Evaluation Of Volcano® Vaporizer For The Efficient Emission Of THC, CBD, CBN And The Significant Reduction And/Or Elimination Of Polynuclear-Aromatic (PNA) Analytes Resultant Of Pyrolysis

REVISED FINAL REPORT

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1.0 Introduction

Investigations of THC, CBD, and CBN evolution by use of vaporizer methodologies were completed using a vaporizer device (Volcano®: Vapormed 78532). The *Cannabis sativa* (marijuana) used in this study was produced by the National Institute on Drug Abuse (NIDA) and obtained from the Drug Detection Laboratory (Sacramento, CA) from a sample submitted to it by a medical marijuana patient in FDA's Compassionate IND program. Approximately 200 mg of finely screened, homogeneous *Cannabis sativa* (marijuana) was "loaded" into the Volcano® atomizing system. The *Cannabis* was heated to a mean temperature of approximately 155-218°C and the evolved vapor collected in the device collection trap (e.g., manufacturer supplied balloon and/or 250 mL Supelco gas trap). Subsequently, the vapor entrapped by the collection apparatus was transferred to a methanol vapor trap and a portion of the concentrated sample quantitatively assayed using High Performance Liquid Chromatograph-Diode Array-Mass Spectrometry (HPLC-DAD-MS) analysis techniques. Gaseous samples obtained from the vaporizer device were additionally assayed using a semi-quantitative Gas Chromatography-Mass Spectrometry (GC-MS) analytical method for the detection of polynuclear aromatic (PNA) compounds as well as additional resolved tentatively identified compounds.

In order to quantitatively and qualitatively evaluate the percent (e.g., w/w) cannabinoids evolved from the plant material using the Volcano® system, additional *Cannabis* samples (e.g., ~ 200 mg) were extracted using solvent (e.g., ethanol) Sohxlet extraction and combustion (e.g., M1) extraction sample preparation techniques.

2.0 Purpose

This study was completed to provide evidence of product (Volcano®) efficacy to MAPS and CaNORML, to be submitted to Dr. Donald Abrams, UC San Francisco, who would subsequently design and seek agency (FDA) approval for the protocol development and initiation of a phase I clinical investigation comparing cannabinoid blood levels in subjects smoking (i.e., pyrolysis) *Cannabis* versus *Cannabis* vaporized with the Volcano® vaporizer system. This concept was initially based on visual observations of patients who used a device to heat versus combust the *Cannabis* material. The desired *Cannabis* effect was obtained. This observation led to a concept, which suggested that by reducing the amount of heat (i.e., < combustion) applied to the *Cannabis* material, the desired cannabinoid compounds can be released and captured in the absence of those compounds which are undesirable (i.e. PNA).

3.0 Abbreviations

API-ES	Atmospheric Pressure Ionization (Electrospray)
ASTM	American Society for Testing and Materials
Avg.	Average
Conc.	Concentration
CV	Coefficient of Variance
DAD	Diode Array Detector
HPLC	High Performance Liquid Chromatograph
LOQ	Limit of Quantitation
MΩ	Mega-ohm
mAU	Milli-Absorbance Units
min	Minutes
MS	Mass Spectrometry
MSD	Mass Selective Detector
m/z	Mass to Charge ratio
NA	Not Applicable
NBS	National Bureau of Standards
ND	Not Detected
R^2	Coefficient of Determination
RRF	Relative Response Factor
RT	Retention Time
Y-Int.	Y-Intercept
λ	Wavelength
w/w	Concentration as a function of weight to weight
NBS	National Bureau of Standards
TIC	Tentatively Identified Compounds
THC	delta9-Tetrahydrocannabinol
CBD	Cannabidiol
CBN	Cannabinol

4.0 Materials and Methods

4.1 Test and Reference Chemicals

Chemic Laboratories, Inc received samples of the following products:

Product	Received From	Received Date	Lot No.	Sample ID No.
delta9-Tetrahydrocannabinol	Sigma Chemical Co.	1/28/2003	092K8800	CON012803-02
Canabinol	Sigma Chemical Co.	1/28/2003	61K4043	CON012803-01
Canabidiol	Sigma Chemical Co.	1/24/2003	071K8805	REF012403-02
Mix:16 PNA reference Stds.	ChemService Co.	5/28/2002	282-148A	REF052802-02
Caffeine	Sigma Chemical Co.	2/13/02	80K1106	REF021302-01
Cannabis sativa	Drug Detection Laboratory	2/13/2003	30100321	CON021303-01

Upon receipt of the test products and reference chemicals, unique sample identification numbers were assigned, as appropriate. The reference standards were stored under secure, controlled, monitored temperatures (e.g., THC & PNA: < 0°C, CBD & CBN: 2-8°C, Caffeine: 18-23 °C). The test sample was stored under secure, controlled, monitored ambient temperatures (18 to 23°C).

4.2 Laboratory Reagents and Equipment

All chemicals and solvents used were at least reagent grade obtained from commercial sources.

Reagent(s) / Equipment	**Manufacturer**	**Lot # or Serial #**
ASTM Type II water	Chemic Laboratories, Inc. *Prepared using a water purification system*	MΩ ≥ 16
Acetonitrile	EMD	43006302
Trifluoroacetic acid	Aldrich	D008180A0
Methanol	Burdick & Jackson	CG596
	Fisher	020390
Toluene	Fisher	015107
Ethyl Alcohol	Aldrich	EA04908DA

Reagent(s) / Equipment	Manufacturer	Lot # or Serial #
Pipette, P200	Gilson	D13600C
Pipette, P1000	Gilson	Q72606L, Q72604L D20535G
~ 2 mm mesh Sieve	NA	NA
Vacuum Pump	VWR Emerson	s/n 0889
Sonicator	Branson Model 5210	RNA9702533D
Class A volumetric pipettes and flasks	NA	NA
Assorted graduated cylinders	NA	NA
Plastic pipettes and beakers	NA	NA
Tygon® tubing (misc. sizes)	NA	NA
Analytical balance	Sartorius	s/n 36110023
Pan balance	Sartorius	s/n 36090199
Soxlett extraction units	Kimble	NA
Soxlett extraction thimbles	Whatman	p/n 2800226
Pre-washed glass wool	NA	NA
Rotary evaporation equipment	Buchi RE 111	1029060
Thermo Couple Thermometer	Metex	M-3850D
Moisture balance	Ohaus-MB45	1121051485
Solvent collection reaction Tube	Quark (100 ml, 24/40 jt.)	QIG-31-4
Rheostat	N/A	N/A
M-1 Volatilizer	Chiro-Tech Inc.	41300 2
Volcano®	Vapormed 78532	NA

High Performance Liquid Chromatograph (HPLC System 8)

Solvent degasser	Hewlett Packard (model 1100)	s/n JP02200597
Solvent delivery system	Hewlett Packard (model 1100)	s/n DE11108372
Sample auto injector	Hewlett Packard (model 1100)	s/n DE11115302
Column compartment	Hewlett Packard (model 1100)	s/n DE11120967
Diode array detector	Hewlett Packard (model 1100)	s/n DE11112413
Mass Selective Detector	Hewlett Packard (model 1100)	s/n US80100427
API-ES Source	Hewlett Packard (model 1100)	s/n US09481775

Gas Chromatograph (GC System 4)

Gas Chromatograph	Hewlett Packard (model 6890)	s/n US00030414
Injector Module	Hewlett Packard (model 6890)	s/n CN13221481
Tray Module	Hewlett Packard (model 6890)	s/n US13312529
Ion Gauge Controller	Hewlett Packard (model 6890)	s/n US60101999
Mass Selective Detector	Hewlett Packard (model 5973)	s/n US81221545

Note: The instrument division of Hewlett Packard is now an independent company known as Agilent Technologies

4.3 Instrumental parameters

Gas Chromatograph-Mass Selective Detector (GC System 4)

Instrument:	Hewlett Packard model 6890 injector module, tray module, ion gauge controller, and model 5973 mass selective detector (SIM & SCAN Mode)
Analytical Column:	DB-xtra low bleed 30M X 0.25 mm, 0.25µm film s/n US2178514H
SIM Ions Monitored	
Group #1:	128, 154, 188, 252 m/z
Group #2:	136, 166, 202, 276 m/z
Group #3:	152, 178, 228, 278 m/z
Dwell	100 msec.
SCAN Mode	50-500 m/z
Injection Port Temperature:	280°C
Injection Volume:	1 µL (liquid injection)
Injection volume:	2 mL (headspace injection)
Carrier Gas:	Helium
Flow Rate:	1 mL/min.
Split ratio:	100/1
GC Temperature Program:	
Initial Temperature / Time:	110°C for 1 min.
Ramp:	Increase 5°C per min. to 320°C Hold for 10.0 min.
Run Time:	53 min.
Retention time (Pyrene):	26.1 min.

High Performance Liquid Chromatography Analysis Conditions

Instrument:	Hewlett Packard model 1100 solvent degasser, solvent delivery system, column compartment, sample auto injector and mass selective detector
Analytical column:	Supelco Discovery C8, 4.6 mm x 250 mm, 5µm, s/n 26893-06
Mobile phase:	75% acetonitrile/ 25% 0.1% TFA/ASTM Type II water
Elution profile:	Isocratic

Assay injection volume:	3.5 µL (15 µL for MS)
Flow rate:	1.00 mL/min
Column temperature:	25°C
Diode array detector:	
Detection wavelength:	205 nm
Band width:	16 nm
Reference wavelength:	425 nm
Bandwidth:	10 nm
Mass selective detector:	API-ES mode
Polarity:	Positive
Scan range	50-1000 m/z
Fragmentor:	50
Gain EMV:	1.0
Gas Temperature:	350°C
Drying Gas:	13.0 L/min
Nebulizer Pressure:	60 psig
VCap (positive):	3500V
VCap (negative):	3500V
Retention Times(s):	
Caffeine:	3.18 min.
CBD:	6.64 min
CBN:	8.56 min.
THC:	9.94 min.
Stop time:	60 min

4.4 HPLC Mobile Phase Preparation

0.10% Trifluoroacetic Acid (TFA) – A 0.10% TFA solution was prepared by combining 1.00 mL of trifluoroacetic acid (>99%) with ASTM Type II water to a total volume of 1000 mL. This solution was then degassed by sonication under vacuum.

Final mobile phase solution was prepared by combining 1000 mL 0.1% TFA with 3000 mL acetonitrile, mixing thoroughly, and degassing via sonication under vacuum, as needed.

4.5 Stock Solution, Calibration, and Quantitation Standard Preparation

4.5.1 Caffeine Stock Solution (Internal Standard):

Approximately 1 g (e.g. 1.002 g) of Caffeine reference standard was quantitatively transferred to a 100 mL volumetric flask and solubilized with approximately 50 mL methanol. Upon complete dissolution of the caffeine, the solution was brought to final volume with methanol. The final concentration of the internal reference stock solution was 10.0 mg/mL.

4.5.2 Tetrahydrocanbinol (THC), Canabidiol (CBD), and Canabinol (CBN) Stock Solutions:

Approximately 9.8 mg (e.g. 0.35 mL of 28 mg/mL reference stock solution) of THC reference standard was quantitatively transferred to a 10.0 mL volumetric flask and solubilized with approximately 5 mL methanol. Upon complete dissolution of the analyte the solution was brought to final volume with methanol. The final concentration of this stock solution was 0.98 mg/mL THC.

Approximately 4.4 mg (e.g., 4.42 mg) of CBN reference standard was quantitatively transferred to a 10.0 mL volumetric flask and solubilized with approximately 5 mL methanol. Upon complete dissolution of the analyte the solutions was brought to final volume with methanol. The final concentration of this stock solution was 0.44 mg/mL CBN.

CBD reference standard was purchased pre-solubilized at a final concentration of 0.99 mg/mL and used as received during preparation of subsequent quantitation standards.

4.5.3 Tetrahydrocanbinol (THC), Canabidiol (CBD), and Canabinol (CBN) quantitation reference standards.

Mixed solution reference standards were prepared by combining individual volumes of each of the three quantitation and internal standards to individual 10.0 mL volumetric flasks and bring to final volume with methanol as described in the following table(s).

Table 1. THC quantitation standard preparation

THC	Chemic ID	Stock Conc. (mg/mL)	Vol_I (µL)	Vol_F (mL)	Standard Conc. (µg/mL)
Mixed Ref STD 1	CON012803-02A-1	0.98	50	10.0	4.90
Mixed Ref STD 2	CON012803-02A-2	0.98	100	10.0	9.80
Mixed Ref STD 3	CON012803-02A-3	0.98	200	10.0	19.6
Mixed Ref STD 4	CON012803-02A-4	0.98	400	10.0	39.2
Mixed Ref STD 5	CON012803-02A-5	0.98	1000	10.0	98.0
Mixed Ref STD 6	CON012803-02A-6	0.98	1250	10.0	122
Mixed Ref STD 7	CON012803-02A-7	0.98	1500	10.0	147

Table 2. CBD quantitation standard preparation

CBD	Chemic ID	Stock Conc. (mg/mL)	Vol_I (µL)	Vol_F (mL)	Sample Conc. (µg/mL)
Mixed Ref STD 1	REF012403-01A	0.99	5	10.0	0.495
Mixed Ref STD 2	REF012403-01B	0.99	10	10.0	0.99
Mixed Ref STD 3	REF012403-01C	0.99	20	10.0	1.98
Mixed Ref STD 4	REF012403-01D	0.99	40	10.0	3.96
Mixed Ref STD 5	REF012403-01E	0.99	100	10.0	9.90
Mixed Ref STD 6	REF012403-01F	0.99	125	10.0	12.38
Mixed Ref STD 7	REF012403-01G	0.99	150	10.0	14.85

Table 3. CBN quantitation standard preparation

CBN	Chemic ID	Stock Conc. (mg/mL)	Vol_I (µL)	Vol_F (mL)	Sample Conc. (µg/mL)
Mixed Ref STD 1	CON012803-01A-1	0.442	100	10.0	4.42
Mixed Ref STD 2	CON012803-01A-2	0.442	200	10.0	8.84
Mixed Ref STD 3	CON012803-01A-3	0.442	400	10.0	17.68
Mixed Ref STD 4	CON012803-01A-4	0.442	800	10.0	35.36
Mixed Ref STD 5	CON012803-01A-5	0.442	2000	10.0	88.40
Mixed Ref STD 6	CON012803-01A-6	0.442	2500	10.0	110.50
Mixed Ref STD 7	CON012803-01A-7	0.442	2000	10.0	88.40

Table 4. Caffeine internal standard preparation

Caffeine	Chemic ID	Stock Conc. (mg/mL)	Vol_I (µL)	Vol_F (mL)	Sample Conc. (µg/mL)
Internal Standard Solution	REF021302-01E-1	10.0	200	10.0	200
	REF021302-01E-2	10.0	200	10.0	200
	REF021302-01E-3	10.0	200	10.0	200
	REF021302-01E-4	10.0	200	10.0	200
	REF021302-01E-5	10.0	200	10.0	200
	REF021302-01E-6	10.0	200	10.0	200
	REF021302-01E-7	10.0	200	10.0	200

4.5.4 PNA reference stock solution

A PNA reference stock solution (prepared in toluene at a concentration of 2,500 µg/mL) was obtained from ChemService Inc. West Chester, PA 19381-0599 that included the following analytes:

Naphthalene	Acenaphthylene
Acenaphthene	Fluorene
Anthracene	Phenanthrene
Chrysene	Pyrene
Benzo(a)pyrene	1,2-Benzanthracene
Benzo(k)fluoranthene	Benzo(b)fluoranthene
1,1,2-Benzoperylene	1,2,4,6-Dibenzanthrancene
Indeno [1,2,3-c,d]pyrene	

Subsequent dilutions were prepared as described below to serve as the quantitation reference standards.

Table 5. PNA quantitation standard preparation

Poly Nuclear Aromatics	Chemic ID	Stock Conc. (µg/mL)	Vol_I (µL)	Vol_F (mL)	Sample Conc. (µg/mL)
Mixed Ref STD 1	REF052802-01H	2500	0.500	10.0	125
Mixed Ref STD 2	REF052802-01H-1	125	6.00	10.0	75.0
Mixed Ref STD 3	REF052802-01H-1-1	75.0	5.00	10.0	37.5
Mixed Ref STD 4	REF052802-01H-1-1-1	37.5	4.00	10.0	15.0
Mixed Ref STD 5	REF052802-01H-1-1-1-1	15.0	5.00	10.0	7.50
Mixed Ref STD 6	REF052802-01H-1-1-1-1-1	7.50	3.00	10.0	2.25

4.6 *Cannabis sativa* (marijuana) preparation

Approximately 1.7 g of *Cannabis sativa* was placed on a medium (approximately 2 mm) sieve screen and (using a stainless steel spatula) gently sifted through the screen unit. Following screening, the resultant material was re-homogenized. The gross and net weight of the test samples were then obtained and recorded and the material transferred to an appropriately screw top glass sample vial. This process was repeated as needed throughout the study.

4.7 Moisture determination

Moisture analysis measurements were completed using an OHAUS MB45 halogen dryer moisture balance according to Chemic Laboratories SOP # 4.62 (*Operation, Maintenance, and Calibration of OHAUS MB45 Moisture Analyzer*) based on the gravimetric principle (e.g., total moisture is determined from the weight loss of a sample dried by heating). Approximately 0.56 g of prescreened, homogenized test sample was directly transferred to an appropriately sized moisture balance sample pan and heated at 140°C until a stabilized value of total moisture loss was obtained (e.g., 30 min). The resultant data was utilized in calculating the total w/w calculations of investigated analytes from the test sample.

4.8 *Cannabis sativa* (marijuana) extraction processes

4.8.1 Analyte extraction using a Soxlett extraction system

Sohxlet extraction of the *Cannabis* material evaluated was completed in order to determine the baseline concentration (w/w) of THC, CBD, and CBN, and to provide a concentration value that can be directly compared to the concentrations of analytes captured during Volcano® and combustion experiments. This was completed by installing four Sohxlet extraction units in a high velocity fume hood (e.g., airflow of approximately 100 CFM). Triplicate 0.20 g portions (0.20 g, 0.20 g, and 0.19 g) of prescreened and homogenized *Cannabis sativa* were transferred to pre-tarred Sohxlet extraction thimbles, each topped with a small portion of pre-washed glass wool, and each thimble transferred individual Sohxlet extraction units. A fourth extraction unit was outfitted with an extraction thimble and pre-washed glass wool only and served as the extraction control sample. To each of the Sohxlet extraction units, an appropriately sized round bottom flask (e.g., 500 mL) containing approximately 250 mL of ethanol was attached. The extraction unit was then heated for approximately two hours, such that a gentle refluxing of the system was maintained. Following the extraction process, the extraction units were removed from the heat and allowed to cool to ambient temperature. The extraction and collection flasks were rinsed with the extraction solvent and the rinsate solution collected in the associated round bottom flask.

In order to evaluate the potential for analyte loss under reduced atmospheric pressure, 1.00 mL of caffeine internal reference standard (5.4.3) was quantitatively transferred to each of four round bottom flasks. Each flask was subjected to rotary evaporation until

approximately 10 mL of final extract was obtained. The resultant concentrates were then quantitatively transferred each of four 50 mL volumetric flasks and brought to final volume with methanol. Approximately 1.5 mL of each sample extract was transferred to individual, actinic glass analysis vials, hermetically sealed with Teflon® lined closures, and assayed using HPLC-DAD-MS analysis parameters as previously described.

4.8.2 Analyte isolation using a M-1 combustion system

In order to directly compare analyte evolution from *Cannabis* at temperatures above 230°C, triplicate 0.20 g portions (0.20 g, 0.20 g, and 0.21 g) of prescreened and homogenized *Cannabis sativa* were transferred to an internal screen housed within the M-1 sample holder(Figure 1) and exposed to the rheostat controlled M-1 heating element(Figure 1) until obvious combustion (e.g., smoldering embers) of the organic material had taken place.

To complete the HPLC-MS investigation, the evolved smoke was transferred via vacuum to a single solvent reservoir containing 50 mL of methanol (serving as the dissolution medium) to which 1.00 mL of caffeine internal reference standard was quantitatively added, yielding an internal standard concentration of approximately 200 µg/mL.

Approximately 1.5 mL of each test sample was transferred to individual actinic glass analysis vials and hermetically sealed with Teflon® lined closures and assayed according to the HPLC-MS parameters previously described. The remaining solutions were transferred to individual appropriately sized actinic glass bottles with hermetically sealed Teflon® lined closures and stored under refrigerated conditions.

To complete the GC-MS investigation, the evolved smoke was transferred via vacuum directly to a 250 mL volatile gas trap. A 2.0 mL portion of the gaseous sample was then transferred via use of a headspace syringe directly onto the chromatographic system and assayed according to the previously described parameters. Additionally, in order to solubilize the resultant gaseous residue that had adhered to the gas trap, 2.0 mL of methanol was added to the volatile gas trap, and collected. Subsequently 1 µL of the solvated residue was directly injected onto the GC-MS system and assayed according to the previously described parameters. The resultant data was directly compared to the headspace sample injection data obtained.

4.8.3 Analyte isolation using a Volcano® vaporizer system

In order to directly compare analyte evolution from *Cannabis* at temperatures approaching 155°C (i.e., surface of heated sample) to 218°C (i.e., screen closest to heating element), triplicate 0.20 g portions (0.20 g, 0.20 g, and 0.20 g) of prescreened and homogenized *Cannabis sativa* was transferred to an internal screen housed within the Volcano® (figure 2) sample holder and each separately exposed to the rheostat controlled Volcano® heating element for a period of approximately 45 sec. The evolved vapor was collected in the Volcano® collection device. A thermocouple unit was introduced to the vaporizer device (above and below the heated test product) in order that the operating temperature could be determined. The collected vapor was then transferred over a period of approximately 15 min via vacuum to a single solvent reservoir containing 50 mL of methanol (serving as the dissolution medium). This process was completed an additional two consecutive times for each *Cannabis* sample and the evolved vapor transferred in the same manner to the identical solvent trap (e.g., composite of 3 evolved vapor samples to a single solvent reservoir).

To the final composited samples (n=3), 1.00 mL of caffeine internal reference standard was quantitatively transferred, yielding an internal standard concentration of approximately 200 µg/mL. Approximately 1.5 mL volumes of each test sample were transferred to individual actinic glass analysis vials and hermetically sealed with Teflon® lined closures and assayed according to the HPLC-MS parameters previously described. The remaining solutions were transferred to individual appropriately sized actinic glass bottles with hermetically sealed Teflon® lined closures and stored under refrigerated conditions.

To complete the GC-MS investigation, the evolved smoke was transferred via vacuum directly to a 250 mL volatile gas trap. A 2.0 mL portion of the gaseous sample was then transferred via use of a headspace syringe directly onto the chromatographic system and assayed according to the previously described parameters. Additionally, in order to solubilize the resultant gaseous residue that had adhered to the gas trap, 2.0 mL of methanol was added to the volatile gas trap, and collected. Subsequently 1 µL of the solvated residue was directly injected onto the GC-MS system and assayed according to the previously described parameters. The resultant data was directly compared to the headspace sample injection data obtained.

4.9 Instrumental system suitability assessment

Prior to test sample extract analysis, the GC-MS and HPLC-DAD-MS analytical systems were initialized with the appropriate analytical column and instrumental conditions as previously described until a stable system baseline is achieved. Injecting control extraction solvent to evaluate the GC and HPLC system for any artifact peaks assessed system stability. Following system stability assessment, analyte linearity was determined using a minimum of five different concentrations (calibration working standards) of the each reference standard. Each standard was injected in duplicate (at a minimum) prior to and following sample analysis. The coefficient of determination (R^2), and the sample replicate relative standard deviation (RSD) and percent coefficient of variation (CV) were determined.

5.0 Results and Discussion

This study was completed to provide quantitative and qualitative data to support the efficacy of the vaporizer unit (Volcano®). Data generated will be utilized by the study sponsor to subsequently seek agency (FDA) approval for the protocol development and initiation of a phase I clinical investigation comparing cannabinoid blood levels in subjects smoking (i.e., pyrolysis) *Cannabis* versus *Cannabis* vaporized using the Volcano® vaporizer system.

The quantitative and qualitative data contained in this report has demonstrated that *Cannabis* heated using the Volcano® system resulted in a release and capture of analytes of interest (e.g., THC, CBD, CBN) in comparable concentrations (w/w); while mitigating the release of those compounds (i.e., PNA) generated as a function of pyrolysis. This conclusion is supported by the following tabular and graphical data.

5.1 Moisture determination

Analysis of approximately 0.56 g of prescreened, homogenized test sample for a period of thirty minutes demonstrated that the percent moisture content was approximately 11.9 % by weight. This conclusion is supported by the following tabular data.

Table 6. Moisture determination

Time Heated (min)	Temp. (°C)	Cumulative weight loss (%)	Time Heated (min)	Temp. (°C)	Cumulative weight loss (%)
2.00	141	9.19	18.00	141	11.17
4.00	141	1.98	20.00	140	11.35
6.00	139	10.09	22.00	140	11.35
8.00	140	10.45	24.00	140	11.53
10.00	140	10.63	26.00	140	11.53
12.00	140	10.81	28.00	140	11.71
14.00	140	10.99	30.00	140	11.89
16.00	140	10.99			
			Mean Temp: (°C):	140	
			Initial weight (g):		0.555
			Final weight (g):		0.489
			Percent loss (%):		11.9%

5.2 HPLC-DAD system suitability assessment

Upon review of the data generated it was experimentally and statistically determined that the analytical methodology was suitable for the quantitative assay of THC, CBD, and CBN at sample concentrations of 4.90 to 147 µg/mL, 0.495 to 14.9 µg/mL, and 4.42 to 88.4 µg/mL respectively. This assessment is supported by the following tabular and graphical data.

Table 7. Precision & Linearity assessment of THC using HPLC-DAD

Standard ID	Dose Conc. (µg/mL)	Replicate	Response (area)	Mean Response	%CV
CON012803-02A-1-1	4.90	A	82.8	83.2	0.62%
	4.90	B	83.2		
	4.90	C	83.2		
	4.90	D	84.0		
CON012803-02A-2-1	9.80	A	156.8	157.7	0.42%
	9.80	B	158.1		
	9.80	C	157.7		
	9.80	D	158.3		
CON012803-02A-3-1	19.6	A	347.8	349.0	0.25%
	19.6	B	349.3		
	19.6	C	349.6		
	19.6	D	349.5		
CON012803-02A-4-1	39.2	A	636.6	640.9	0.53%
	39.2	B	644.6		
	39.2	C	642.3		
	39.2	D	640.1		
CON012803-02A-5-1	98.0	A	1790.7	1789.4	0.21%
	98.0	B	1786.4		
	98.0	C	1786.5		
	98.0	D	1794.1		
CON012803-02A-6-1	123	A	2103.4	2112.3	0.33%
	123	B	2110.5		
	123	C	2118.3		
	123	D	2117.2		
CON012803-02A-7-1	147	A	2610.0	2610.7	0.40%
	147	B	2596.8		
	147	C	2621.3		
	147	D	2614.7		

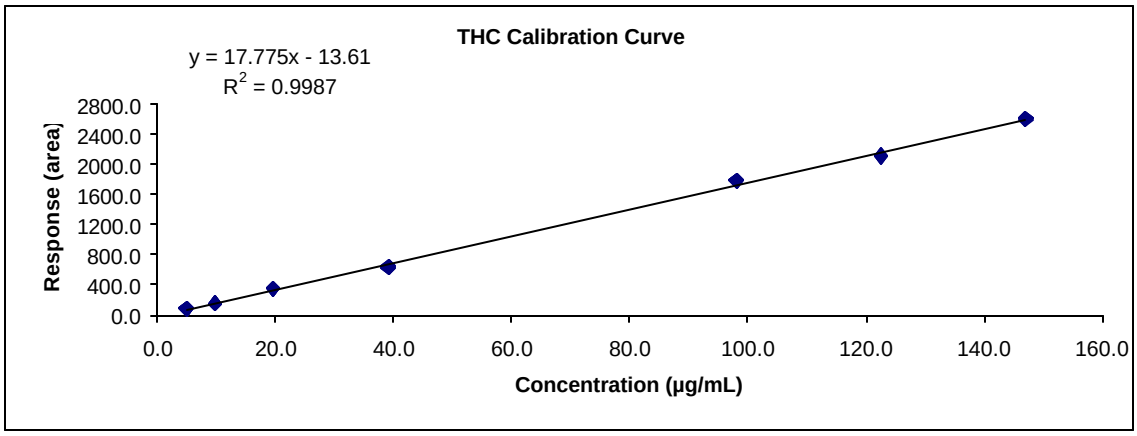


Table 8. Precision & Linearity assessment of CBD using HPLC-DAD

Standard ID	Dose Conc. (µg/mL)	Replicate	Response (area)	Mean Response	%CV
REF012403-01A-1	0.495	A	10.2	10.3	5.42%
	0.495	B	11.1		
	0.495	C	9.9		
	0.495	D	10.0		
REF012403-01B-1	0.99	A	24.5	24.4	0.48%
	0.99	B	24.3		
	0.99	C	24.5		
	0.99	D	24.3		
REF012403-01C-1	1.98	A	45.3	45.0	0.50%
	1.98	B	45.1		
	1.98	C	45.0		
	1.98	D	44.7		
REF012403-01D-1	3.96	A	86.0	86.2	0.50%
	3.96	B	86.2		
	3.96	C	86.8		
	3.96	D	85.8		
REF012403-01E-1	9.90	A	230.5	229.8	0.29%
	9.90	B	228.9		
	9.90	C	230.1		
	9.90	D	229.8		
REF012403-01F-1	12.4	A	324.1	325.6	0.48%
	12.4	B	324.5		
	12.4	C	327.5		
	12.4	D	326.1		
REF012403-01G-1	14.9	A	355.7	355.8	0.38%
	14.9	B	354.1		
	14.9	C	357.5		
	14.9	D	355.9		

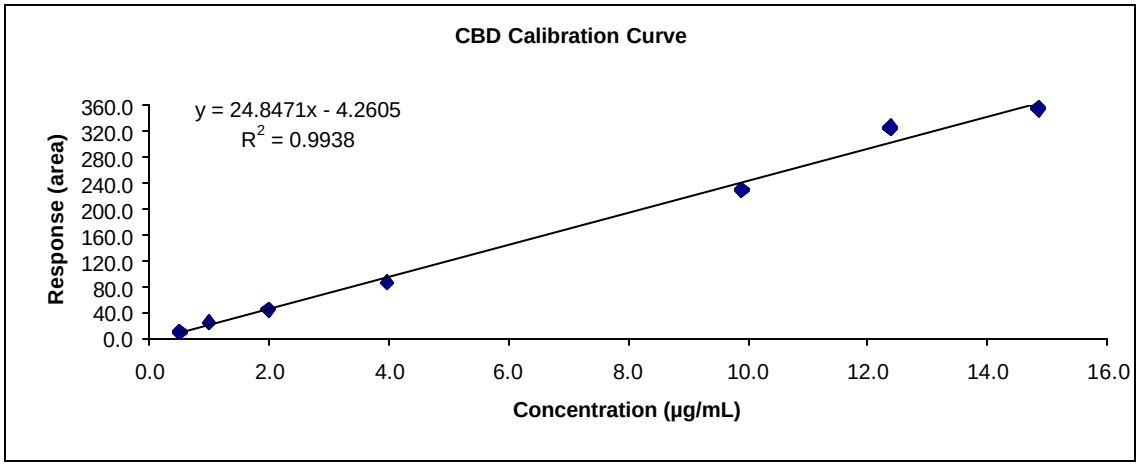


Table 9. Precision & Linearity assessment of CBN using HPLC-DAD

Standard ID	Dose Conc. (µg/mL)	Replicate	Response (area)	Mean Response	%CV
CON012803-01A-1-1	4.42	A	82.5	83.3	0.79%
	4.42	B	82.9		
	4.42	C	83.7		
	4.42	D	83.9		
CON012803-01A-2-1	8.84	A	146.7	147.1	0.44%
	8.84	B	148.1		
	8.84	C	146.9		
	8.84	D	146.8		
CON012803-01A-3-1	17.68	A	287.0	287.7	0.33%
	17.68	B	289.1		
	17.68	C	287.6		
	17.68	D	287.2		
CON012803-01A-4-1	35.36	A	615.0	618.7	0.54%
	35.36	B	622.1		
	35.36	C	621.0		
	35.36	D	616.8		
CON012803-01A-5-1	88.4	A	1527.4	1525.8	0.16%
	88.4	B	1522.7		
	88.4	C	1525.3		
	88.4	D	1527.9		
CON012803-01A-6-1	111	A	1877.1	1886.4	0.38%
	111	B	1884.7		
	111	C	1892.9		
	111	D	1891.1		
CON012803-01A-7-1	88.4	A	1463.3	1462.0	0.39%
	88.4	B	1454.0		
	88.4	C	1467.5		
	88.4	D	1463.3		

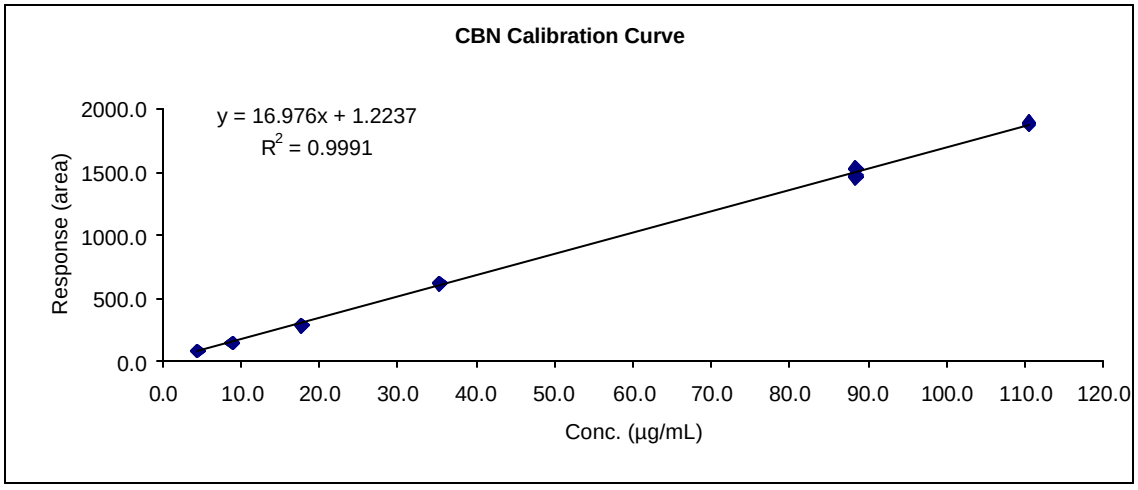


Table 10. Precision assessment of Caffeine internal standard using HPLC-DAD

Standard ID	Dose Conc. (µg/mL)	Replicate	Response (area)	Mean Response	%CV	Mean RRF
REF021302-01E-1-1	200	A	5416.9	5630.9	1.59%	0.03559
	200	B	5454.6			
	200	C	5432.0			
	200	D	5445.7			
REF021302-01E-2-1	200	A	5688.9			
	200	B	5690.1			
	200	C	5667.0			
	200	D	5723.8			
REF021302-01E-3-1	200	A	5651.9			
	200	B	5650.9			
	200	C	5691.3			
	200	D	5692.0			
REF021302-01E-4-1	200	A	5636.3			
	200	B	5654.6			
	200	C	5641.6			
	200	D	5629.8			
REF021302-01E-5-1	200	A	5591.1			
	200	B	5620.3			
	200	C	5629.0			
	200	D	5603.2			
REF021302-01E-6-1	200	A	5688.1			
	200	B	5690.7			
	200	C	5692.5			
	200	D	5707.6			
REF021302-01E-7-1	200	A	5635.1			
	200	B	5599.7			
	200	C	5672.6			
	200	D	5768.8			

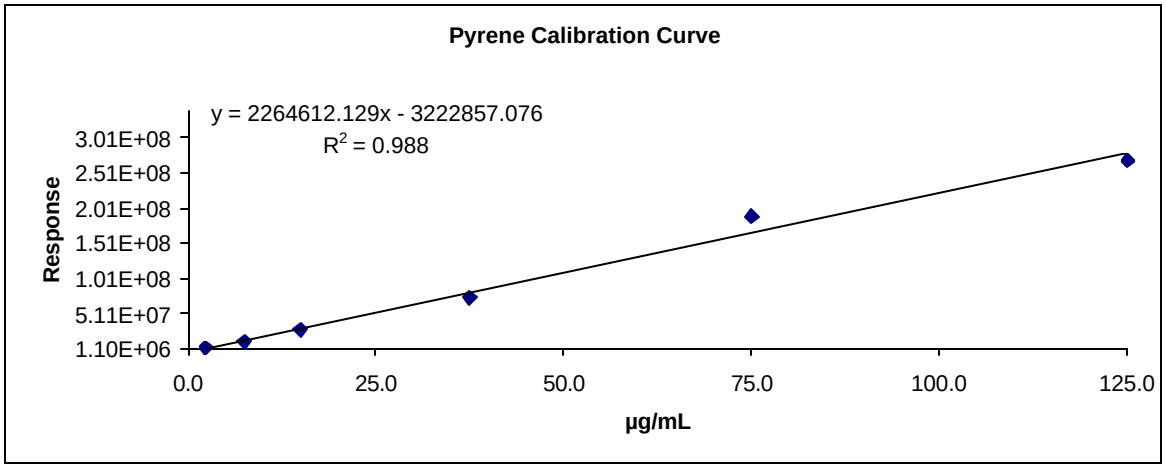
5.3 GC-MS system suitability assessment

Upon review of the data generated it was experimentally and statistically determined that the analytical methodology was suitable for the semi-quantitative and qualitative assay of polynuclear aromatic compounds at sample concentrations of 2.25 to 125 µg/mL. Semi-quantitative evaluation of the isolated PNA compounds were calculated and expressed as total Pyrene. Representation of the data in this manner allows for direct estimation of total PNA compounds isolated. This assessment is supported by the following tabular and graphical data.

Table 11. Precision & Linearity assessment of Pyrene using GC-MSD

Standard ID	Dose Conc. (µg/mL)	Replicate	Response (area)	Mean Response	%CV
REF052802-01H-1-1-1-1-1	2.25	1	3860621	3162150	31.24%
	2.25	2	2463678		
REF052802-01H-1-1-1-1	7.50	1	8694389	10743718	17.88%
	7.50	2	11032803		
	7.50	3	12503963		
REF052802-01H-1-1-1	15.0	1	21196490	28308049	21.90%
	15.0	2	32567008		
	15.0	3	31160649		
REF052802-01H-1-1	37.5	1	70679877	73871668	3.81%
	37.5	2	76012703		
	37.5	3	74922424		
REF052802-01H-1	75.0	1	159518341	189613308	13.76%
	75.0	2	205658907		
	75.0	3	203662677		
REF052802-01H	125	1	268368945	268858495	0.38%
	125	2	268163416		
	125	3	270043125		

One data point from the 2.25 µg/mL standard was eliminated as an experimental outlier.



5.4 *Cannabis sativa* extract analysis: HPLC-DAD-MS

5.4.1 THC analysis

Upon review of the data generated it was experimentally determined that the isolation and assay (e.g., 4.15 ± 0.17 %w/w) of THC, from *Cannabis sativa* using traditional alcoholic solvent extraction was within referenced expectations (e.g., ~4%w/w).

Additionally it has been experimentally determined that the isolation of THC using pyrolysis techniques (e.g., M-1 Combustion) and vaporization (e.g., Volcano®) result in 78.1% and 46.9% efficiency respectively. This assessment is supported by the following tabular data.

Table 12. HPLC-DAD-MS quantitative results: THC

Sample ID	Sample Weight (g)	Rep	THC (area)	Caffeine (area)	THC Total mass (µg)	THC Final Conc. (mg/g)	THC Final Conc. (% w/w)	Mean THC Final Conc. (% w/w)	Standard deviation (% w/w)
Solvent Sohxlet Extraction									
CON021303-01A-1-1	0.20	A	2803.3	5245.4	8670	43.4	4.3	4.15	0.17
	0.20	B	2808.7	5253.2	8674	43.3	4.3		
CON021303-01A-2-1	0.20	A	2611.4	5111.4	8288	41.4	4.1		
	0.20	B	2631.2	5149.2	8290	41.4	4.1		
CON021303-01A-3-1	0.19	A	2364.7	5106.8	7512	39.5	4.0		
	0.19	B	2370.5	5110.1	7526	39.6	4.0		
Volcano® Vaporization (1)									
CON021303-01A-5	0.20	A	1590.1	5148.7	5110	25.5	2.6	1.95	0.49
	0.20	B	1600.5	5194.5	5099	25.4	2.5		
CON021303-01B-1	0.20	A	983.4	5444.6	2989	14.9	1.5		
	0.20	B	975.5	5415.4	2981	14.9	1.5		
CON021303-01B-2	0.20	A	1183.9	5387.9	3636	18.1	1.8		
	0.20	B	1172.6	5324.1	3645	18.2	1.8		
M-1 Combustion									
CON021303-01B-3	0.20	A	2059.4	5032.4	6772	33.8	3.4	3.24	0.11
	0.20	B	2050.5	5060.8	6705	33.5	3.4		
CON021303-01B-4	0.20	A	1998.1	5087.1	6500	32.4	3.2		
	0.20	B	1981.6	5073.9	6463	32.3	3.2		
CON021303-01B-5	0.21	A	2021.3	5077.2	6588	31.3	3.1		
	0.21	B	2003.4	5077.4	6529	31.0	3.1		

(1) Variance in replicate sample results is suspected as being due to product heating. Sponsor suggested refining technique for future studies.

5.4.2 CBD analysis

Upon review of the data generated it was experimentally determined that the isolation and assay (e.g., 0.075 ± 0.04 % W/W) of CBD, from *Cannabis sativa* using traditional alcoholic solvent extraction was precise.

Additionally it has been experimentally determined that the isolation of CBD using pyrolysis techniques (e.g., M-1 Combustion) and vaporization (e.g., Volcano®) resulted in 200% and 121% efficiency respectively. The increased mass isolated is suspected as being due to thermal conversion of cannabinoid species to CBD under the conditions investigated. This assessment is supported by the following tabular data.

Table 13. HPLC-DAD-MS quantitative results: CBD

Sample ID	Sample Weight (g)	Rep	CBD (area)	Caffeine (area)	CBD Total mass (µg)	CBD Final Conc. (µg/g)	CBD Final Conc. (% w/w)	Mean CBD Final Conc. (% w/w)	Standard deviation (% w/w)
Solvent Sohxlet Extraction									
CON021303-01A-1-1	0.20	A	39.8	5245.4	92	459	0.046	0.075	0.040
	0.20	B	36.4	5253.2	84	419	0.042		
CON021303-01A-2-1	0.20	A	46.2	5111.4	109	547	0.055		
	0.20	B	46.9	5149.2	110	551	0.055		
CON021303-01A-3-1	0.19	A	102.3	5106.8	242	1275	0.128		
	0.19	B	100.7	5110.1	239	1255	0.126		
Volcano® Vaporization									
CON021303-01A-5	0.20	A	103.4	5148.7	248	1239	0.12	0.091	0.026
	0.20	B	104.8	5194.4	249	1246	0.12		
CON021303-01B-1	0.20	A	60.4	5444.5	137	685	0.068		
	0.20	B	60.7	5415.3	138	692	0.069		
CON021303-01B-2	0.20	A	70.3	5387.9	161	806	0.081		
	0.20	B	69.8	5324.0	162	809	0.081		
M-1 Combustion									
CON021303-01B-3	0.20	A	128.0	5032.4	314	1570	0.16	0.15	0.016
	0.20	B	126.4	5060.7	308	1542	0.15		
CON021303-01B-4	0.20	A	132.3	5087.0	321	1606	0.16		
	0.20	B	130.6	5073.9	318	1589	0.16		
CON021303-01B-5	0.21	A	109.1	5077.2	265	1263	0.13		
	0.21	B	109.0	5077.4	265	1262	0.13		

5.4.3 CBN analysis

Upon review of the data generated it was experimentally determined that the isolation and assay (e.g., 0.094 ± 0.007 %w/w) of CBN, from *Cannabis sativa* using traditional alcoholic solvent extraction was precise.

It has been experimentally determined that the isolation of CBD using pyrolysis techniques (e.g., M-1 Combustion) and vaporization (e.g., Volcano®) resulted in 202% efficiency. The increased mass isolated is suspected as being due to thermal conversion of cannabinoid species to CBN under the conditions investigated. Additionally, it has been experimentally determined that the isolation of CBN using solely vaporization techniques (e.g., Volcano®) resulted in 86.2% efficiency. This assessment is supported by the following tabular data.

Table 14. HPLC-DAD-MS quantitative results: CBN

Sample ID	Sample Weight (g)	Rep	CBN (area)	Caffeine Response (area)	CBN Total mass (µg)	CBN Final Conc. (µg/g)	CBN Final Conc. (% w/w)	Mean CBN Final Conc. (% w/w)	Standard deviation (% w/w)
Solvent Soxlett Extraction									
CON021303-01A-1-1	0.20	A	68.0	5245.4	206	1032	0.10	0.094	0.007
	0.20	B	66.7	5253.2	202	1010	0.10		
CON021303-01A-2-1	0.20	A	60.0	5111.4	187	935	0.093		
	0.20	B	59.3	5149.2	183	917	0.092		
CON021303-01A-3-1	0.19	A	54.0	5106.8	168	886	0.089		
	0.19	B	52.5	5110.1	164	861	0.086		
Volcano® Vaporization									
CON021303-01A-5	0.20	A	70.8	5148.7	223	1117	0.11	0.081	0.025
	0.20	B	72.4	5194.5	226	1132	0.11		
CON021303-01B-1	0.20	A	40.0	5444.6	119	597	0.060		
	0.20	B	39.0	5415.4	117	585	0.059		
CON021303-01B-2	0.20	A	45.7	5387.9	138	690	0.069		
	0.20	B	46.7	5324.1	143	713	0.071		
M-1 Combustion									
CON021303-01B-3	0.20	A	121.2	5054.5	389	1947	0.19	0.19	0.005
	0.20	B	120.4	5060.8	386	1932	0.19		
CON021303-01B-4	0.20	A	116.1	5087.1	371	1854	0.19		
	0.20	B	114.6	5073.9	367	1835	0.18		
CON021303-01B-5	0.21	A	120.5	5077.2	385	1835	0.18		
	0.21	B	119.8	5077.4	383	1824	0.18		

5.5 *Cannabis sativa* extract analysis: GC-MS

5.5.1 Representative analysis of 1 µL of Volcano® generated gaseous headspace, re-dissolved in 2 mL methanol

Upon review of the data generated it was experimentally determined that the isolation and assay of 3 tentatively identified compounds (TIC), inclusive of cannabinoid related compounds, were isolated using the Volcano® vaporizer system from the gaseous headspace, re-dissolved and concentrated in 2 mL of methanol. Approximately 3.6 mg of TIC (as Pyrene) was isolated from 200 mg of treated *Cannabis sativa,* equivalent to approximately 1.8%w/w. Those compounds identified via comparison with the NBS mass spectral that demonstrated greater than 70% match quality were reported as positively identified isolated compounds.

It is recognized that the reduced recovery of cannabinoid related substances isolated in the Volcano®-GC-MS investigation (e.g. 3.3 mg/g) versus Volcano®-HPLC-DAD-MS investigation (e.g., 19.5 mg/g) was the result of the mathematical representation of the analytes as Pyrene as well as the reduced volatility of the cannabinoid related analytes. The observation most notable is the lack of production of significant numbers of pyrolyticly induced analytes. This assessment is supported by the following tabular and graphical data.

Table 15. GC-MS semi-quantitative results: solvated extract analysis; Volcano®

Retention time (min)	Response (area)	Tentatively Identified Compound (TIC)	NBS Library Mach quality	Recovered conc. as Pyrene (mg/g)	Recovered % of total recovered
30.62	4961669	2-methyl-2, 4 (2H-1-Benzopyran-5-ol)	81	0.065	1.90%
32.55	246510987	Dronabinol	99	3.2	94.3%
33.62	9875017	Cannabinol	94	0.13	3.78%

Total recovered mass as Pyrene (mg):	3.4
Weight extracted (mg):	200
% recovered:	1.8%

5.5.2 Representative analysis of 2 mL of Volcano® generated gaseous headspace

Upon review of the data generated it was experimentally determined that the isolation and assay of 5 tentatively identified compounds (TIC), inclusive of cannabinoid related, were isolated using the Volcano® vaporizer system from the gaseous headspace. Approximately 0.079 mg of TIC (as Pyrene) was isolated from 200 mg of treated *Cannabis sativa* equivalent to approximately 0.04% w/w. Those compounds identified via NBS mass spectral match greater than 70% match quality were reported as positively identified isolated compounds.

It is recognized that the reduced recovery of cannabinoid related substances isolated in the Volcano®-GC-MS investigation (e.g. 0.075 mg/g) versus Volcano®-HPLC-DAD-MS investigation (e.g. 19.5 mg/g) is resultant of the mathematical representation of the analytes as Pyrene as well as the reduced volatility of the cannabinoid related analytes. The observation most notable is the lack of production of significant numbers of pyrolyticly induced analytes. This assessment is supported by the following tabular and graphical data.

Table 16. GC-MS semi-quantitative results: gaseous headspace analysis; Volcano®

Retention time (min)	Response (area)	Tentatively Identified Compound (TIC)	Percent NBS Library Match Quality	Recovered conc. as Pyrene (mg/g)	Recovered % of total recovered
9.33[1]	1221726	Caryophyllene	78	0.0010	1.3%
30.62[2]	2417494	2-methyl-2, 4 (2H-1-Benzopyran-5-ol)	81	0.0020	2.5%
32.56	85295887	Dronabinol	99	0.070	89.1%
33.62	5487650	Cannabinol	81	0.0045	5.7%
42.97*	1289703	5-[(acetyl Benz [e] azulene-3,8-dione	86	0.0011	1.3%

Total recovered mass as Pyrene (mg): 0.079
Weight extracted (mg): 200
% Recovered: 0.04%

1 – "Photogenic sesquiterpinoid essential oil commonly found in Cannabis". Ethan Ruso, M.D., Montana Neurobehavioral Specialists, Missoula, MT 59802
2 – 2-methyl-2,4(2H-1-Benzopyran-5-ol is a "suspected breakdown product of CBD or a component that the plant is using to build CBD". Aidan Hampson, Ph.D., Cortex Pharmaceuticals, Inc. Irvine, CA 92618.
* - Polynuclear aromatic hydrocarbon.

5.5.3 Representative analysis of 1 µL of M-1 (combustion) generated gaseous headspace, re-dissolved in 2 mL methanol.

Upon review of the data generated it was experimentally determined that the isolation and assay of 37 tentatively identified compounds (TIC), inclusive of cannabinoid related, were isolated using the M-1 combustion system from the gaseous headspace. Approximately 14 mg of TIC (as Pyrene) was isolated from 200 mg of treated *Cannabis sativa* equivalent to approximately 9% w/w. Those compounds identified via NBS mass spectral match greater than 70% match quality were reported as positively identified isolated compounds. It is noted that approximately 7 mg (~11% W/W) is associated with non-cannabinoid analytes. Those analytes of known to be directly associated with poly nuclear aromatic hydrocarbons as well as TIC are presented within the following tabular data. This assessment is supported by the following tabular and graphical data.

Table 17. GC-MS semi-quantitative results: solvated combustion extract analysis; M1

Retention time (min)	Response (area)	Best match	NBS Library Match % Quality	Recovered Conc. as Pyrene (mg/g)	Recovered % of Total recovered
4.27	5371404	Phenol, 4-ethyl-	91	0.071	0.10%
4.46	4820930	1H-Indene, 1-methyl-	91	0.063	0.09%
4.62	11975267	1,2-Benzennediol	74	0.157	0.23%
5.01	28398562	Naphthalene	91	0.373	0.53%
5.17*	33292637	Benzofuran, 2,3-dihydro-	72	0.437	0.63%
6.91	21443444	Indole	87	0.282	0.40%
7.14	5635171	Naphthalene, 2-methyl-	95	0.074	0.11%
7.45	5932574	Naphthalene, 2-methyl-	93	0.078	0.11%
7.72	4757806	1,4-Benzenedoil, 2-methyl-	91	0.062	0.09%
8.99	11013411	1H-Indole, 4-methyl-	90	0.145	0.21%
9.32	60797737	Caryophyllene	99	0.798	1.15%
9.71	4674849	1,6,10-Dodetatriene, 7,11-dimethyl-	96	0.061	0.09%
9.97*	2209752	Naphthalene, 1,2,3,5,6,7,8,8a-octah	89	0.029	0.04%
10.20	18874442	4,7,10-Cycloundecatriene	99	0.248	0.36%
11.12	2060913	1H-3a,7-methanoazulene, octahydro-1	90	0.027	0.04%
11.20	2094526	Cylohexene, 1-methyl-4-(5-methyl-1	86	0.027	0.04%
12.14*	13696523	Naphthalene, decahydro-4a-methyl-1-	92	0.180	0.26%
12.33*	16059454	Naphthalene, 1,2,3,5,6,7,8,8a-octah	98	0.211	0.30%
13.50	17021514	Caryophyllene Oxide	96	0.223	0.32%
13.59	4347127	1H-Cyclopropa [a]naphthalene, 1a,2,3	98	0.057	0.08%
14.75	2271757	10,10-Dimethylenebicyc	89	0.030	0.04%
15.33	2173568	5-Azulenemethanol, 1,2,3,3a,4,5,6,7	86	0.029	0.04%
15.67	26178775	.alpha.-Bisabolol	87	0.344	0.49%
15.85	9580620	1-Decene	90	0.126	0.18%
18.37	32298240	6-Octen-1-ol, 3,7-domethyl-, acetat	78	0.424	0.61%
18.70	2422132	Diphenylethyne	90	0.032	0.05%
21.24	4388527	Hexadecanoic acid	92	0.058	0.08%
29.16	3509363	Glaucyl alcohol	86	0.046	0.07%
30.63*	69664748	2H-1-Benzopyran-5-ol, 2-methyl-2-(4	95	0.915	1.31%
30.73	75367485	Resorcinol, 2-pmemtha-1,8-dien-3-y	98	0.990	1.42%
31.84	4625532	.Delta.8-Tetrahydrocannabinol	91	0.061	0.09%
32.59*[1]	4408666746	Dronabinol	98	57.9	83.04%
33.07*[1]	2029605	Dronabinol	91	0.027	0.04%
33.63	334263844	Cannabinol	97	4.389	6.30%
37.34	3583356	Docosane	96	0.047	0.07%
41.22	25609584	Vitamine E	89	0.336	0.48%
45.39	28142178	.beta.-Amyrin	95	0.369	0.53%

* - Polynuclear aromatic hydrocarbons.
1 – Significantly increased response resulting in peak splitting; thus 2 consecutive retention times.

Total recovered (mg):	14
Weight extracted (mg):	200
% recovered:	9%

5.5.4 Representative analysis of 2 mL of M-1 (combustion) generated gaseous headspace

Upon review of the data generated it was experimentally determined that the isolation and assay of 111 tentatively identified compounds (TIC), inclusive of cannabinoid related, were isolated using the M-1 combustion system from the gaseous headspace. Approximately 17 mg of TIC (as Pyrene) were isolated from 200 mg of treated *Cannabis sativa* equivalent to approximately 8.5% w/w. Those compounds identified via NBS mass spectral match greater than 70% match quality were reported as positively identified isolated compounds. It is noted that approximately 15 mg (~8% W/W) is associated with non-cannabinoid analytes. Those analytes of known to be directly associated with polynuclear aromatic hydrocarbons as well as TIC are presented within the following tabular data. This assessment is supported by the following tabular and graphical data.

Table 18. GC-MS semi-quantitative results: gaseous headspace analysis; M-1

Retention time (min)	Response (area)	Best match	NBS Library Mach quality	Recovered conc. as Pyrene (mg/g)	Recovered % of total recovered
4.30	32935726	Benzeneacetonitrile	91	0.027	0.16%
4.60	2310571	1-chloro-octadecane	91	0.002	0.01%
4.99	18390657	Naphthalene	90	0.015	0.09%
5.18*	69332076	2,3-dihydro-Benzofuran	86	0.057	0.34%
6.21	4465468	2,6,10,14-tetramethyl-Hexadecane	90	0.004	0.02%
6.91	86166759	Indole	90	0.071	0.42%
7.12	7925421	1-methyl-naphthalene	93	0.007	0.04%
8.52	35115397	1,1'-oxybis-Octane	83	0.029	0.17%
8.69	12256513	2,6,10-trimethyl-tetradecane	83	0.010	0.06%
9.00	23982131	3-methyl-1H-Indole	81	0.020	0.12%
9.32	116897251	Caryophyllene	98	0.096	0.57%
10.15	313228545	Cyclododecane	97	0.257	1.52%
10.74	4799627	Pentadecane	97	0.004	0.02%
10.85	146804387	Heptadecane	98	0.120	0.71%
11.35	950013208	Nonadecene	86	0.780	4.60%
11.95*	90056152	2,2'-diethyl-1,1'-Biphenyl	94	0.074	0.44%
12.63	154063760	Hexadecanal	76	0.126	0.75%
13.10	2964842	Hexadecane	90	0.002	0.01%
13.50	35308265	Caryophyllene Oxide	95	0.029	0.17%
14.13*	33918891	2,2'-diethyl-1,1'-Biphenyl	80	0.028	0.16%
14.82	296612752	tetradecanoic	99	0.243	1.44%
15.12	42131403	(Z)-3-Hexadecene	98	0.035	0.20%
15.47	295232200	Octadecane	98	0.242	1.43%
16.18	4653356	2-Dodecen-1-yl (-) succinic anhydride	89	0.004	0.02%
16.28	3384476	2-methyl-1-Hexadecanol	78	0.003	0.02%
16.32	5094990	1-Pentadecene	92	0.004	0.02%
17.33	34270249	2-Heptadecanol	78	0.028	0.17%

Retention time (min)	Response (area)	Best match	NBS Library Mach quality	Recovered conc. as Pyrene (mg/g)	Recovered % of total recovered
17.52	34215482	2-(tetradecyloxy)-Ethanol	81	0.028	0.17%
17.74	13953740	Hexadecane	90	0.011	0.07%
17.87	18906884	Heneicosane	87	0.016	0.09%
18.08	85618813	Pentadecanoic acid	97	0.070	0.41%
18.19	151994108	1,2-Benzenedicarboxylic acid, bis (2)	86	0.125	0.74%
18.50	2213315118	Cyclohaxadecane	99	1.816	10.71%
18.65	45837144	Nonadecane	96	0.038	0.22%
18.77	42293352	1-Nonadecene	90	0.035	0.20%
19.00	199692334	2-Hexadecanol	90	0.164	0.97%
19.17	76550515	2-Heptadecanone	87	0.063	0.37%
19.37	103194224	Caffeine	94	0.085	0.50%
19.77	14872741	Docosane	86	0.012	0.07%
20.02	102125171	1-Octadecene	97	0.084	0.49%
20.20	96794873	1-Hexadecanol	86	0.079	0.47%
20.39	57493519	3-Eicosene	97	0.047	0.28%
20.91	2933718734	Dibutyl Phthalate	83	2.407	14.20%
21.24	114002736	Nonadecane	90	0.094	0.55%
21.49	9672077	1-Nonadecene	86	0.008	0.05%
21.76	122401077	1-Octadecene	99	0.100	0.59%
22.43	51345191	3,5,6,7-tetrah-s-Indacen-1(2H)-one	81	0.042	0.25%
22.54	4913720	Octadecane	95	0.004	0.02%
22.63	33563860	1-Nonadecene	86	0.028	0.16%
23.03	32829703	N-methyl-N-[4-[4-methoxy-Acetamide	90	0.027	0.16%
23.15	82313597	2,3,5,6-tetra-s-Indacene-1,7-dione	76	0.068	0.40%
23.48	857664501	5-Octadecene	97	0.704	4.15%
24.01	15554319	Octadecane	90	0.013	0.08%
24.35	140996042	16-methyl-, met Heptadecanoic acid	96	0.116	0.68%
24.52*	95037913	5-dodecyldihydro-2 (3H)-Furanone	83	0.078	0.46%
24.66	32387060	1-Henricosyl Formate	90	0.027	0.16%
25.01	14710926	(Z)-9-Tricosene	91	0.012	0.07%
25.79	32371423	2-Hexyl-1-decanol	86	0.027	0.16%
25.86	200623444	Hexadecanamide	93	0.165	0.97%
26.00	32616620	1-Nonadecene	99	0.027	0.16%
26.33	53218271	2-Dodecen-1-yl (-) succinic anhydride	86	0.044	0.26%
26.65	7339051	2-Dodecen-1-yl (-) succinic anhydride	89	0.006022	0.04%
27.09	56583135	Cis-11-Hexadecen-1-yl acetate	81	0.046430	0.27%
27.21	129242826	1-Phenantthrenecarboxylic acid, 7-et	96	0.106053	0.63%
27.36	10625426	1-Phenantthrenecarboxylic acid, 7-et	92	0.008719	0.05%
27.51	17570838	Tricosane	98	0.014418	0.09%
27.58	156887637	1-Nonadecene	98	0.128737	0.76%

Retention time (min)	Response (area)	Best match	NBS Library Mach quality	Recovered conc. as Pyrene (mg/g)	Recovered % of total recovered
28.37	69739203	1,2,1-Phenanthrenecarboxylic acid	92	0.057226	0.34%
28.73	20887801	Hexanedioic acid Dioctyl ester	90	0.017140	0.10%
28.95	98593890	1-Phenantthrenecarboxylic acid, 7-et	86	0.080903	0.48%
29.10	627678209	1,2,1-Phenanthrenecarboxylic acid	99	0.515053	3.04%
29.26	380114163	2-[(2-bu Cyclopropanenanoic acid	92	0.311910	1.84%
30.65*	70574444	2H-1-Benzopyran-5-ol, 2-methyl-2-(4	94	0.057911	0.34%
30.75	85939990	Resocinol, 2-p-mentha-1,8-dien-3-y	98	0.0705	0.42%
31.07	125006268	Tricosane	93	0.103	0.61%
31.66	21935407	Acetamide, N-methyl-N-[4-[4-4methoxy	91	0.0180	0.11%
31.83	432784246	Hexadecanoic acid, 2,3-dihyroxypro	74	0.355	2.10%
32.46	10236345	Cyclotetradecane, 1,7,11-trimethyl-	91	0.00840	0.05%
32.58	2219980004	Dronabinol	99	1.82	10.75%
32.72	63820716	Hexacosane	96	0.0524	0.31%
33.23	27548366	1,3-Benzenediol,2-(3,7-dimethyl-2,	90	0.0226	0.13%
33.43	33550885	Acetamide, N-methyl-N-[4-[4-4methoxy	94	0.0275	0.16%
33.63	240628731	Cannabinol	95	0.197	1.16%
34.09	13044163	Cyclohexane, 1-(1,5-dimethylhexyl)-	86	0.0107	0.06%
34.32	125757721	Heptacosane	99	0.103	0.61%
34.52	197356583	1-Octdecanethiol	87	0.162	0.96%
35.17	243624195	Octadecanoic acid, 2,3-dihydroxypro	86	0.200	1.18%
35.86	69273621	Tricosane	92	0.0568	0.34%
36.15	1676695684	Squalene	94	1.38	8.12%
37.29	34686159	3-Eicosene, (E)-	91	0.0285	0.17%
37.34	71189968	Heneicosane	96	0.0584	0.34%
38.77	62069103	Heptacosane	95	0.0509	0.30%
39.10	20150673	2-Dodecen-1-yl (-) succinic anhydride	94	0.0165	0.10%
40.16	67270687	Heptacosane	97	0.0552	0.33%
40.96	109391601	9-Hexadecenoic acid, eicosyl ester	76	0.0898	0.53%
41.04	9230053	Cyclotetradecane, 1,7,11-trimethyl-	83	0.00757	0.04%
41.50	30676052	Eicosane	91	0.0252	0.15%
41.79	1169213328	Cholesterol	99	0.959	5.66%
42.27	45017056	9-Hexadecenoic acid, eicosyl ester	72	0.0369	0.22%
42.61	16741293	Cholesteryl acetate	97	0.0137	0.08%
42.69	4624026	Heneicosane, 3-methyl-	91	0.00379	0.02%
42.80	36515665	Eicosane	90	0.0300	0.18%

Retention time (min)	Response (area)	Best match	NBS Library Mach quality	Recovered conc. as Pyrene (mg/g)	Recovered % of total recovered
43.00	4896647	Heneicosane, 3-methyl-	91	0.00402	0.02%
43.22	61362365	Cholesta-3,5-dien-7-one	96	0.0504	0.30%
43.32	28641892	Cholesteryl acetate	99	0.0235	0.14%
43.58	130345192	9-Hexadecenoic acid, eicosyl ester	91	0.107	0.63%
43.86	206844252	Hexadecanoicacid, hexadecyl ester	95	0.170	1.00%
44.15	31783685	Eicosane	83	0.0261	0.15%
46.70	150517876	9-Hexadecenoic acid, eicosyl ester	83	0.124	0.73%
47.02	108047194	1-Octadecanethiol	84	0.0887	0.52%
50.91	86165775	9-Hexadecenoic acid, eicosyl ester	83	0.0707	0.42%

* - Polynuclear aromatic hydrocarbons

Total recovered (mg): 17.0
Weight extracted (mg): 200
% recovered: 8.5%

6.0 Figures

Figure 1. M-1 combustion unit

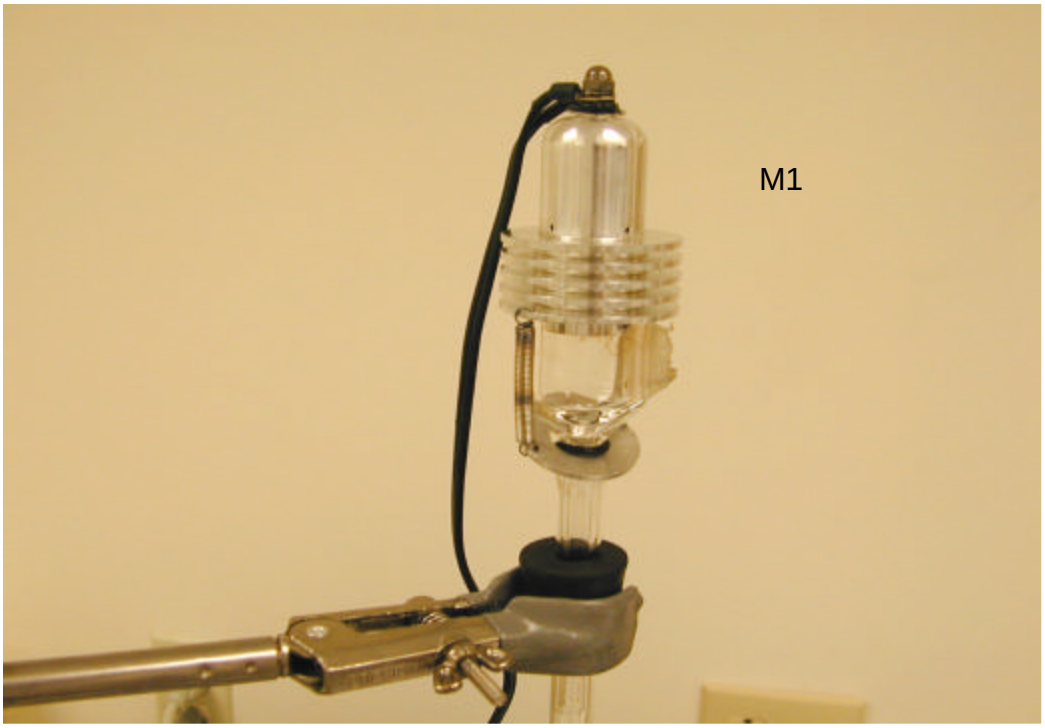


Figure 2. Volcano® vaporizer

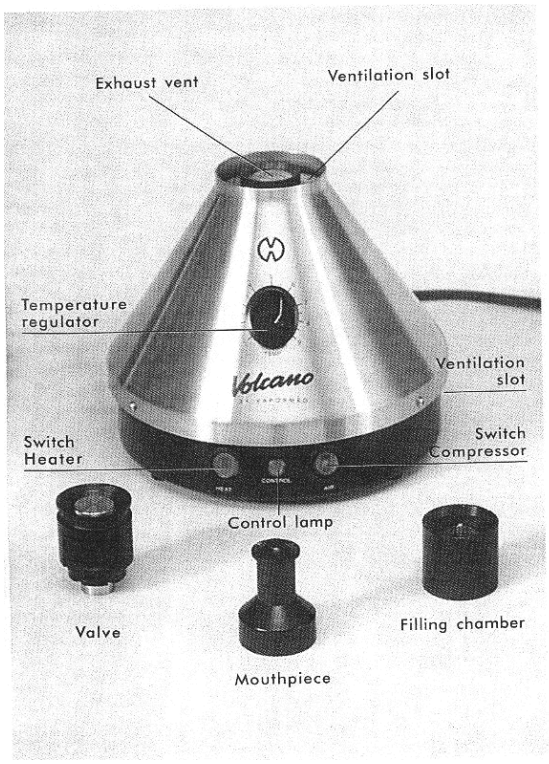
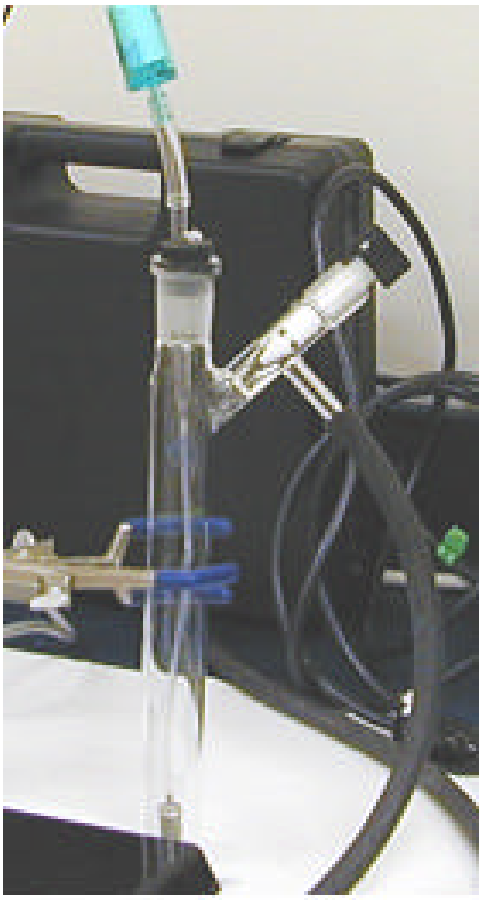


Figure 3. Quark solvent collection reaction Tube



7.0 Representative Chromatograms and Spectra

Figure 4. Representative HPLC-DAD-MS chromatogram: Method control

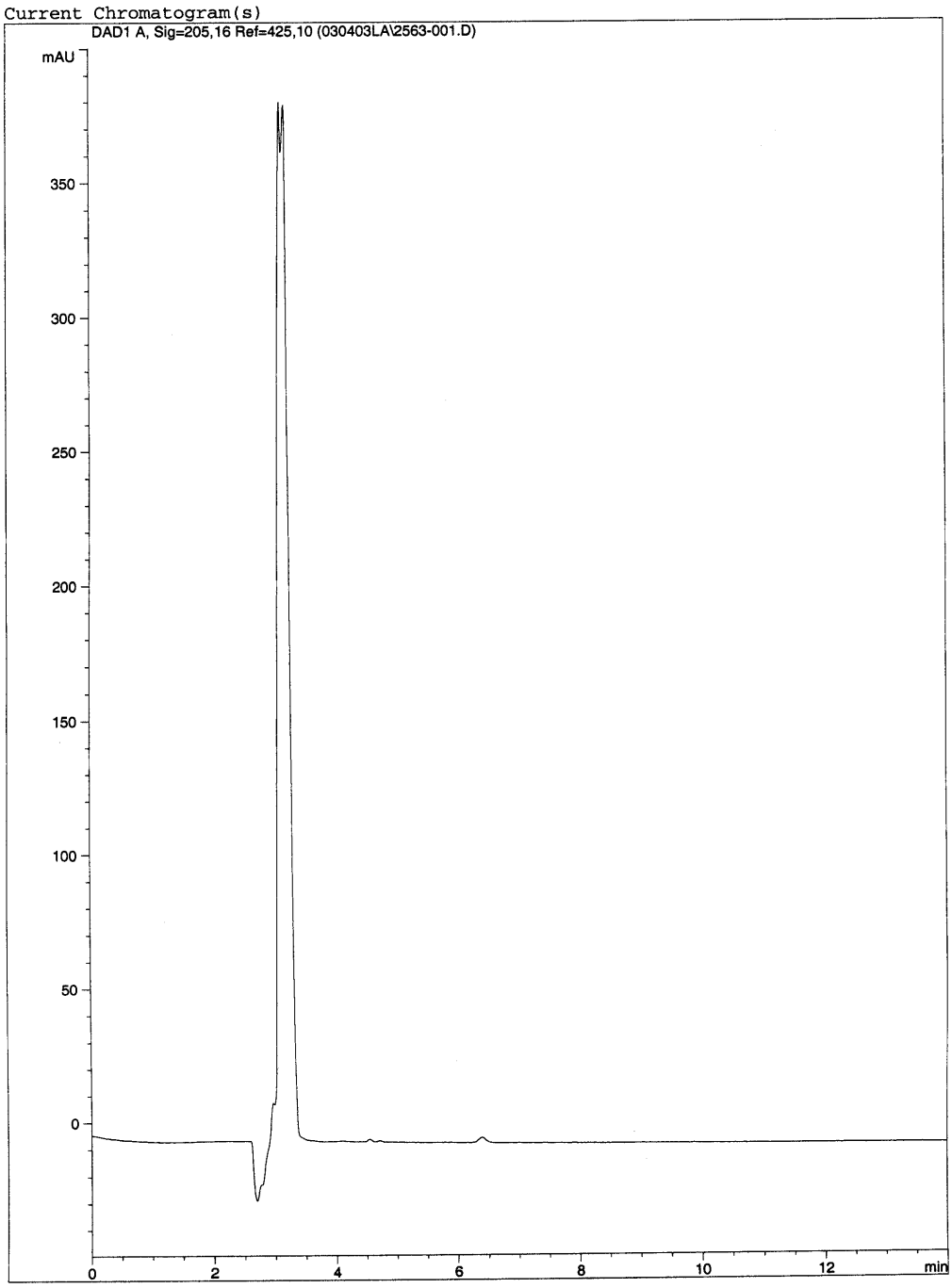


Figure 5. Representative HPLC-DAD chromatogram: Cannabinoid reference standard (4.90 µg/mL THC, 0.495 µg/mL CBD, 4.42 µg/mL CBN).

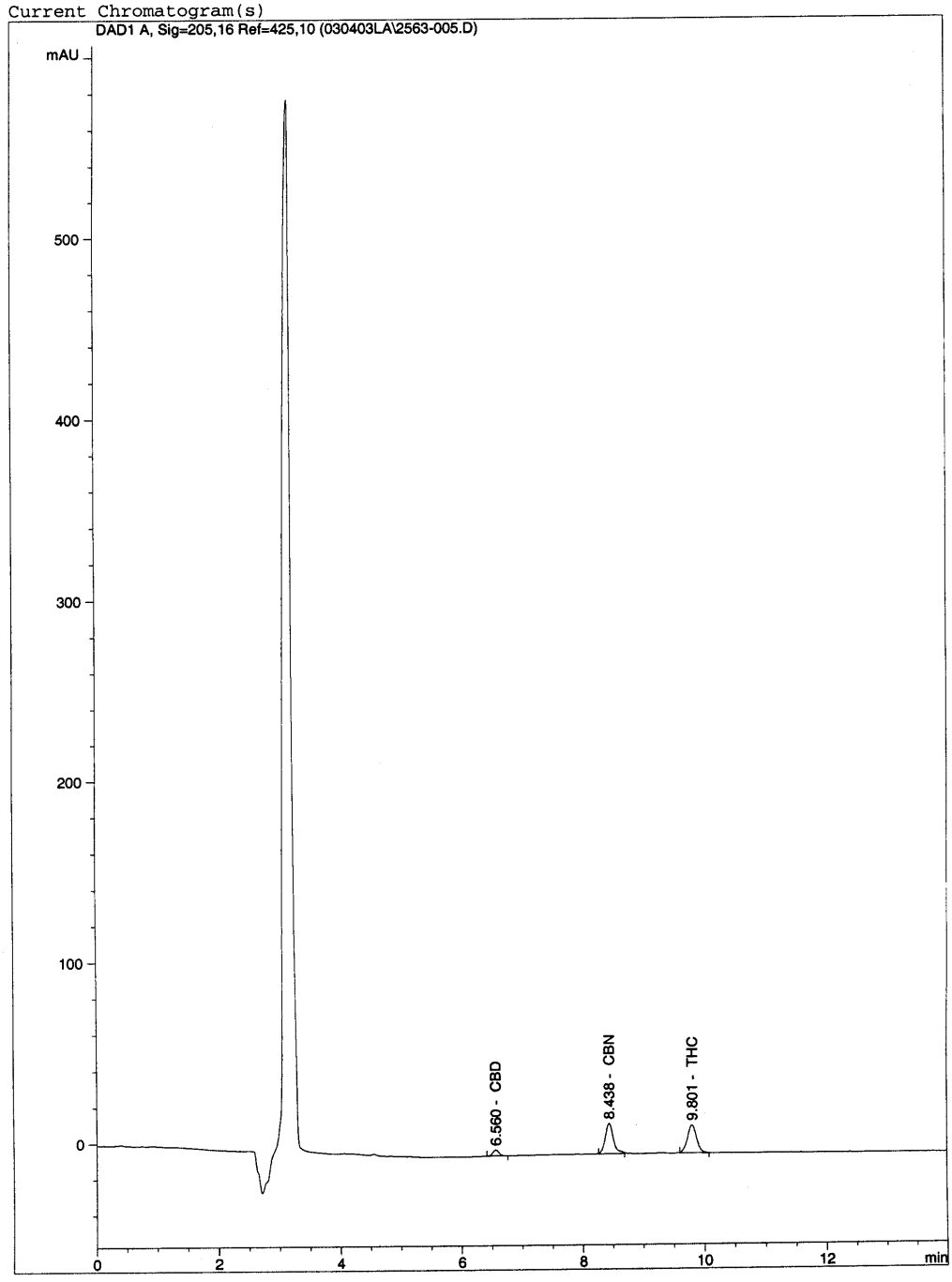


Figure 6. Representative MS spectrum: *Cannabis sativa* THC Sample

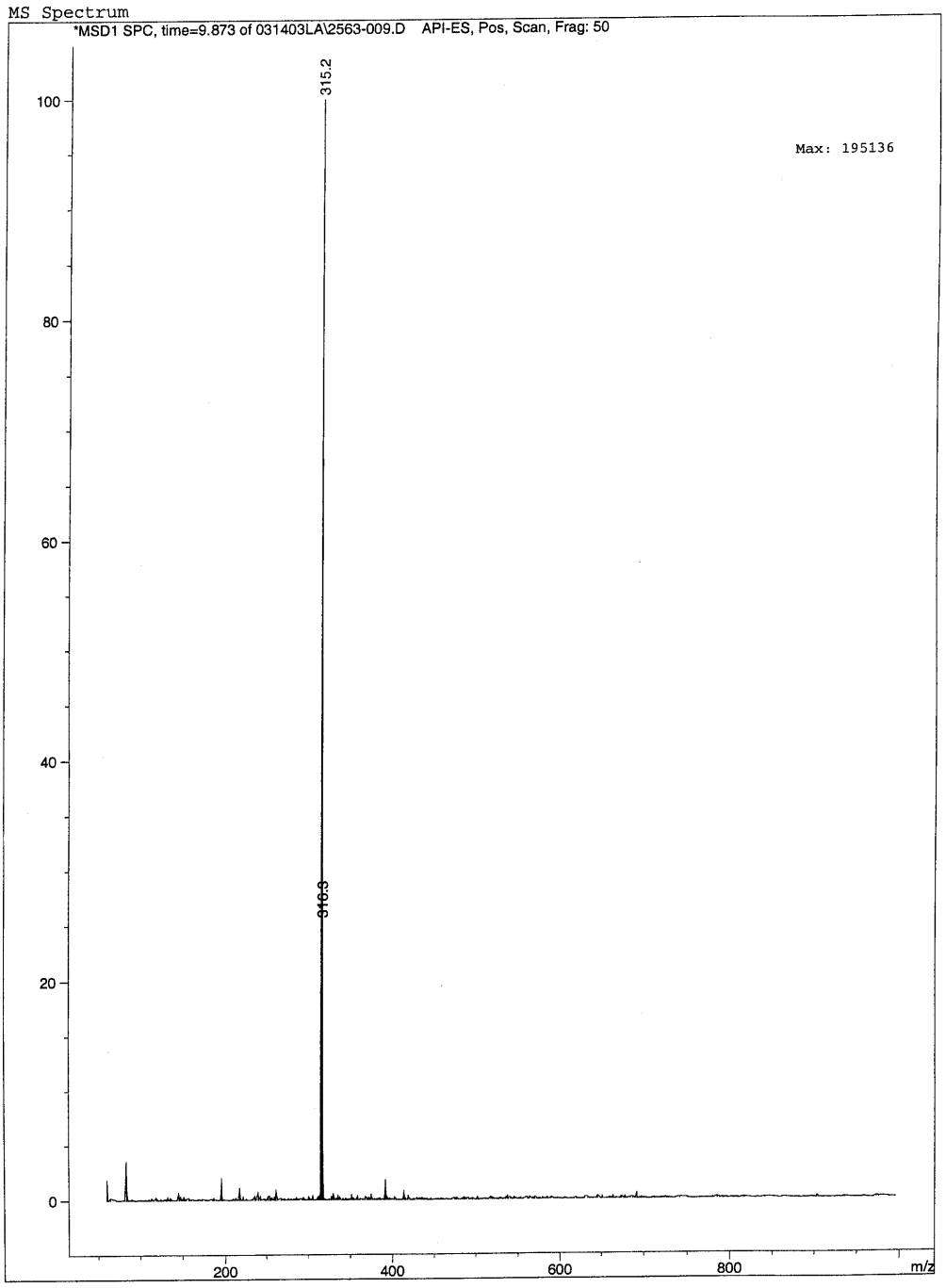


Figure 7. Representative MS spectrum: *Cannabis sativa* CBD Sample

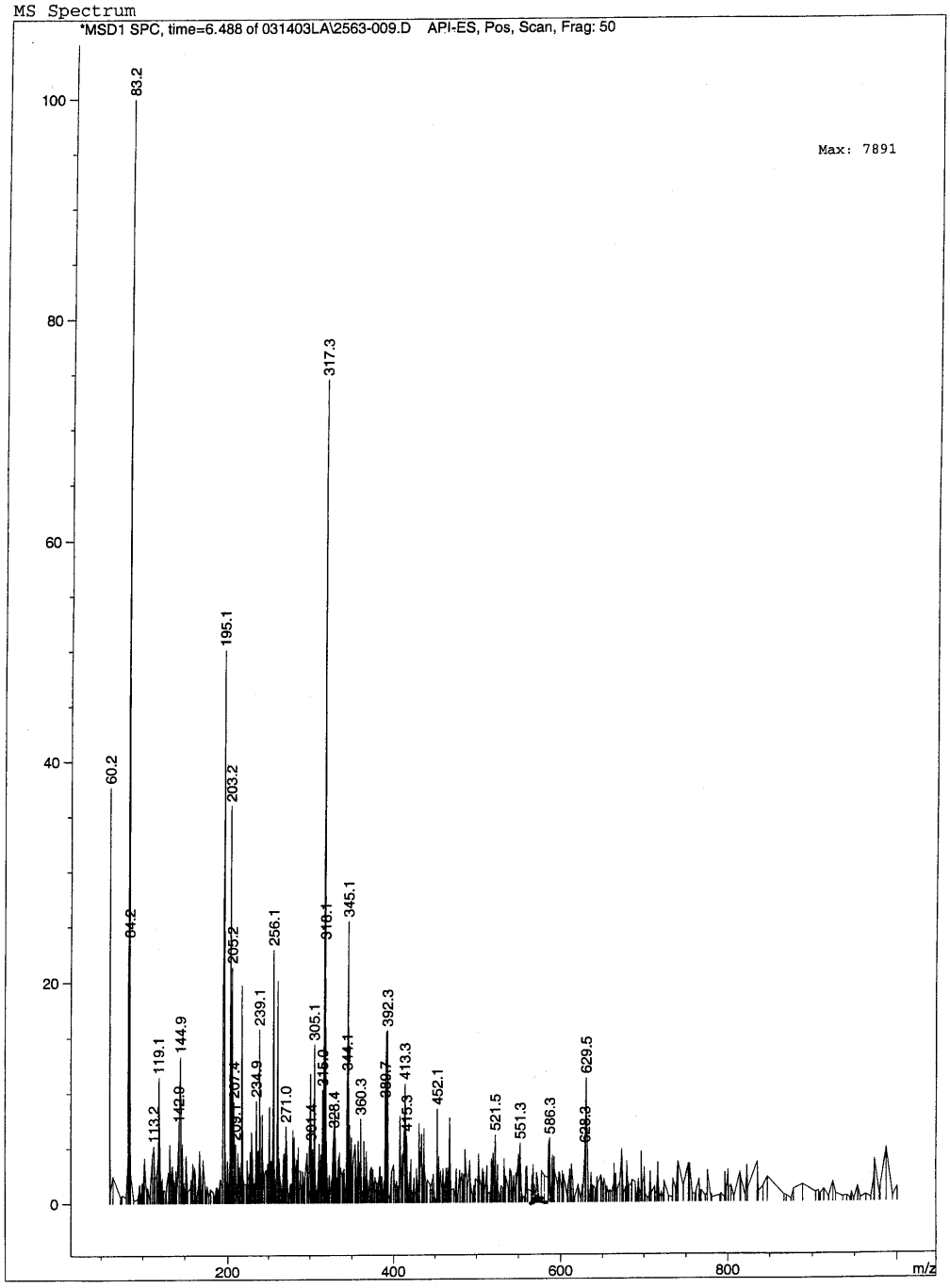


Figure 8. Representative MS spectrum: *Cannabis sativa* CBN Sample

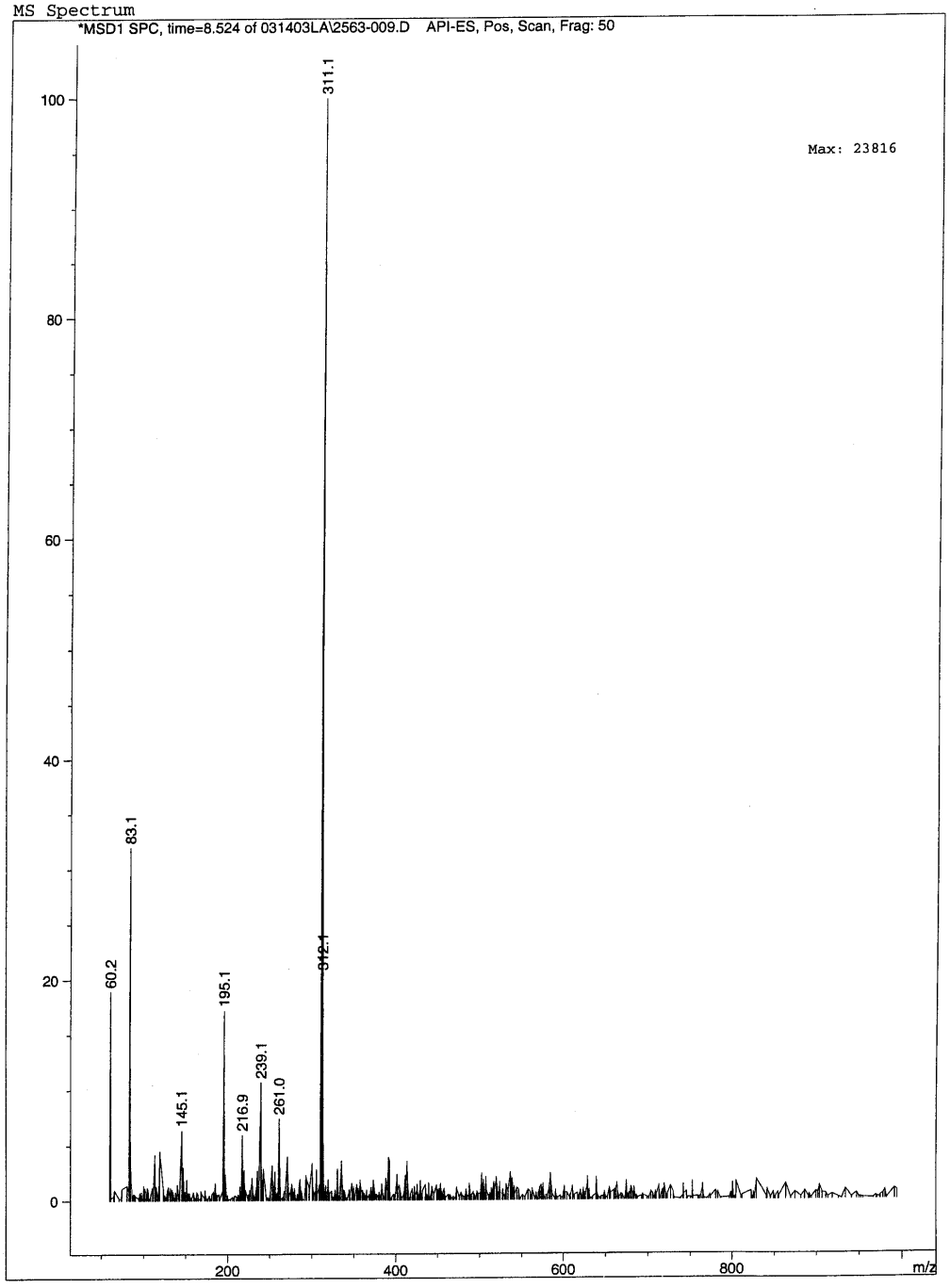


Figure 9. Representative HPLC-DAD-MS chromatogram: *Cannabis sativa* extract analysis via solvent Soxlett extraction.

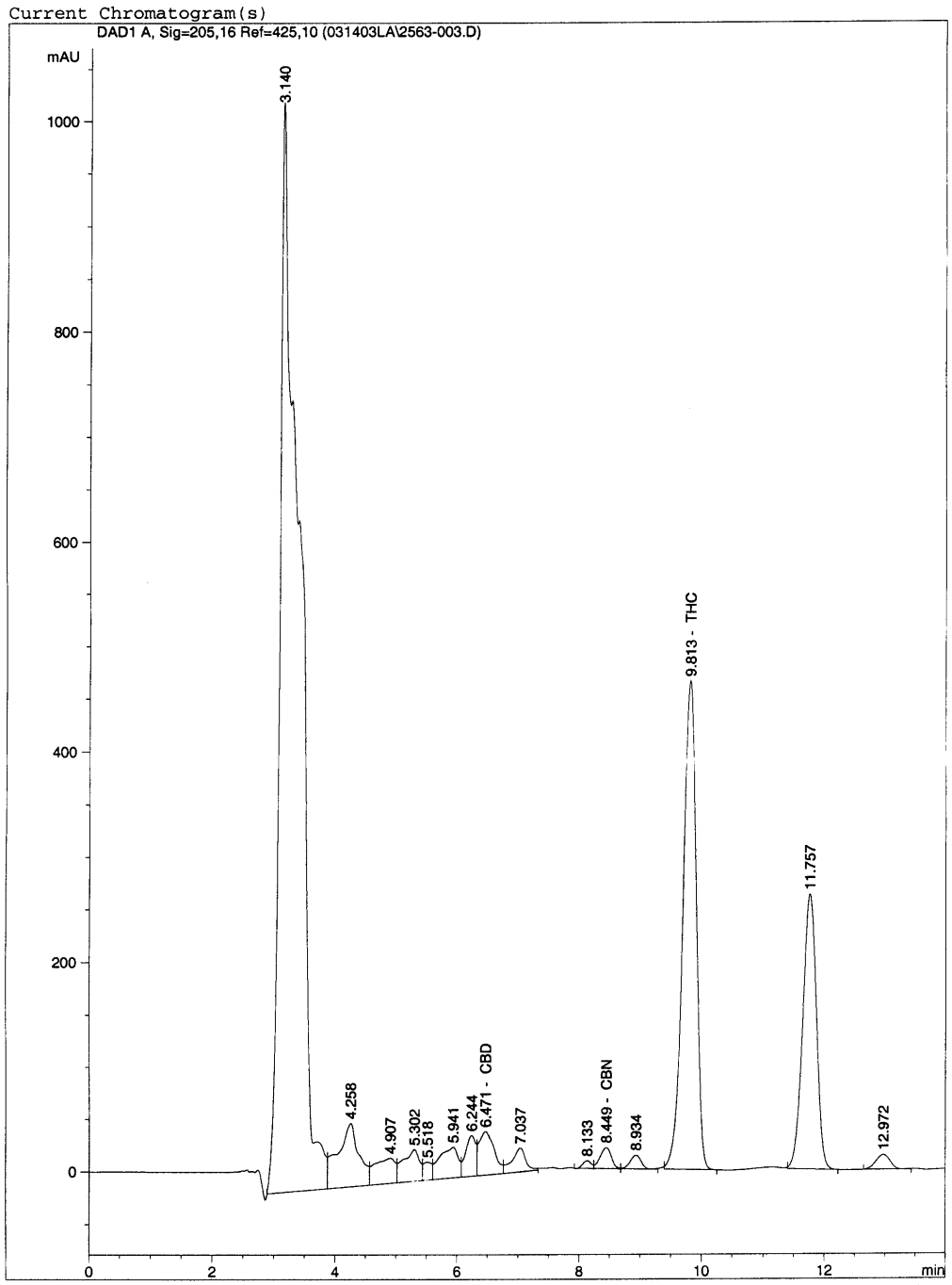


Figure 10. Representative HPLC-DAD-MS chromatogram: *Cannabis sativa* extract analysis via Volcano® vaporization.

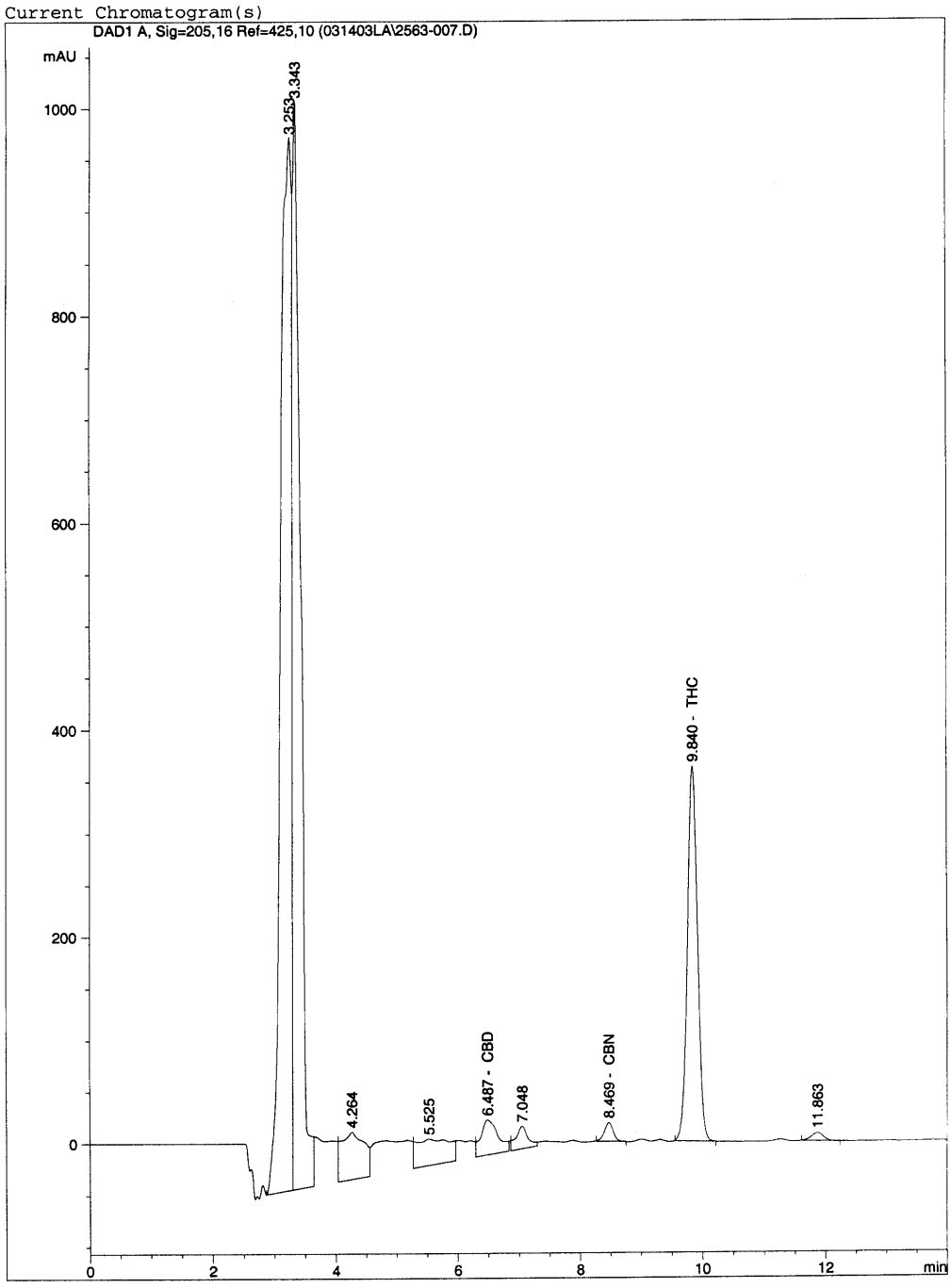


Figure 11. Representative HPLC-DAD-MS chromatogram: *Cannabis sativa* extract analysis via M-1 combustion extraction.

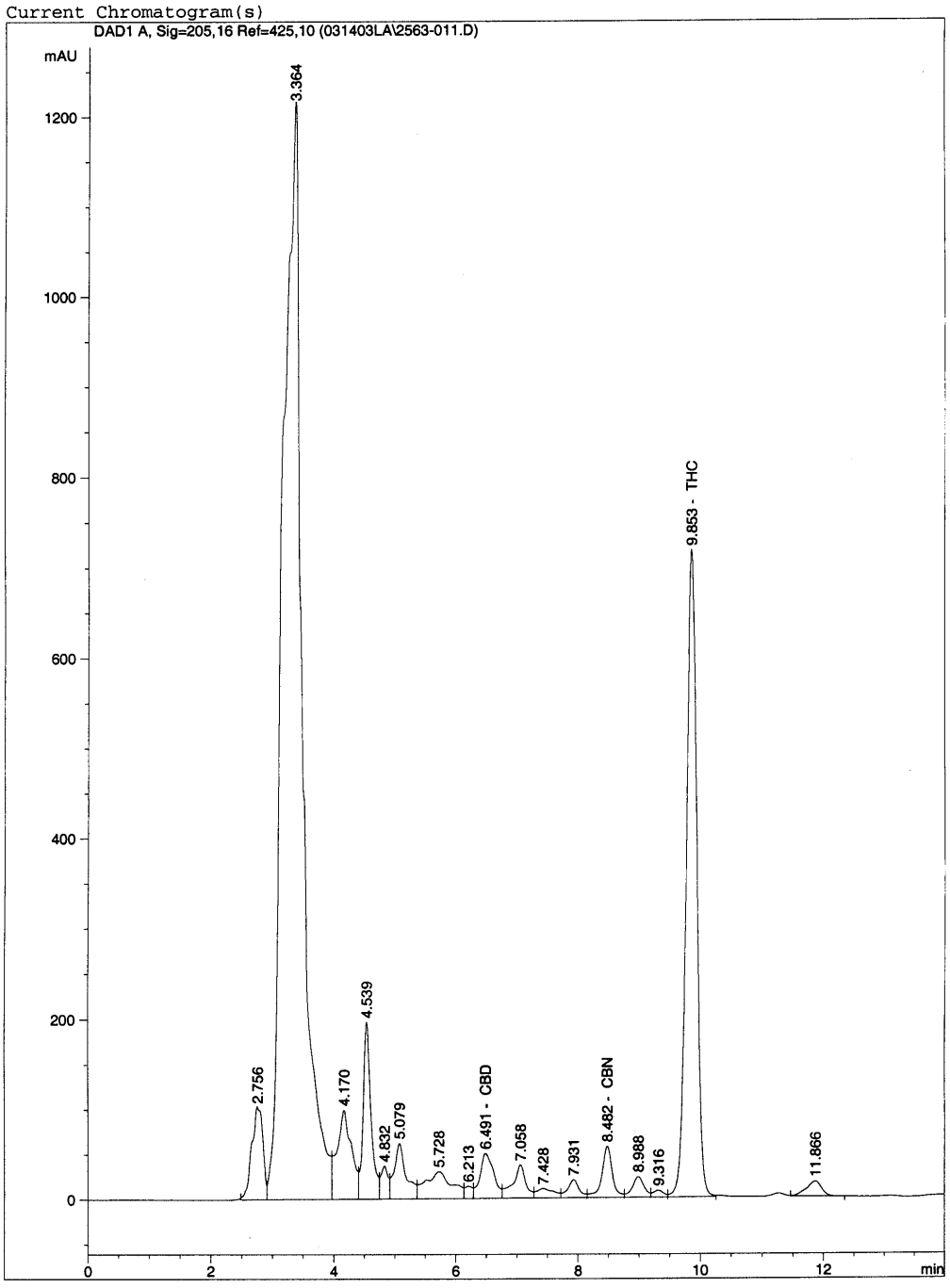


Figure 12. Representative GC-MS chromatogram: Method control

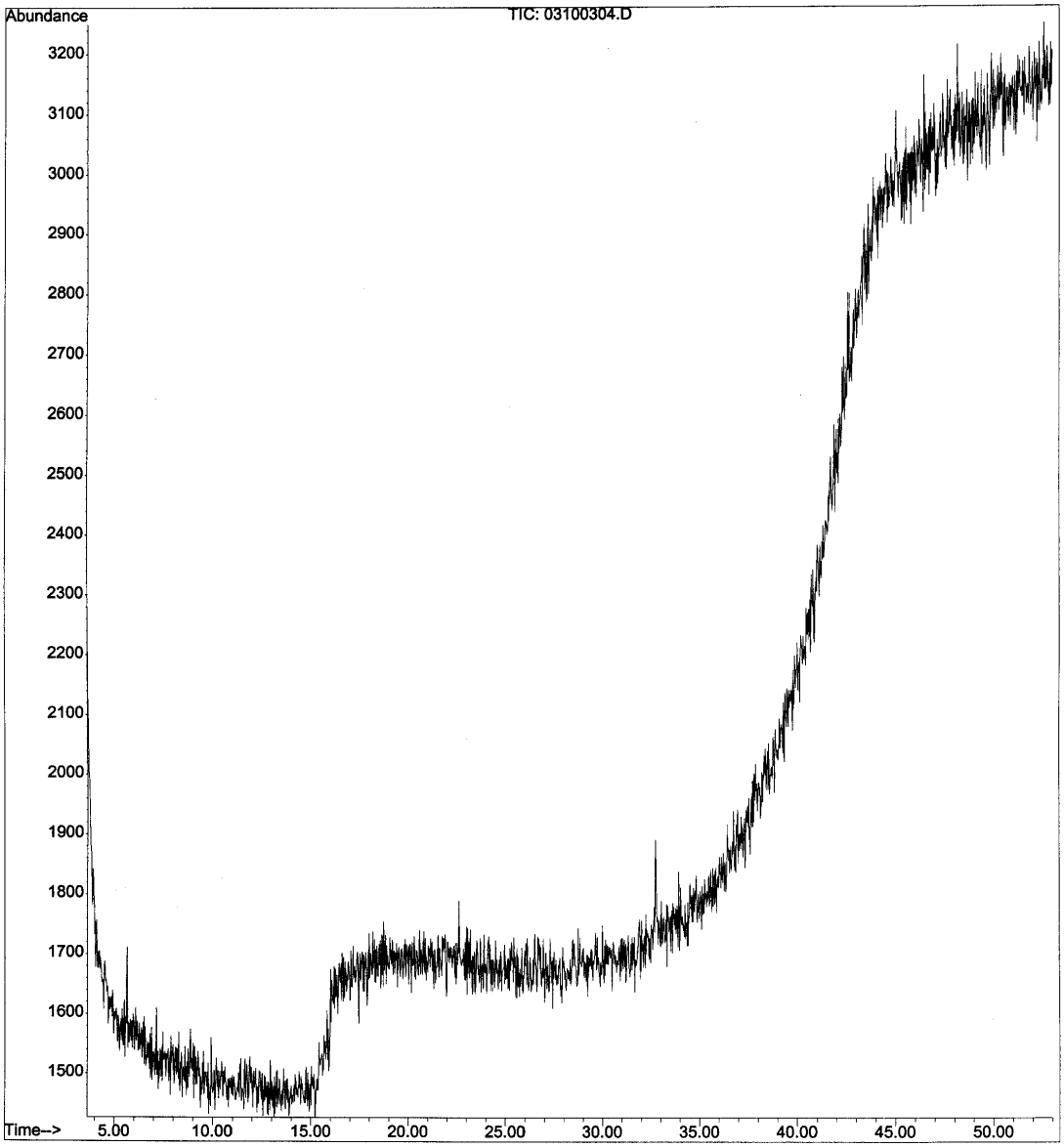


Figure 13. Representative GC-MS chromatogram: PNA reference standard (2.25 µg/mL mixed PNA)

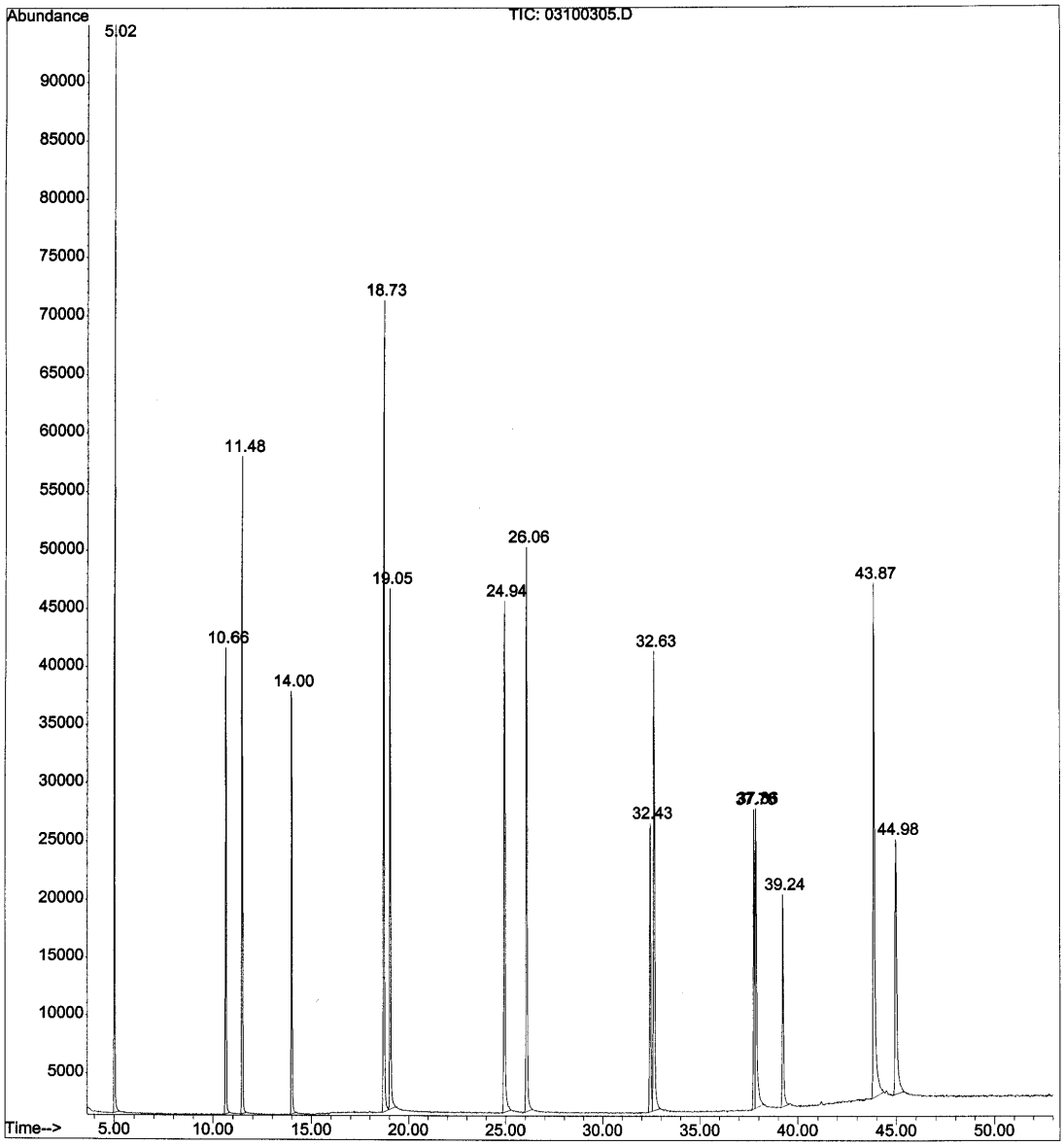


Figure 14. Representative MS spectrum: PNA reference standard (2.25 µg/mL pyrene)

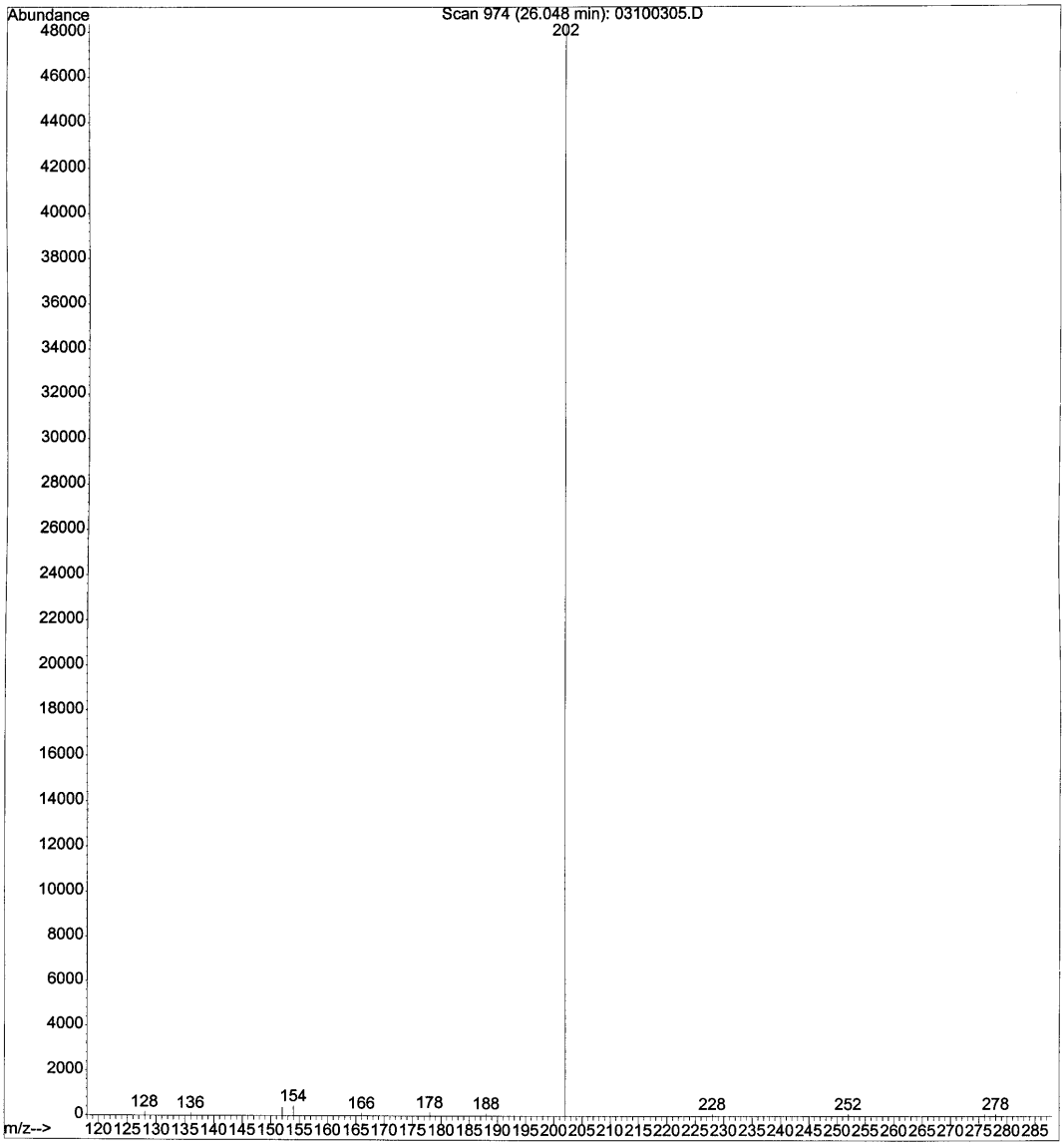


Figure 15. Representative GC-MS chromatogram: *Cannabis sativa* extract headspace gas sample analysis via Volcano® vaporization.

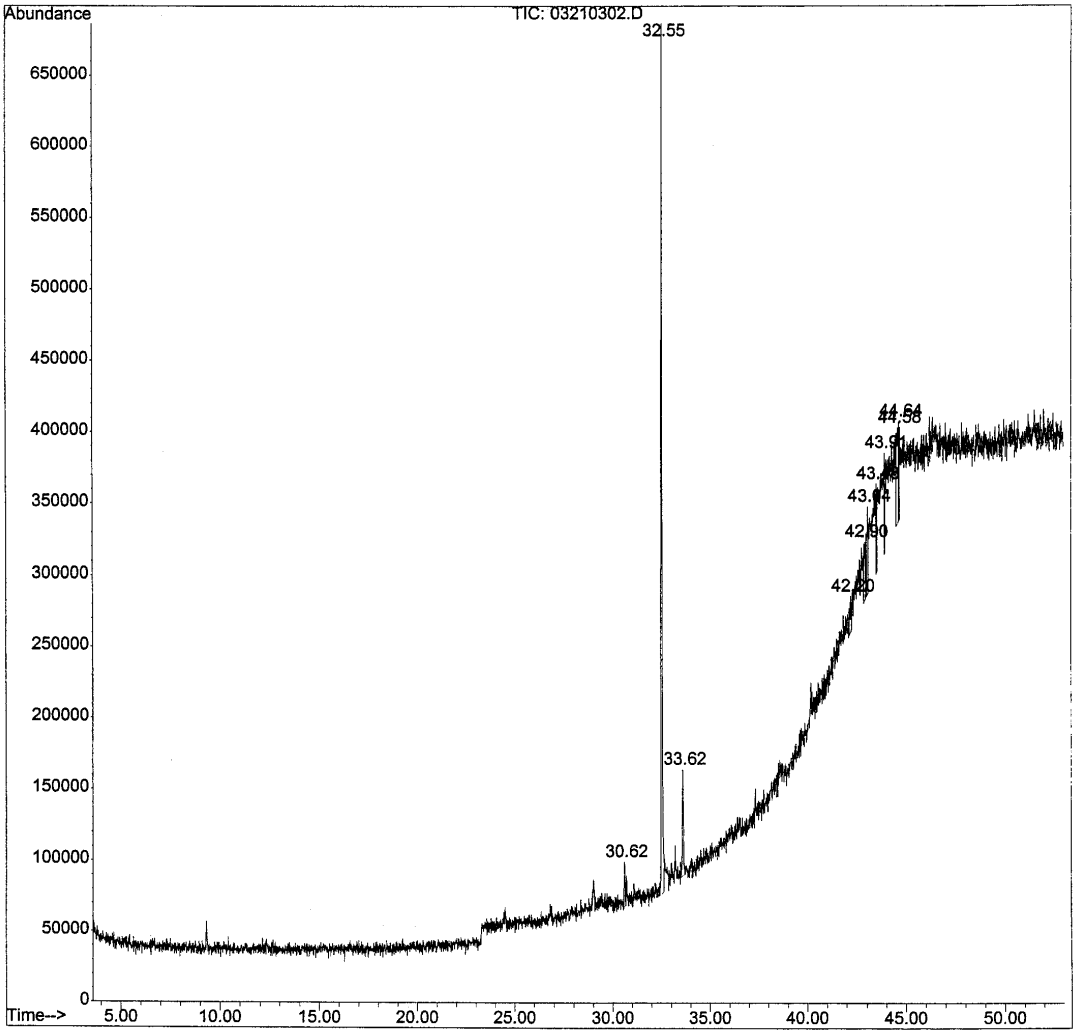


Figure 16. Representative GC-MS chromatogram: *Cannabis sativa* extract solvent sample analysis via Volcano® vaporization.

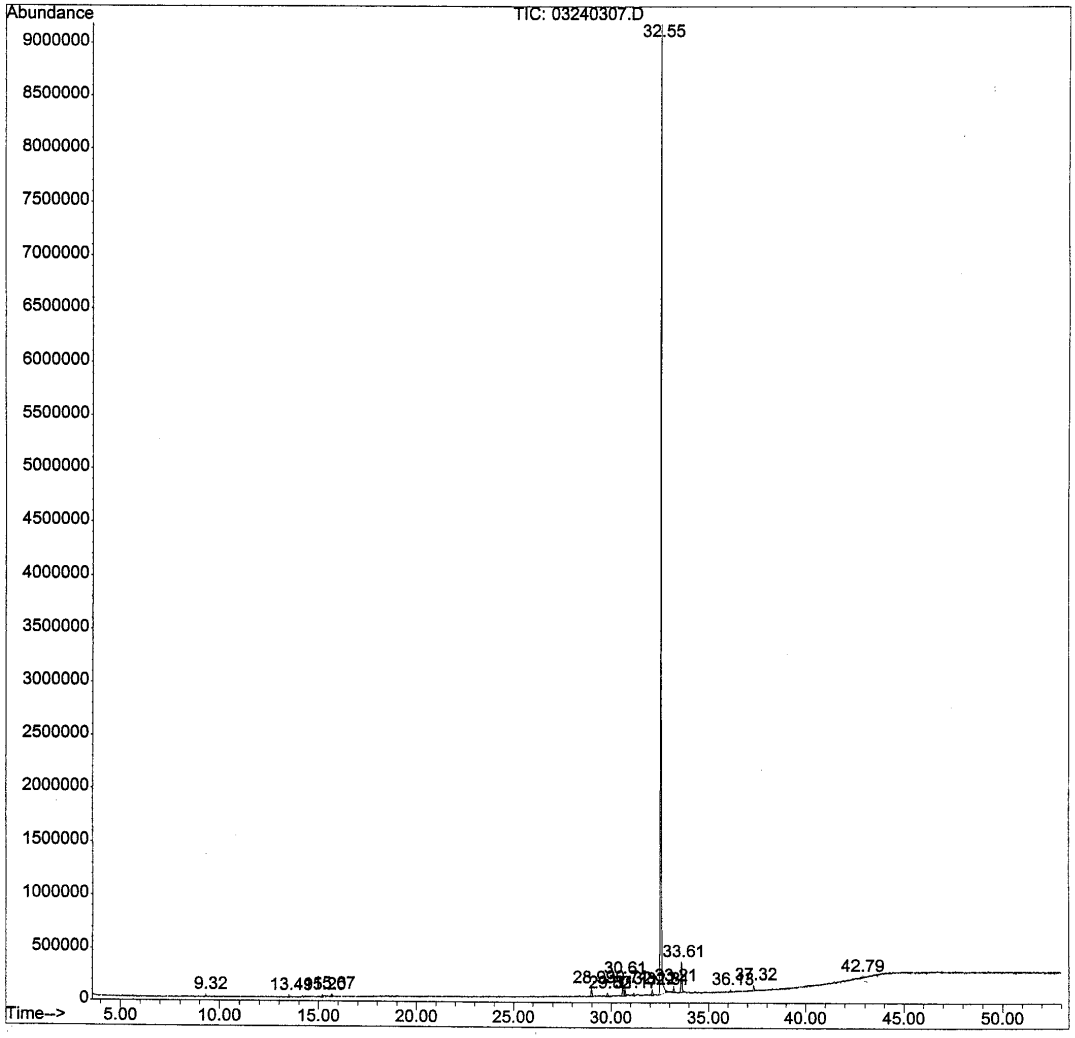


Figure 17. Representative HPLC-DAD-MS chromatogram: *Cannabis sativa* extract headspace gas sample analysis via M-1 combustion extraction

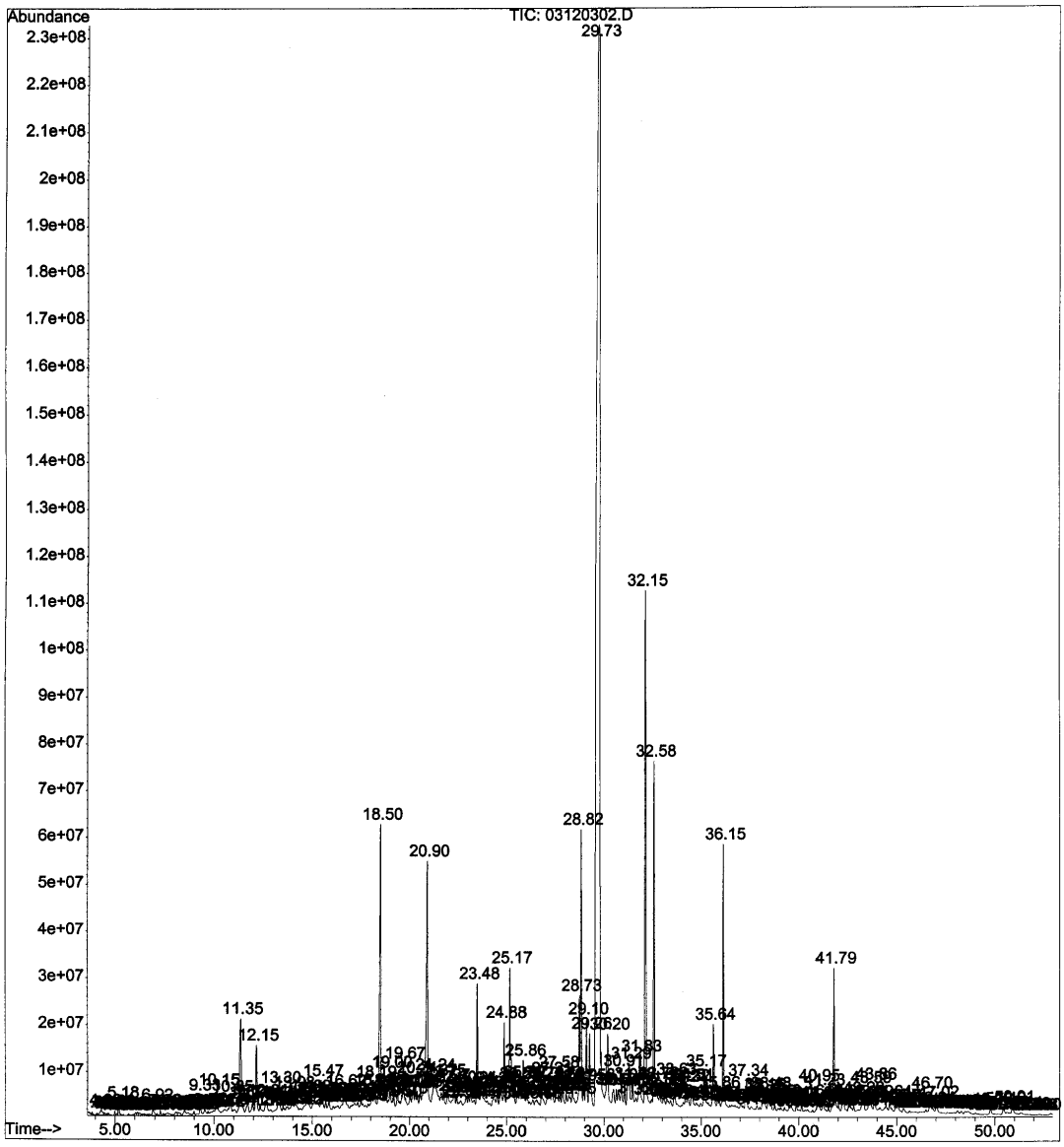
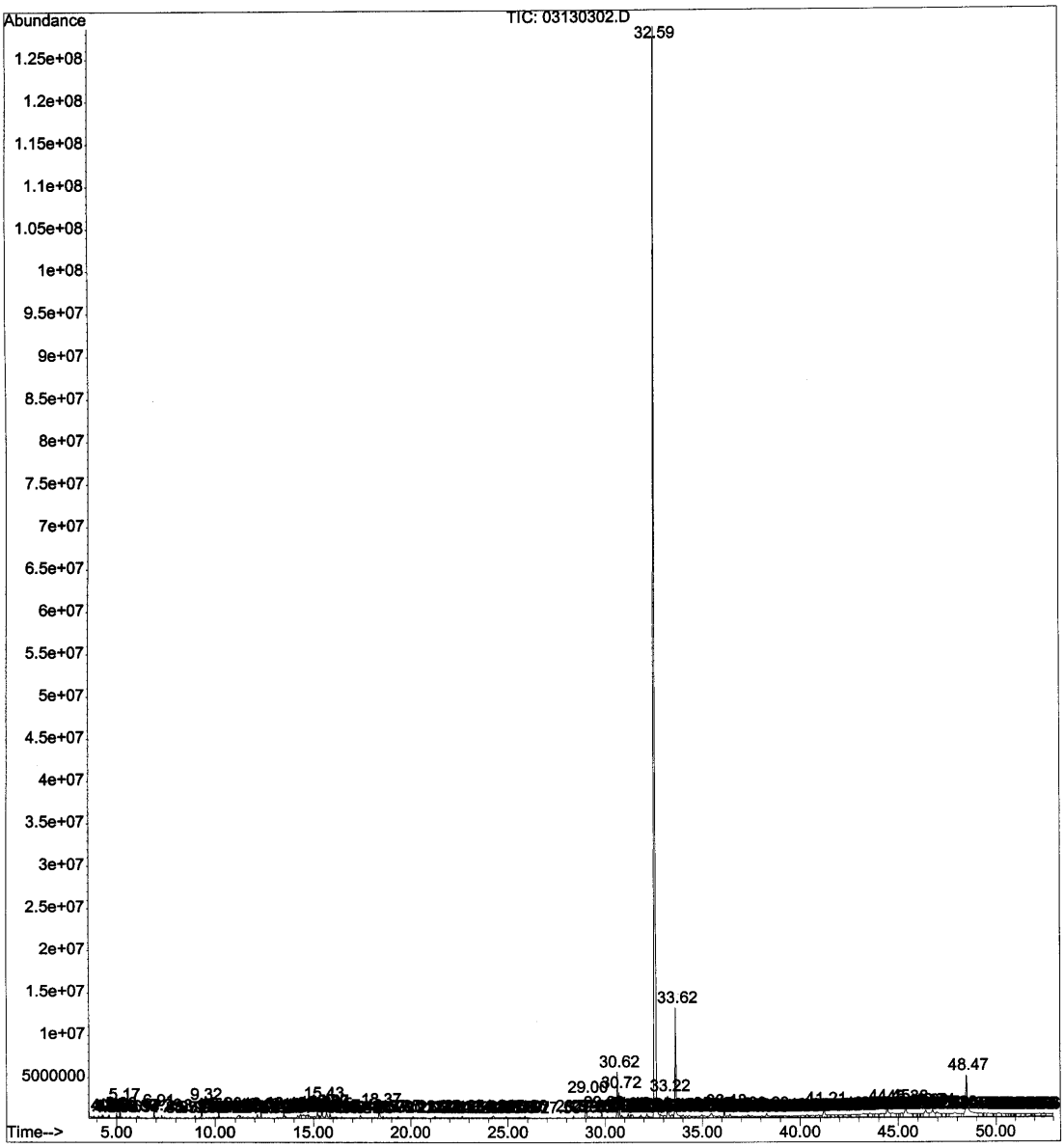


Figure 18. Representative GC-MS chromatogram: *Cannabis sativa* extract solvent sample analysis via M-1 combustion extraction.



Quality Assurance

GLP and cGMP Compliance Statement

The data presented herein were performed under Good Laboratory Practices, as defined in 21 CFR 58, and are in compliance with the Current Good Manufacturing Practices, as defined in 21 CFR 210 and 211, with the following exceptions, actual or potential:

- This study was performed in accordance with protocol number 2563 - *Evaluation of Volcano® Vaporizer for the efficient emission of THC, CBD, CBN, and the significant reduction and/or elimination of Polynuclear-aromatic (PNA) analytes resultant of pyrolysis* (Attachment 1), as well as Chemic Laboratories, Inc. standard operating procedures (SOPs). The Quality Assurance Unit (QAU) ensures that all laboratory work is conducted in accordance with all pertinent SOPs, project specific protocols and/or study profiles. SOPs have been prepared for all laboratory functions including those for the QAU, test chemical receipt, storage, handling and disposal, health and safety, instrument and equipment procedures, laboratory procedures, data interpretation and data acquisition. Procedures are in place to document transgressions from approved protocols and SOPs.

- The study director was not identified for this study.

- No in-life phase inspections were performed, although the data, report, and facilities were audited.

_____

Amy G. Fox
Manager, Quality Control / Quality Assurance

8.0 Signatures and Approval:

Submitted by :

Chemic Laboratories, Inc.
480 Neponset Street, Bldg. 7C
Canton, MA 02021

Data/ Report, Reviewed and Approved:

_____

Joseph P. St. Laurent, Date
President, CSO
Chemic Laboratories, Inc.

_____

Amy G. Fox, Date
Director, Regulatory Affairs
Chemic Laboratories, Inc.

This report was revised to add details regarding product origin and study purpose, as directed by the client, as well as correcting the analytical column used during the LC-MS assay. In addition, specific details of the methods and discussion were elaborated upon as per the client's comments.

In order to ensure proper data interpretation, this report may not be reproduced except in its full content as per Chemic Laboratories Quality Assurance Plan.

This report has been signed in accordance with Chemic Laboratories, Inc. SOP No. 1.14

All raw data and a copy of the final report produced during this assay are stored in Chemic Laboratories, Inc. archives according to standard operating procedure 1.15.

Report Received and Acknowledged:

_____

Rick Doblin, Ph.D. Date
Multidisciplinary Association for

Psychedelic Studies (MAPS)