CAN METHOD 100

Method for the determination of Cannabinoids and Organochlorine Pesticides in marijuana, hashish, and THC infused edible products by Gas Chromatography with PID and BCD detectors in series. This method is suitable for laboratory, or on-site analysis.

1. Scope and Application

1.1 This method may be used to determine the concentrations of various Cannabinoids and Organochlorine Pesticides in extracts from solid and liquid matrices, using gas chromatography with fused-silica; open-tubular, capillary columns and Photoionization (PID) and Bromide Chloride Detector (BCD) detectors run in series. The following parameters may be determined by this method:

Parameter	CAS Registry No.
Cannabanoids:	
Cannabidiol (CBD)	13956-24-1
Cannabinol (CBN)	521-35-7
Delta^{9}-Tetrahydrocannabinol (THC)	1972-08-3
Organochlorine Pesticides:	
Aldrin	309-00-2
α-BHC	319-84-6
β-BHC	319-85-7
γ-BHC (Lindane)	58-89-9
δ-BHC	319-86-8
α-Chlordane	5103-71-9
γ-Chlordane	5103-74-2
Chlordane - not otherwise specified (n.o.s.)	57-74-9
Chlorobenzilate	510-15-6
1,2-Dibromo-3-chloropropane (DBCP)	96-12-8
4,4'-DDD	72-54-8
4,4'-DDE	72-55-9
4,4'-DDT	50-29-3
Diallate	2303-16-4
Dieldrin	60-57-1
Endosulfan I	959-98-8
Endosulfan II	33213-65-9
Endosulfan sulfate	1031-07-8
Endrin	72-20-8
Endrin aldehyde	7421-93-4
Endrin ketone	53494-70-5
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
Hexachlorobenzene	118-74-1
Hexachlorocyclopentadiene	77-47-4
Isodrin	465-73-6
Methoxychlor	72-43-5
Toxaphene	8001-35-2

1.2 This is gas chromatographic (GC) method applicable to the determination of the compounds listed above in marijuana and THC infused edible products. Sample extracts are analyzed by direct injection into the GC.

1.3 The method detection limit (MDL, defined in Section 12.1) for each parameter is listed in Section 10.1. The MDL for a specific extract may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 Any modification of this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the operation of a gas chromatograph and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A composite sample is homogenized in a mechanical or electric grinder. A 0.1g sample is accurately weighed and transferred to a 4mL vial. 3mL of laboratory grade methanol is added to the sample. The vial is then tightly capped and the sample is vigorously agitated for 2 min to extract the compounds from the matrix. The extract is then left to settle for 2 min and then an aliquot of the extract is injected into a gas chromatograph equipped with a 30m capillary column for the separation of the compounds and PID and BCD detectors for the identification of cannabinoids and pesticides, respectively.

3. Interferences

3.1 Impurities in the sample matrix account for the majority of contamination problems. Samples with an oily or tar nature can contaminate the front of the column. The analytical system must be demonstrated to be free from contamination under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.3.

3.2 Contamination by carry-over can occur whenever high level and low level samples are sequentially analyzed. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of reagent solvent to check for cross contamination.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this view point, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets
should also be made available to all personnel involved in the chemical analysis

5. Apparatus and Materials

5.1 Sampling equipment, for discrete sampling.

5.1.1 Vial—4 mL capacity or larger (Restek #24654 or equivalent), equipped with a Teflon faced septa and a screw cap (Restek #21743 or equivalent).

5.1.2 Vial—2 mL capacity (Restek #24473 or equivalent), equipped with a Teflon faced septa and a screw cap.

5.2 Grinder—The composite sample may be homogenized in a grinder. A small electric coffee grinder is suitable as long as the sides and blades can be wiped clean in between uses (Mr. Coffee, Braun or equivalent).

5.3 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, and detectors, and data system for measuring peak areas. (DPS Companion 2 #500-C2-107).

5.3.1 Column 1—30m x 0.53mm ID MXT-5, 1micron film thickness fused silica capillary (Restek #70155, or equivalent). This column was used to develop the method performance statements in Section 12.

5.3.2 Detector—Photoionization detector. This type of detector has proven effective in the analysis of cannabanoids for the parameters listed in the scope (Section 1.1), and was used to develop the method performance statements in Section 12.

5.3.3 Detector—Bromide Chloride detector. This type of detector is non-radioactive, highly sensitive to chloride ions, and has proven effective in the analysis of organochlorine pesticides for the parameters listed in the scope (Section 1.1), and was used to develop the method performance statements in Section 12.

5.4 Syringes—5 mL, glass, or plastic hypodermic with Luerlok tip (SGE #5MDR-LL-GT, or equivalent).

5.5 Micro syringes—10 µL, fixed needle (SGE #002105, or equivalent).

5.6 Balance—Analytical, capable of accurately weighing 0.01 g.

6. Reagents

6.1 Methanol—Reagent grade (JT Baker #JT9070-13, or equivalent).

6.2 Stock standard solutions—Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions. Commercially prepared stock
standards may be used at any concentration if they are certified by the manufacturer or by an independent source. Prepare stock standard solutions in reagent grade methanol.

6.2.1 Cannabidiol (CBD), (Restek #34011, 1mg/mL, or equivalent)
Cannabinol (CBN), (Restek #34010, 1mg/mL, or equivalent)

Tetrahydrocannabinol (THC), (Restek #34067, 1mg/mL, or equivalent) Organochlorine Pesticide Mix AB #1, (Restek #32291, 200µg/mL, or equivalent). Prepare a working stock solution by diluting 1:10 in methanol, 20ug/mL.

6.3 Using a 500µL syringe to prepare the working analytical standard as follows:

6.3.1 Transfer 100µL of CBD into a 2mL screw cap vial.
6.3.2 Transfer 100µL of CBN
6.3.3 Transfer 500µL of THC
6.3.4 Transfer 2µL of Pesticides (working stock solution 20ug/mL)
6.3.5 Add 298µL of methanol, to make a 1mL working standard.

7. Calibration

7.1 The gas chromatograph must be operated using temperature and flow rate conditions equivalent to those as follows:

7.1.1 – Column temperature: 270^{O}C isothermal
Helium carrier gas
Pressure program: 150 kPa (hold for 3min), ramp at 50 kPa to 400 kPa.

7.1.2 – PID detector:

Detector temperature: 250^{O}C
High Voltage: 650V
Collector: -100V
Gain: 4

7.1.3 – BCD detector:

Detector temperature: 250^{O}C
Cell temperature 800^{O}C
Collector: -100V
Gain: 1

7.2 External standard calibration procedure

7.2.1 Using the analytical standard prepared in section 6.3, inject 1µL into the injection port on-column. Only a one point calibration is performed in this method, because the commercially available stock standard solutions for THC are low in concentration.

7.2.2 Analyze the calibration standard according to Section 10, and tabulate peak area responses versus the concentration of the standard. The results can be used to prepare a calibration curve for each compound.

7.3 The working calibration curve must be verified on each working day by the measurement of a QC check standard.

7.4 Analyze the QC check sample according to Section 10.

7.5 For each parameter, compare the response (Q) with the corresponding calibration acceptance criteria. The single point calibrations acceptance should be within +/- 10%. If the responses for all parameters of interest fall within the designated range, analysis of actual samples can begin. If any individual Q falls outside the range, a new calibration curve must be prepared for that parameter according to Section 7.2.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and an ongoing analysis of spiked samples to evaluate and document data quality. The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a quality control check standard must be analyzed to confirm that the measurements were performed in an in-control mode of operation.

8.1.1 The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of advances that are occurring in chromatography, the analyst is permitted certain options (detailed in Section 10.1) to improve the separations or lower the cost of measurements. Each time such a modification is made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 Each day, the analyst must analyze a methanol blank to demonstrate that interferences from the analytical system are under control.

8.1.4 The laboratory must, on an ongoing basis, spike and analyze a minimum of 5% of all samples to monitor and evaluate laboratory data quality. This procedure is described in Section 8.3.

8.1.5 The laboratory must, on an ongoing basis, demonstrate through the analyses of quality control check standards that the operation of the measurement system is in control. This procedure is described in Section 8.4. The frequency of the check standard analyses is equivalent to 10% of all samples analyzed but may be reduced if spike recoveries from samples (Section 8.3) meet all specified quality control criteria.

8.1.6 The laboratory must maintain performance records to document the quality of data that is generated. This procedure is described in Section 8.2.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 A quality control (QC) check sample concentrate is required containing each parameter of interest. The QC check sample concentrate must be prepared

by the laboratory using stock standards prepared independently from those used for calibration.

8.2.2 Analyze four aliquots QC check sample according to Section 10.

8.2.3 Calculate the average recovery, and the standard deviation of the recovery for each parameter using the four results.

8.2.4 For each parameter compare s and with the +/- 10% corresponding acceptance criteria for precision and accuracy. If s and for all parameters of interest meet the acceptance criteria, the system performance is acceptable and analysis of actual samples can begin. If either s exceeds the precision limit or falls outside the range for accuracy, the system performance is unacceptable for that parameter. Locate and correct the source of the problem and repeat the test for each compound of interest.

8.3 As part of the QC program for the laboratory, field duplicates may be analyzed to assess the precision of the measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column or mass spectrometer must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 All samples must be stored in sealed containers to prevent cross contamination. All samples must be composited prior to extraction. For plant material take 15-20 small portions from the entire surface of the sample for the composite. Duplicate samples are composited in the same manner.

9.1.1 Homogenize the plant material sample using the electric grinder.

9.1.2 For edible samples, composite and grind a large enough portion to represent the entire sample.

9.1.3 For liquid samples, mix thoroughly prior to weighing.

9.2 All samples are prepared by accurately weighing 0.10 g of the composite sample in a 4mL vial. Add 3.0 mL of reagent grade methanol. Secure the screw cap and agitate vigorously for 2 min. After the sample is extracted it should be stored in a cool, dark place and analyzed within 14 days.

10. Procedure

10.1 Section 7.1 summarizes the recommended operating conditions for the gas chromatograph. The MDL for the cannabinoids is 0.01 %. The MDL for total pesticides is 500ng/ml (ppb). The areas for all of the individual pesticides are combined and calibrated together. If an individual pesticide compound is detected, it is quantified against the calibration curve of total pesticides. In this way, the results of the pesticide analysis are generally reported as ND (Not Detected) < 500ppb. The individual pesticides can be determined by this method, if necessary. The temperature program of the GC oven would need to be changed to better separate the individual compounds.

10.2 Calibrate the system daily as described in Section 7.

10.3 Inject an aliquot of the sample extract through the injection port of the GC directly on-column.

10.4 Identify the parameters in the sample by comparing the retention times of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11. Calculations

11.1 Determine the concentration of individual compounds in the sample.

11.1.1 Calculate the concentration of the parameter being measured from the peak response using the calibration curve determined in Section 7.3.

11.2 Report results in % for the cannabinoids, and ppb for the pesticides without correction for recovery data. All QC data obtained should be reported with the sample results.

12. Method Performance

12.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations were obtained using reagent grade methanol. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

12.2 This method is recommended for the concentration range from the MDL to 5000 x MDL. Dilution techniques should be used to measure concentration levels above 5000 x MDL.

13. Sample Chromatograms

13.1 Sample chromatograms for the cannabinoids using a PID detector, and organochlorine pesticides using a BCD detector are as follows. Chromatograms are: (1) cannabinoids and pesticide standard, (2) representative sample, and (3) methanol blank.

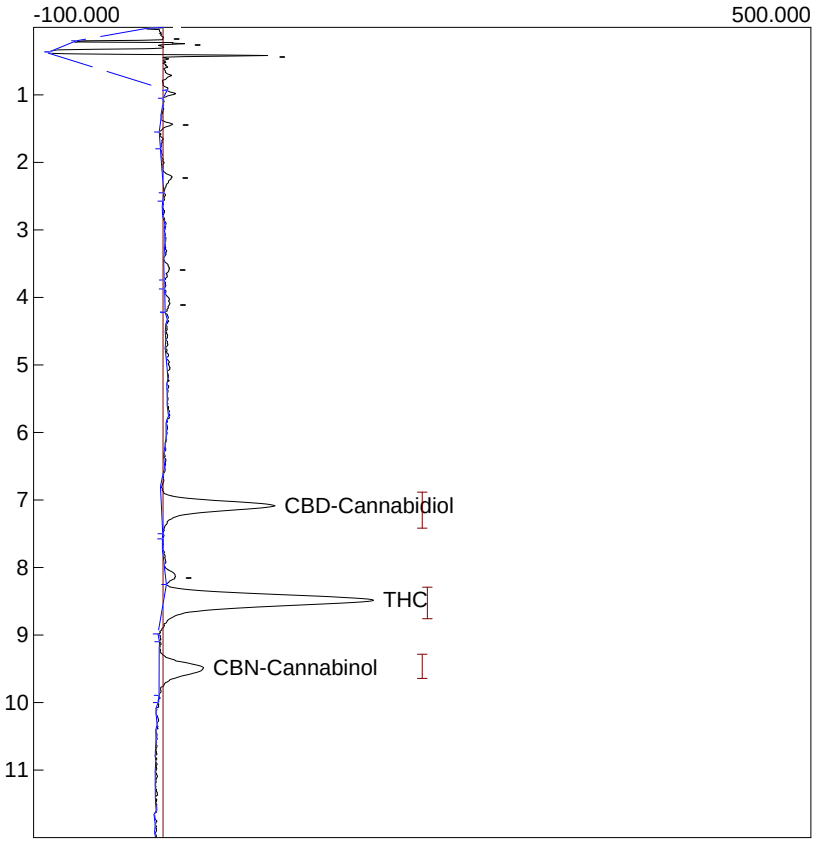
Lab name: DPS Instruments
Analysis date: 12/17/2010 12:37:30
Method: 1uL Direct Injection
Description: PID
Column: 30m X 0.53mm MXT-5
Carrier: Helium @ 150 kPa
Temp. prog: THC.tem
Events: ZERO.evt
Components: THC.cpt
Data file: PID135.CHR ()
Sample: THC Standard
Operator: Dave Pierce
Comments: PID Detector
HV = 650
Gain = 5
Collector = -100V
Detector Temp = 250C
Range 0-5V
Pressure Program= 150kPa 4min, ramp 50kPa to 400kPa

Temperature program:

Init temp	Hold	Ramp	Final temp
270.00	12.000	10.000	270.00

Events:

Time	Event
0.000	ZERO
0.000	H ON ()
0.000	INTEG NONE
1.000	INTEG BASED



Component	Retention	Area	External	Units
CBD-Cannabidiol	7.083	942.7450	0.3000	%
THC	8.483	1994.2900	1.5000	%
CBN-Cannabinol	9.483	495.6330	0.3000	%
		3432.6680	2.1000	

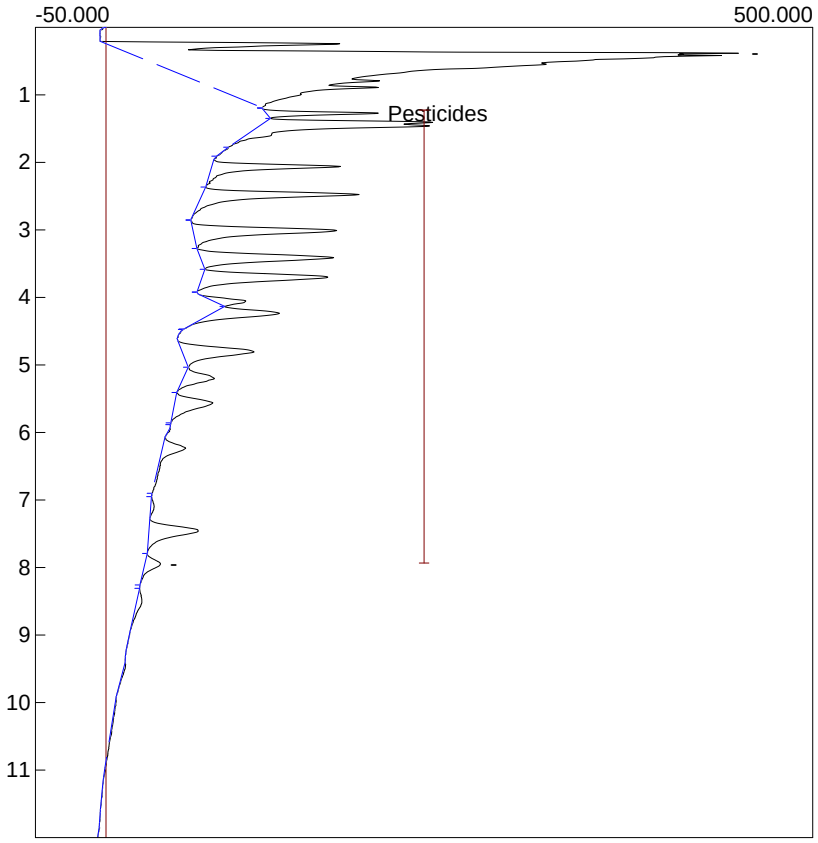
Lab name: DPS Instruments
Analysis date: 12/17/2010 12:37:30
Method: 1uL Direct Injection
Description: BCD
Column: 30m X 0.53mm MXT-5
Carrier: Helium @ 150 kPa
Temp. prog: THC.tem
Events: ZERO.evt
Components: PEST.cpt
Data file: BCD135.CHR ()
Sample: THC Standard
Operator: Dave Pierce
Comments: BCD Detector
Cell = 800 C
Gain = 1
Collector = -100V
Detector Temp = 250C
Range 0-5V
Pressure Program= 150kPa 4min, ramp 50kPa to 400kPa

Temperature program:

Init temp	Hold	Ramp	Final temp
270.00	12.000	10.000	270.00

Events:

Time	Event
0.000	ZERO
0.000	H ON ()
0.000	INTEG NONE
1.000	INTEG BASED



Component	Retention	Area	External	Units
Pesticides	1.275	6452.7590	24.0000	ppm
		6452.7590	24.0000	

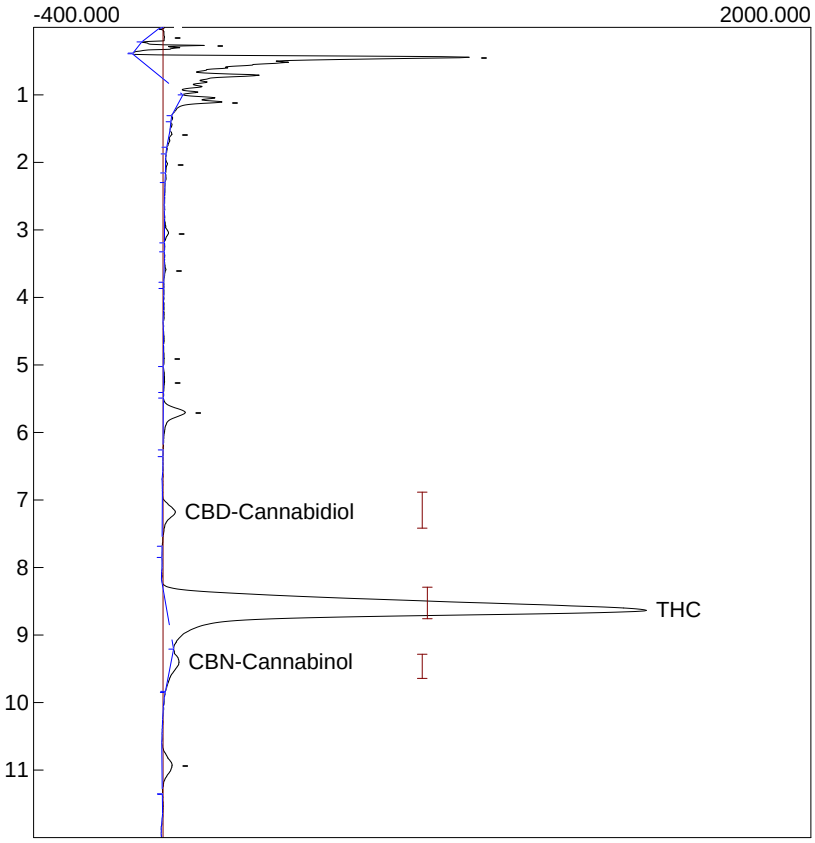
Lab name: DPS Instruments
Analysis date: 12/17/2010 13:21:33
Method: 1uL Direct Injection
Description: PID
Column: 30m X 0.53mm MXT-5
Carrier: Helium @ 150 kPa
Temp. prog: THC.tem
Events: ZERO.evt
Components: THC.cpt
Data file: PID138.CHR ()
Sample: Jillybean
Operator: Dave Pierce
Comments: PID Detector
HV = 650
Gain = 5
Collector = -100V
Detector Temp = 250C
Range 0-5V
Pressure Program= 150kPa 4min, ramp 50kPa to 400kPa
0.1g / 3ml Methanol

Temperature program:

Init temp	Hold	Ramp	Final temp
270.00	12.000	10.000	270.00

Events:

Time	Event
0.000	ZERO
0.000	H ON ()
0.000	INTEG NONE
1.000	INTEG BASED



Component	Retention	Area	External	Units
CBD-Cannabidiol	7.175	610.4900	0.1943	%
THC	8.633	22890.8110	17.2173	%
CBN-Cannabinol	9.400	406.3165	0.2459	%
		23907.6175	17.6575	

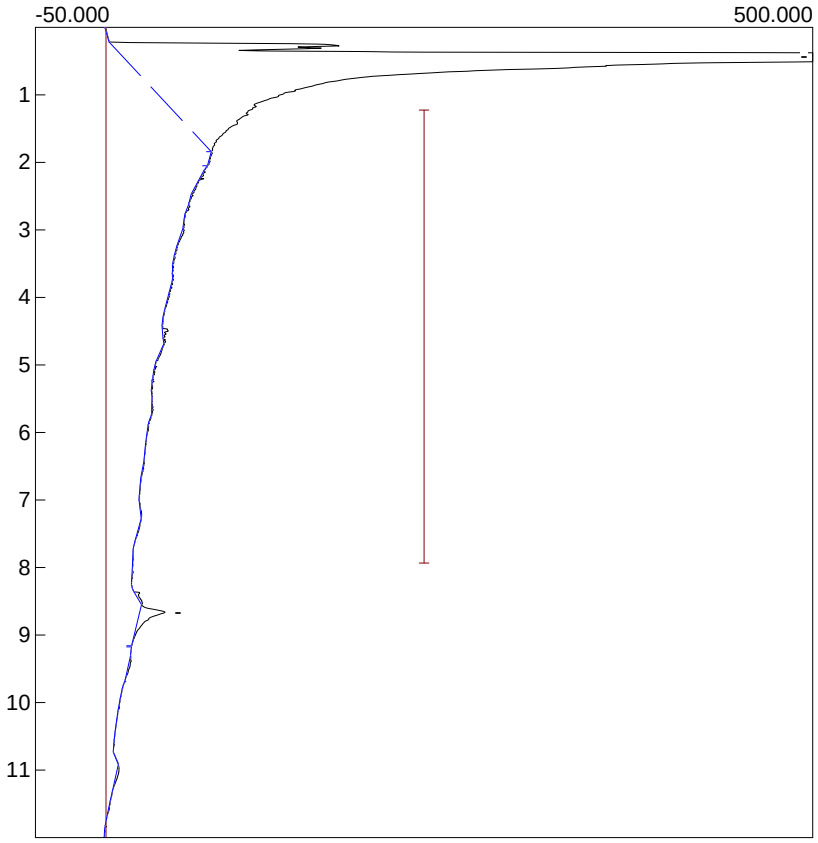
Lab name: DPS Instruments
Analysis date: 12/17/2010 13:21:33
Method: 1uL Direct Injection
Description: BCD
Column: 30m X 0.53mm MXT-5
Carrier: Helium @ 150 kPa
Temp. prog: THC.tem
Events: ZERO.evt
Components: PEST.cpt
Data file: BCD138.CHR ()
Sample: Jillybean
Operator: Dave Pierce
Comments: BCD Detector
Cell = 800 C
Gain = 1
Collector = -100V
Detector Temp = 250C
Range 0-5V
Pressure Program= 150kPa 4min, ramp 50kPa to 400kPa
0.1g / 3ml Methanol

Temperature program:

Init temp	Hold	Ramp	Final temp
270.00	12.000	10.000	270.00

Events:

Time	Event
0.000	ZERO
0.000	H ON ()
0.000	INTEG NONE
1.000	INTEG BASED



Component	Retention	Area	External	Units
		0.0000	0.0000	

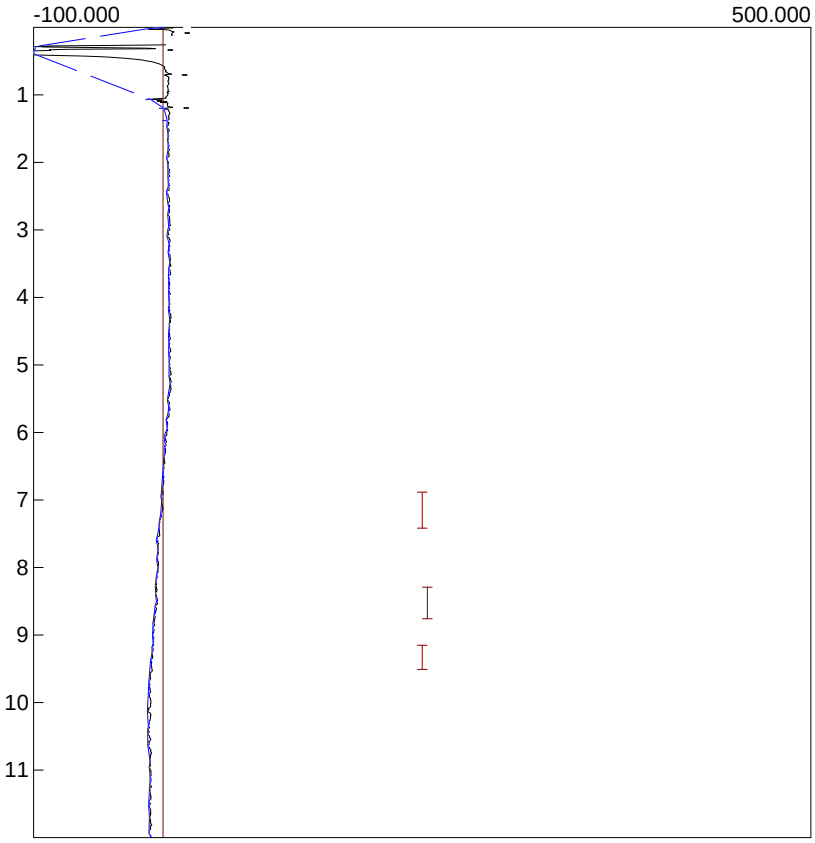
Lab name: DPS Instruments
Analysis date: 12/17/2010 16:50:09
Method: 1uL Direct Injection
Description: PID
Column: 30m X 0.53mm MXT-5
Carrier: Helium @ 150 kPa
Temp. prog: THC.tem
Events: ZERO.evt
Components: THC.cpt
Data file: PID142.CHR ()
Sample: Methanol Blank
Operator: Dave Pierce
Comments: PID Detector
HV = 650
Gain = 5
Collector = -100V
Detector Temp = 250C
Range 0-5V
Pressure Program= 150kPa 4min, ramp 50kPa to 400kPa

Temperature program:

Init temp	Hold	Ramp	Final temp
270.00	12.000	10.000	270.00

Events:

Time	Event
0.000	ZERO
0.000	H ON ()
0.000	INTEG NONE
1.000	INTEG BASED



Component	Retention	Area	External	Units
		0.0000	0.0000	

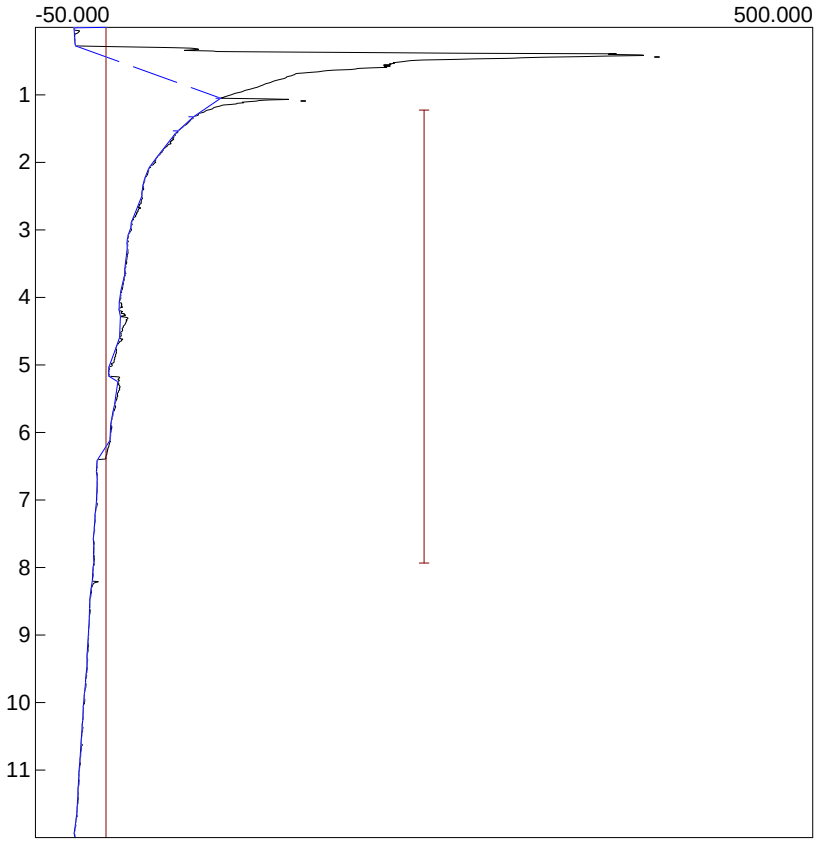
Lab name: DPS Instruments
Analysis date: 12/17/2010 16:50:09
Method: 1uL Direct Injection
Description: BCD
Column: 30m X 0.53mm MXT-5
Carrier: Helium @ 150 kPa
Temp. prog: THC.tem
Events: ZERO.evt
Components: PEST.cpt
Data file: BCD142.CHR ()
Sample: Methanol Blank
Operator: Dave Pierce
Comments: BCD Detector
Cell = 800 C
Gain = 1
Collector = -100V
Detector Temp = 250C
Range 0-5V
Pressure Program= 150kPa 4min, ramp 50kPa to 400kPa

Temperature program:

Init temp	Hold	Ramp	Final temp
270.00	12.000	10.000	270.00

Events:

Time	Event
0.000	ZERO
0.000	H ON ()
0.000	INTEG NONE
1.000	INTEG BASED



Component	Retention	Area	External	Units
		0.0000	0.0000	