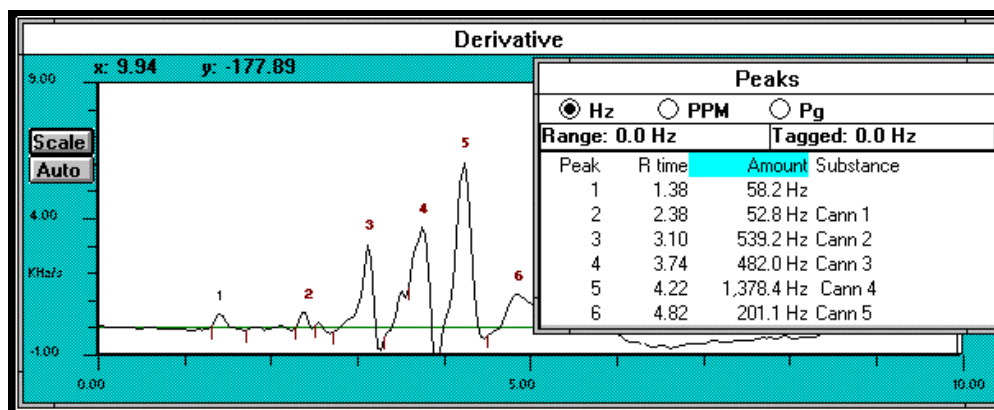


Figure 3- Cannabis Sample Labeled 'HG-NY'

Chromatograms displayed in Figure 4 and Figure 5 show samples labeled M1 and M2. In these chromatograms a detector scale factor of 10Hz/picogram has been applied and the area of each peak is given in picograms (pg). It was found that this scale factor gave a reliable estimate of the relationship between frequency response in the instrument and pg of analyte perceived by the SAW detector.

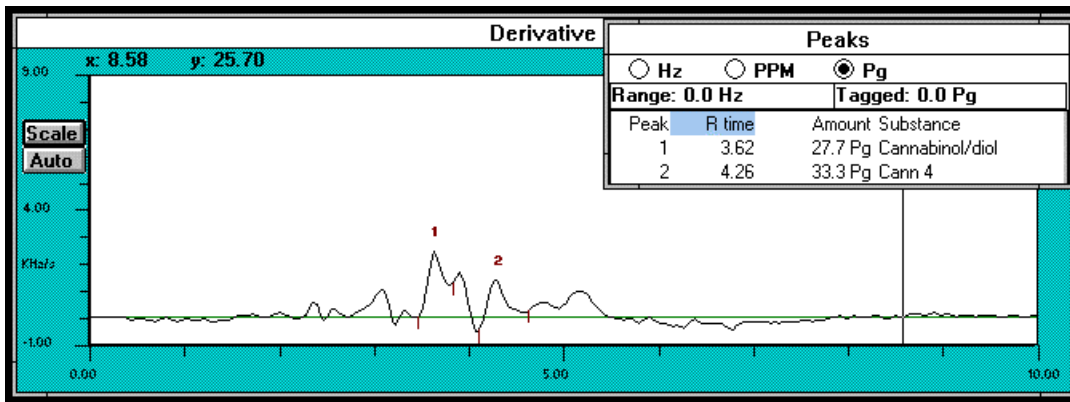


Figure 4- M 1 Cannabis sample at RT showing small volatile component

It can be Figure 2 and Figure 3 that some samples of cannabis are nearly identical, even though the samples were obtained from different sources. In other instances, as seen in Figure 4 and Figure 5, the samples can have differing profiles with the presence or absence of significant peaks.

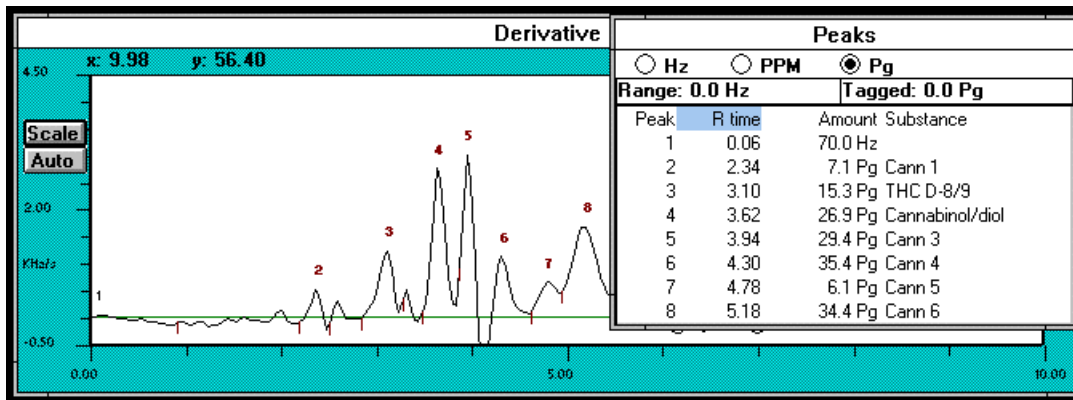


Figure 5- M2 cannabis sample

Sample M1 was one of the oldest samples available and can be seen by its peak listing to have a relatively poor yield of volatile components. The relative potency of each sample of cannabis can be measured by the quantity of volatiles present. Sample M2 shown in Figure 5 was fresher and produced a larger volatile peak profile. Older samples produce smaller quantities of the early eluting more volatile components one of which, as will be shown is THC.

Comparative Analysis

The SAW/GC software can easily compare one fragrance or scent to another by overlaying one trace over another. Using this feature both similarities and differences between different samples of marijuana can be observed. An example is the overlay comparison of samples M1 and M2 shown in Figure 6 and the overlay comparisons of AP and NY-HG shown in Figure 7. In each case, although from different sources, these samples show essentially the same fragrance since they have the same peaks.

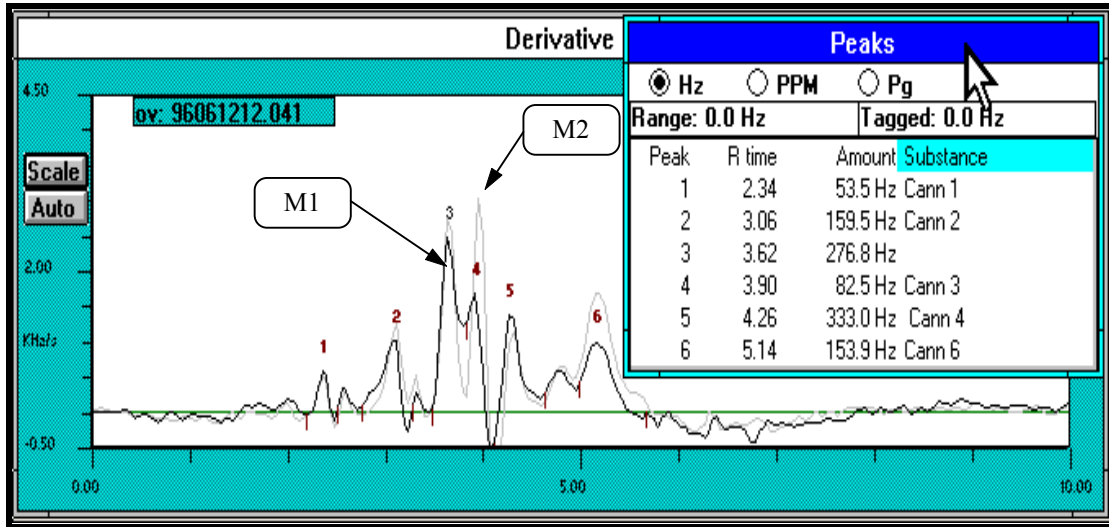


Figure 6- Overlay of Cannabis Samples M1 and M2 .

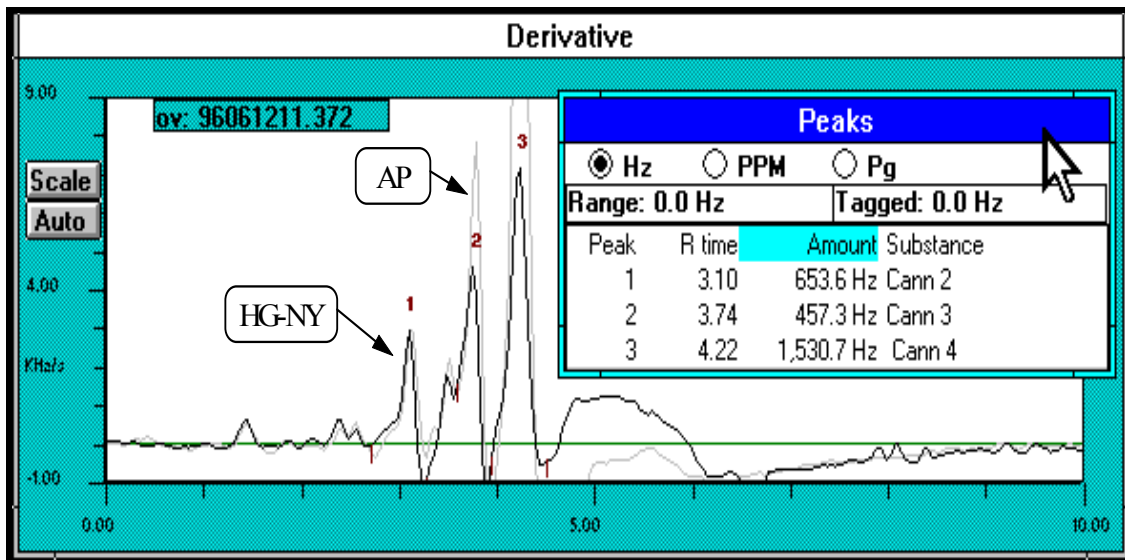


Figure 7- Overlay plot of 'HG-NY' and 'AP' cannabis fragrance

In other instances, as shown in Figure 8 and Figure 9, the samples can have very different fragrances. Sample M2 is distinctly different from HG-NY. In addition, sample M2 shows an additional peak at approximately 4 seconds which is not present in the HG-NY sample.

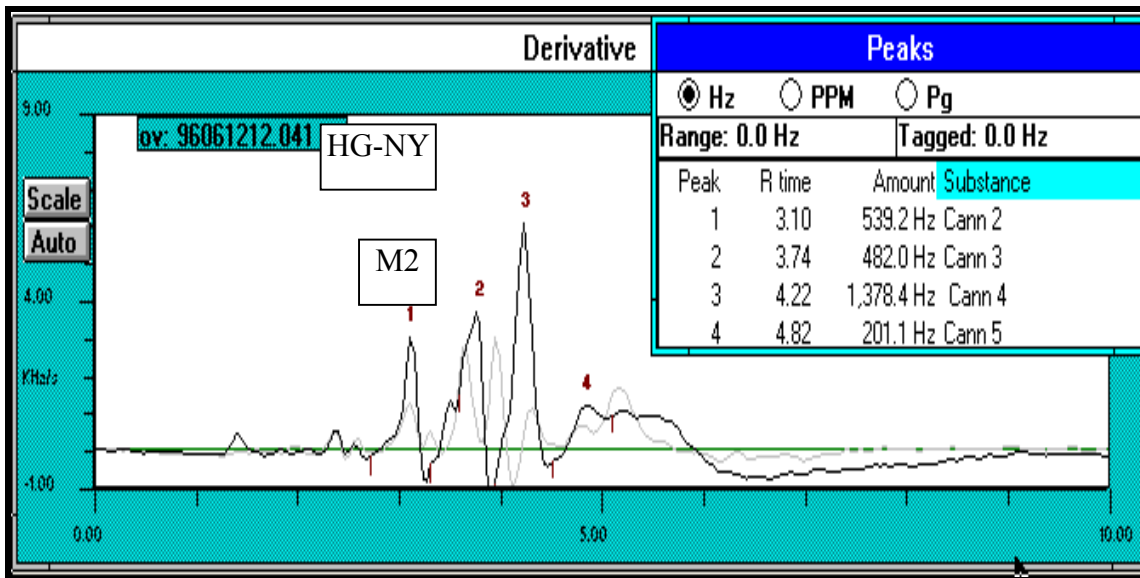


Figure 8- Comparison of M2 and HG-NY Cannabis

Figure 9 shows a comparison of Jamaican and HG-NY cannabis. The Jamaican shows almost no volatile components compared to the cannabis from New York.

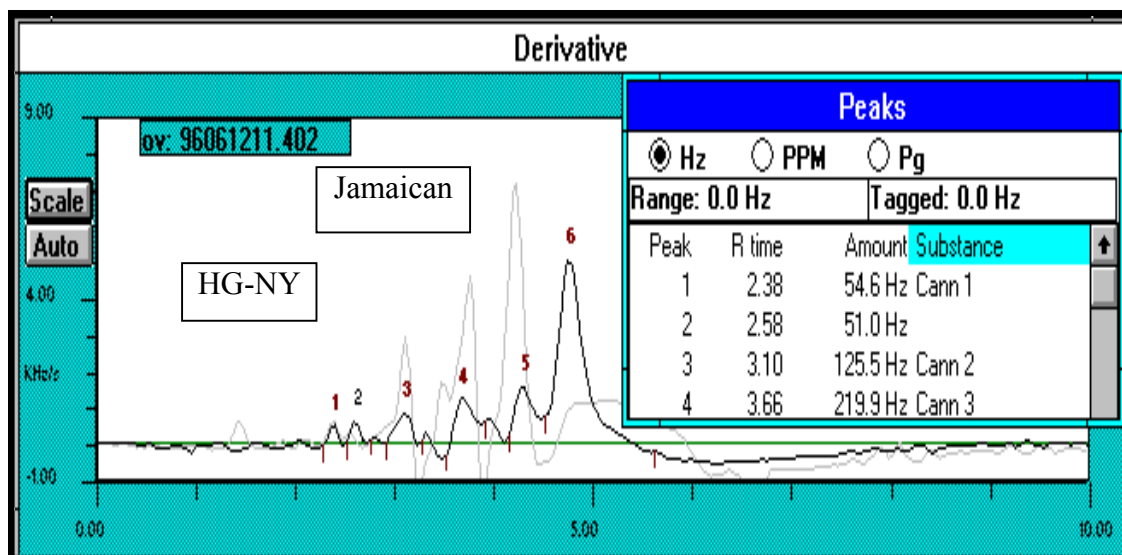


Figure 9- Jamaican Cannabis (JC) compared with HG-NY Cannabis.

Comparisons with Chemical Standards

To determine which peaks in the chromatograms were actually Cannabinol and THC, chemical standards were purchased from Sigma Chemical Co. and used to calibrate the SAW/GC. Actual chromatograms obtained with these samples are shown in Figure 10 and Figure 11.

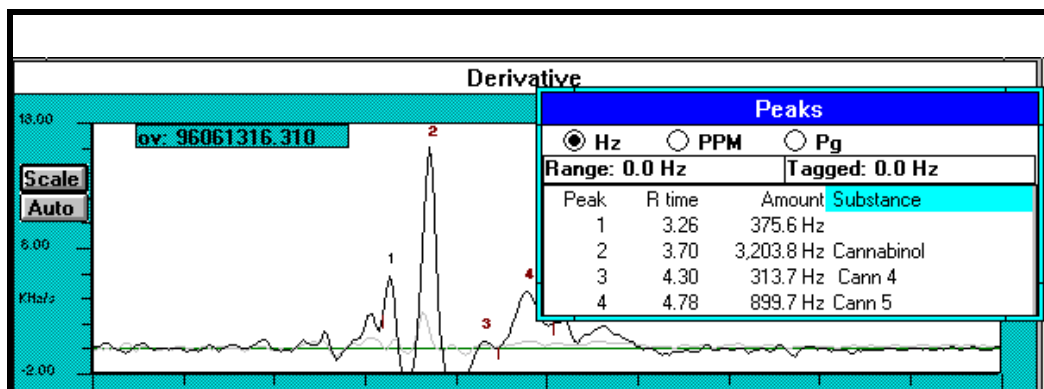


Figure 10- Cannabinol standard at 40°C and 50°C (overlay).

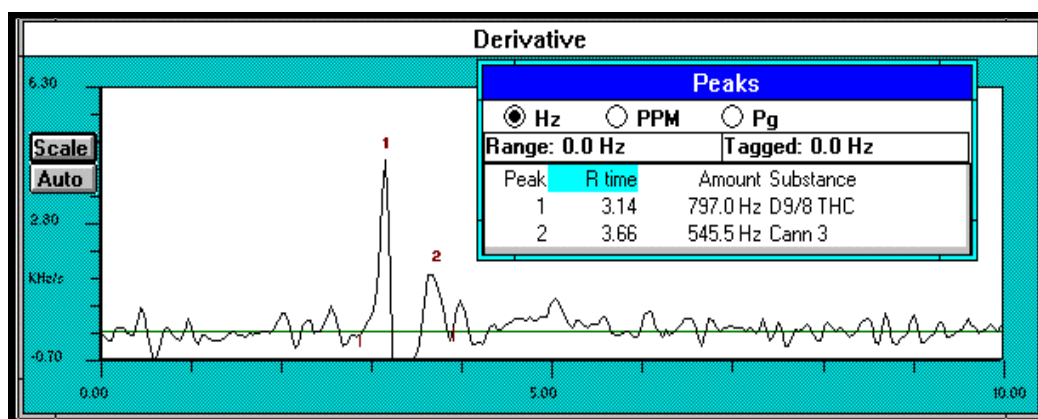


Figure 11- Δ^9 Tetrahydrocannabinol Standard run at 35°C

Δ^9 Tetrahydrocannabinol has a retention time of 3.14 seconds. Under these conditions, the other isomer, Δ^8 , which is also physiologically active and differs only in the position of a double bond, is not separable. The retention time of cannabinol was 3.66 seconds. The last standard to be tested was cannabinadiol in which the THC cyclic ether has been ring opened to the reduced form of diol. This compound co-elutes with cannabinol and has a retention time of 3.66 seconds. Retention times for the four standards tested are listed in Table I (See Page 2).

Using the above results for retention times of the chemical standards, a peak information dialog window is filled out using the system software. An example of the peak identification information is shown in Figure 13. In this listing five peaks, labeled Cann 1, Cann 3, Cann 4, Cann 5, and Cann 6 correspond to unidentified compounds commonly found in marijuana vapors. Their retention times are listed and a 50 Hz alarm

is set. Using known standards, the THC Δ-8/9 and Cannabinol/diol peaks are also entered into the peak identification table with the same 50 Hz alarm. Scale factors for all peaks are set to 10Hz/pg which provides a convenient, but un-calibrated, benchmark for peak amplitudes in lieu of full calibration based upon known vapor pressures. With a full

File							
File Description							
Cannabis Profiles & Standards							
Units to Display				Peak Sum Range		Re-Index	
<input checked="" type="radio"/> Hz <input type="radio"/> PPM <input type="radio"/> Pg Sample Flow (ccm) <input type="text" value="0.0"/>				From: <input type="text" value="0.0"/> To: <input type="text" value="0.0"/>			
Retention Time	Percent Spread	Substance	Alarm Level	Converted Alarm Level	Hz/ (PPM*CC)	Hz/ Pg	Tag
2.340	2.500	Cann 1	50.00 Hz	50.00	0.0000	10.0000	↑
3.100	5.000	THC D-8/9	50.00 Hz	50.00	0.0000	10.0000	
3.660	2.500	Cannabinol/diol	50.00 Hz	50.00	0.0000	10.0000	
3.940	2.500	Cann 3	50.00 Hz	50.00	0.0000	10.0000	
4.220	2.500	Cann 4	50.00 Hz	50.00	0.0000	10.0000	
4.780	2.500	Cann 5	50.00 Hz	50.00	0.0000	10.0000	
5.180	2.500	Cann 6	50.00 Hz	50.00	0.0000	10.0000	↓

Figure 13- Peak identification file for cannabis and cannabis standards

calibration the SAW/GC can provide an accurate vapor pressure measurement in parts per billion or parts per million units. Alternately the amplitude measurement can be in mass units of picograms (pg) or nanograms (ng).. The practical quantification limits of the instrument are typically in ppb or less than 1 pg of a compound by weight.

A chromatogram of the Cannabinol standard with the THC standard chromatogram overlaid in the background is shown in Figure 12. The retention time difference of these two standards is 0.56 seconds.

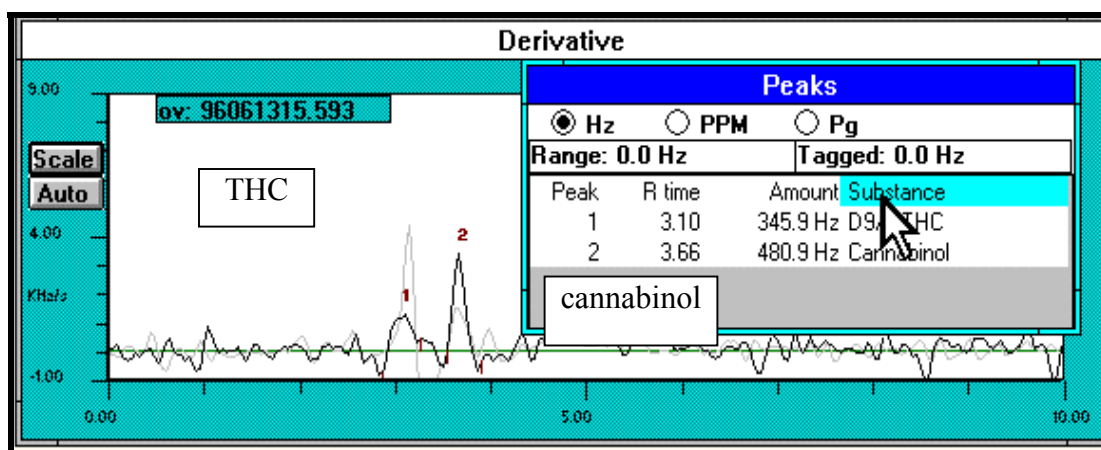


Figure 12- Cannabinol Standard Overlain by THC Standard .

Of interest is to re-examine the previously tested samples using the new peak identification file and overlays of the THC and cannabinol standards. Figure 14 shows a chromatogram of M2 cannabis overlain by the THC standard chromatogram. Note that THC vapors are not detected, however, cannabinol vapors are detected and in this

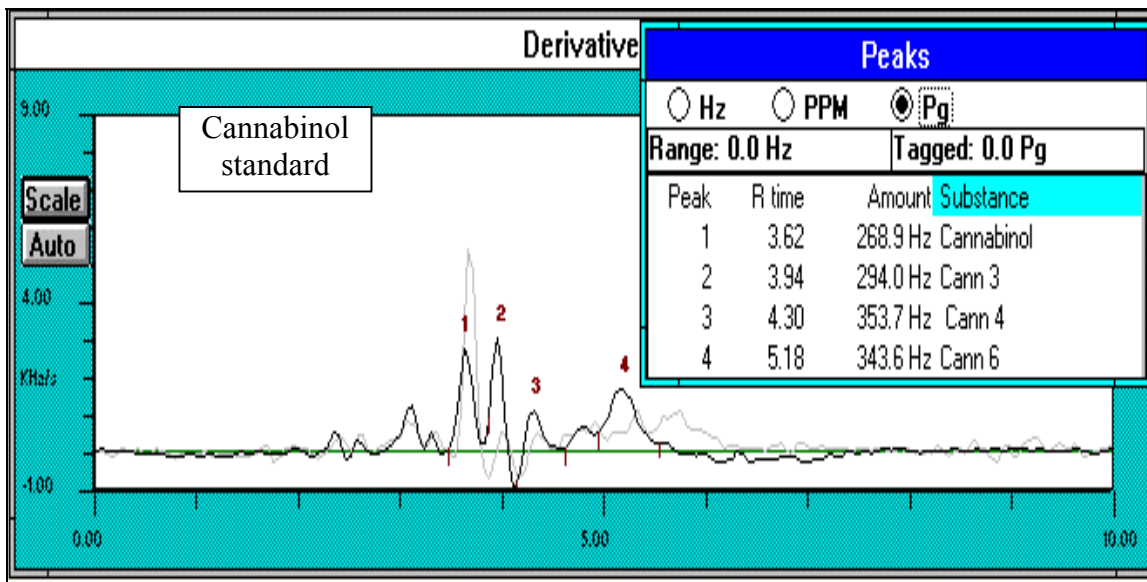


Figure 14- M2 Cannabis overlain by cannabinol standard

sample.

This result is in contrast to the HG-NY sample shown in Figure 15. Here the THC standard overlay shows that a high THC content as well as cannabinol are present in the sample vapors. This procedure can be used to identify and quantify the psycho-active components any cannabis sample. The entire procedure takes less than 15 seconds. Five seconds are used to collect and preconcentrate the vapors and 10 seconds is required to

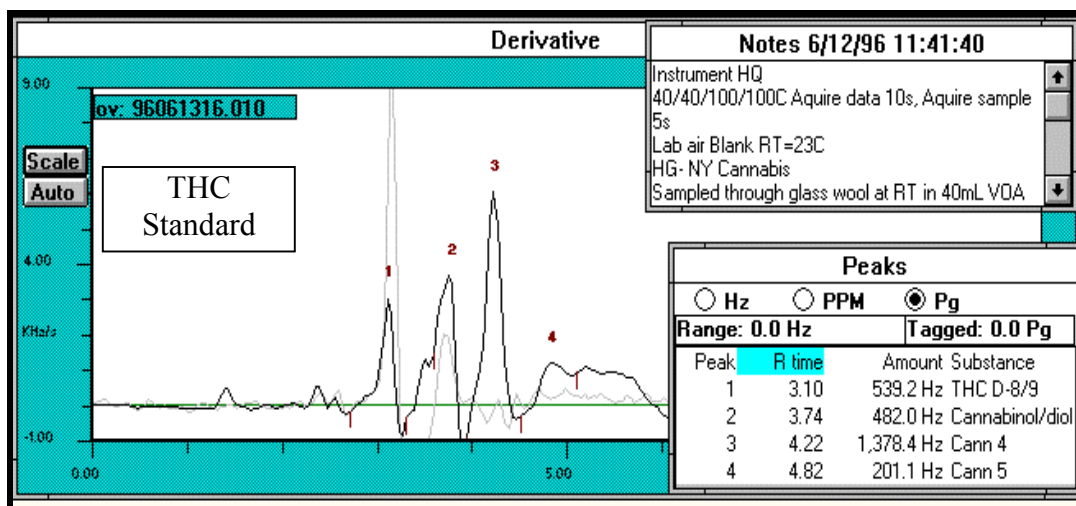


Figure 15- NY-HG Cannabis sample showing peak ID table and overlaid THC standard

create the chromatogram of the vapor.

Conclusion

The distinguishing features of multi-compound fragrances associated with material suspected of containing cannabis may be verified if it contains the physiologically active ingredients such as THC. A peak identification or pattern recognition algorithm coupled with a fast SAW/GC allows suspect samples to be screened at rapid rate. Information as to the physical composition of each sample may also be used to quantify the potency and possible origin of the contraband material.

The SAW/GC contraband screening instrument is equipped with a special user interface for unskilled users. The new user interface transforms the analytical chromatogram picture into a simple array of detection alarms as shown in Figure 16. Each compound or peak is assigned a bar-alarm and audio visual keys are used to inform the user that a positive recognition signal has been detected. Using relationships between signal-to-noise and alarm threshold, the user interface provides estimates for probability of detection and probability of false alarm. As a screening tool the SAW/GC typically operates with less than 1% probability of false alarm and 95% probability of detection.

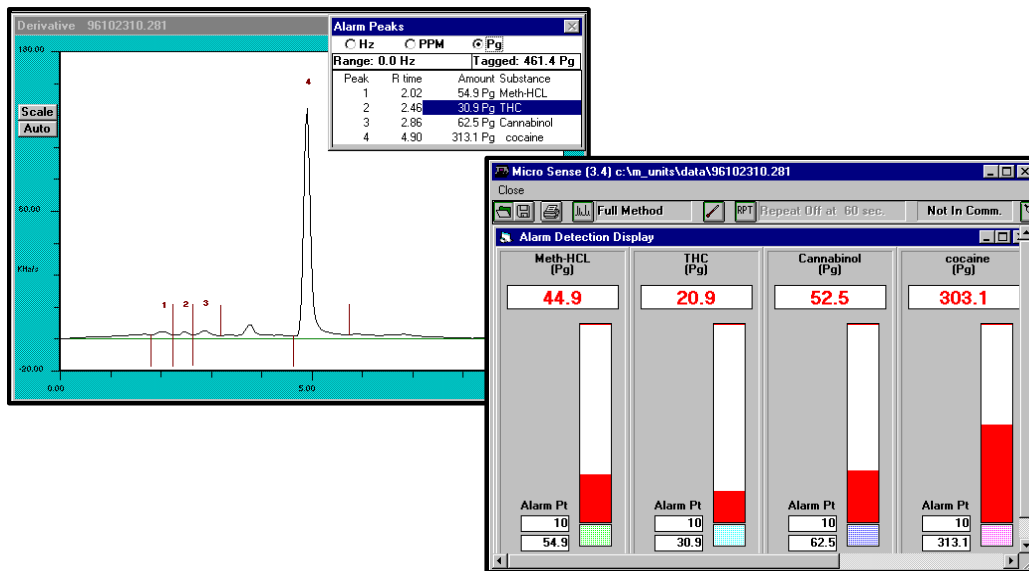


Figure 16- Chromatograms can be replaced with Simplified User Interface

The SAW/GC screening tool performance has been field tested in applications involving drugs of abuse for the U.S. Customs and the Drug Enforcement Agency (DEA-Figure 17), explosives for the Federal Aviation Administration (Figure 18), and currency detection for the U.S. Postal Inspection Service. These examples represent only a small fraction of law enforcement applications. The ability to screen environmental vapors and document the results provides law enforcement officers with new and important capabilities which enhance that already provided by canine searches. The SAW/GC does not replace the canine as much as it enhances their performance.



Figure 17- Vapor screening by Law Enforcement.



Figure 18- Vapor screening for Aviation Security