

Study report

The Volcano® herbal vaporizer: Variability of repeated balloon filling



Product Isolation from Nature
P.O.Box 506
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Performed this study for:

Storz&Bickel GmbH
Tuttlingen Germany

Prisna project #:

2008-016

Study period:

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The Volcano vaporizer by Storz&Bickel GmbH (Tuttlingen, Germany) is a herbal vaporizer that can be used for the inhalation of (medicinal) vapors from a variety of plants. It is a sophisticated device that utilizes a temperature -controlled heatflow to vaporize the volatile components out of plant materials. The vapor is collected in a heat-resistant plastic bag where it can cool down to a comfortable temperature. After filling, the bag is removed from the heat source and connected to a mouth piece for inhalation.

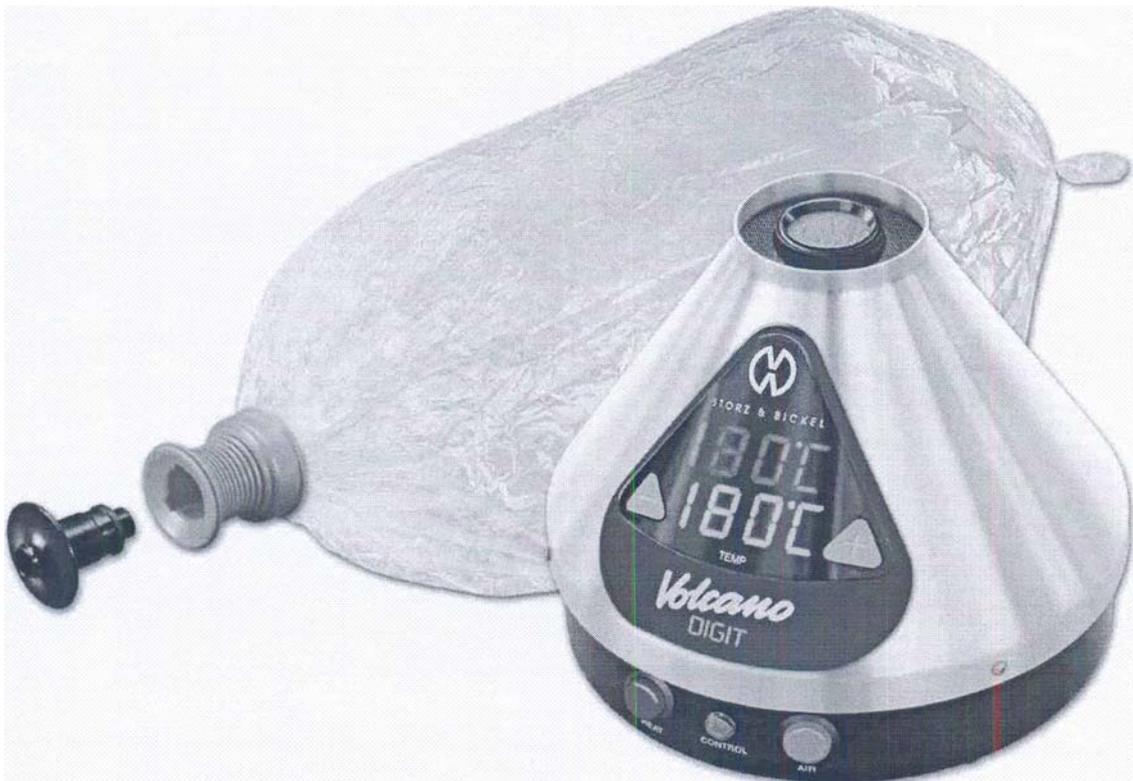
One of the applications of the Volcano vaporizer is the medical administration of the main active components (cannabinoids) of the Cannabis plant. In several clinical trials, vaporizing with the Volcano was shown to be a reliable and reproducible method for the administration of THC [Abrams 2007, Zuurman 2008]. In the Netherlands, medicinal grade cannabis is available through pharmacies for the treatment of a variety of chronic diseases, including multiple sclerosis, chronic neuropathic pain, and Tourette syndrome [OMC 2009]. In other countries, including Germany, Italy, Canada and the United Kingdom, medicinal cannabis is gradually being introduced, either in the form of herbal material, or as extract. For the specific use in combination with medicinally used cannabis, the Volcano Medic is currently under development by Storz& Bickel.

The Volcano Medic is intended for use with herbal Cannabis, and Dronabinol (purified THC). The balloon is the part of the Volcano that stores the vapor until the user is ready for inhalation. According to the manual this should be done within 10 minutes after filling of the balloon is completed. The balloon is not intended for single-use but may be used up to 2 weeks with an average use of 5 times each day. However, during the use of the balloon, cannabinoids and other components of the vapor were shown to stick to the inside of the balloon, as a result of condensation [Hazekamp 2006a]. After the repeated use of a single balloon, the inside will become increasingly contaminated with this condensated material. As a consequence, the possibility may exist that a) the increased 'stickiness' of the inside of the balloon will increase the condensation of vapor components during later balloon fillings (i.e.: the vapor will contain increasingly less active components), or b) the condensated material will, at some point, release from the balloon and add to the vapor present in the bag (i.e.: the vapor will suddenly be much stronger than anticipated). It is clear that such phenomena would make to repeated use of a single balloon unadvisable.

In order to determine if the phenomena mentioned above really take place, this study was performed. A single balloon was used on 14 different days, and filled 5 times each day, so the same balloon was exposed to 70 fillings. The experiment was performed with Cannabis plant material (at 185°C), and then it was repeated with pure THC (at 210°C). Vaporizing was done according to the manual of the Volcano. Evaporation temperatures were selected based on the experience of Storz&Bickel. The vapor in each balloon was analyzed for its cannabinoid content, with a special focus on the most biologically active cannabinoid THc.

- A)
 - B) Preparing standardized Cannabis plant material for performing the experiments with plant material;
 - C) Isolation of THC from standardized Cannabis plant material for performing the experiments with THC;
 - D) Repeated filling experiments on 14 separate days, with 5 fillings each day; preparation of vapor extracts;
 - E) Analysis of vapor extracts by validated UPLC method; E) Statistical evaluation of UPLC results

The final goal of this study is to determine if a single balloon can be used for 14 days, with an average use of 5 times daily.



All organic solvents were analytical grade and obtained from Merck Biosolve Ltd. Valkenswaard, The Netherlands.

Cannabis plant material (female flowertops, variety 'Bedrocan®') was medical grade and obtained from Bedrocan BV (Veendam, The Netherlands). Batch number was 07.65.260907 and harvest date was 24 December 2007. Plants were cultivated under standardized conditions according to the requirements of Good Agricultural Practice (GAP) [Hazekamp 2006b, OMC 2009]. The same cannabis material is officially dispensed through Dutch pharmacies under the Dutch medicinal cannabis program, supervised by the Office of Medicinal Cannabis (OMC). After harvest, the plant material was air-dried in the dark under constant temperature and humidity for 1 week. The variety 'Bedrocan' has a THC content of ca. 18% (w/w) and a water content of ca. 5% (w/w).

For this study, 10 grams of cannabis was grinded (in small portions) with the Storz&Bickel Grinder, as delivered with the Volcano device, and homogenized. Material was stored at roomtemperature in the dark, in a air-tight container until used. Under these conditions shelflife is at least 1 year [OMC 2009]. For each experiment 100 mg grinded material was accurately (+/- 1 mg) weighed on a calibrated analytical balance and placed in the standard filling chamber of the Volcano. The filling chamber was placed onto a Volcano apparatus set at 185°C and the balloon was filled. Balloon was then removed from the Volcano and kept for 10 min at room temperature before extraction of the vapor.

Delta-9-tetrahydrocannabinol (THC) was isolated from the Cannabis plant material described above. The methods for isolation [Hazekamp, 2004a] and quantification [Hazekamp, 2004b] have been published. Total yield was 820 mg and final Purity was 99.5%. The Certificate of Analysis is available at Prisma BV. Finally, an ethanolic stock solution was prepared of 39.1 mg THC per ml.

For the THC solution the 'liquid pad' was used, as provided by Storz&Bickel. This is a removable disc made of tightly packed stainless steel wire mesh, capable of holding a certain volume of liquid. Its reliability was previously shown in a clinical trial [Zuurman 2008]. A new liquid pad was used for each experiment. For each balloon filling, exactly 250 microliter of the stock solution was brought onto the liquid pad, which equals 9.8 mg of pure THC.

Ethanol was removed by placing the filling chamber (with liquid pad) onto a Volcano apparatus at a setting of 100°C for 20 sec. At this temperature, THC will not evaporate [Hazekamp 2006a] but ethanol will. Subsequently, the filling chamber was placed onto another Volcano apparatus set at 210°C and the balloon was filled. Balloon was then removed from the Volcano and kept for 10 min at roomtemperature before extraction

Preparation of vapor extracts

Cannabinoids were recovered from the vapor inside the balloon by condensation onto glass fiber filters, designed to capture particles >0.1 microns. Glass fiber filters (Cambridge type, borosilicate glass, 92 mm diameter) and tightly fitting filter holders for vapor extraction were obtained from Borgwaldt Technik GmbH (Hamburg, Germany). With the use of a vacuum pump, the vapor was aspirated through the glass fiber filter with a constant flow of 30L/min. The filter was then placed in a 50ml plastic tube and extracted with ethanol (15ml, 10 min, under constant agitation). Extraction was repeated three times and extracts were combined. Ethanol was added to a final volume of exactly 50ml. The ethanolic samples (vapor extracts) were accurately diluted to an appropriate concentration, and analyzed by UPLC to determine the cannabinoid composition.

Cannabinoid analysis by UPLC

Quantitative analysis was performed with a Waters Acquity Ultra Performance LC (UPLC) system. The UPLC method used for the quantitative analysis of cannabinoids present in the vapor extracts has been fully validated according to ICH guidelines. This method is part of the official Dutch Monography for the quality control of medicinal cannabis distributed through Dutch pharmacies [OMC 2009].

The UPLC system consisted of a Solvent Delivery Pump (Serial number: J05UPB 162M), an Auto Sampler (Serial number: J05UPS 062M), and a Photodiode Array Detector (Serial number: J05UPD 449M). Equipment control, data acquisition and integration were performed with Water Empower 2 software. Chromatographic separation was achieved using a Waters C₁₈ analytical column (1.7µm, 2.1 x150 mm) protected by a Waters C₁₈ guard column. The mobile phase consisted of acetonitrile and water, both acidified with 0.1 % formic acid. The gradient elution is shown below.

Total runtime was 12.5 minutes. Flow-rate was set to 0.3 ml/min, the injection volume was 10µL, and detection wavelength was 228 nm. For identification of cannabinoids, full UV-spectra were recorded in the range of 200-400nm. All experiments were carried out at a column temperature of 30°C.

UPLC gradient:

t(min.)	% water	% acetonitrile
0.0	30	70
6.0	30	70
10.5	0	100
10.7	0	100
11.0	30	70
12.5	30	70

Processing of the data

The primary result of the UPLC analysis is the peak area at 228nm for each cannabinoid analyzed. In the experiment performed with herbal cannabis, the following cannabinoids were determined:

CBG	cannabigerol
THCV	tetrahyd rocannabivarin
CBN	cannabinol
THC	tetrahydrocannabinol
CBC	cannabichromene
THCA	tetrahydrocannabinolic acid

Other cannabinoids were below the treshhold of detection. In the experiments with pure THC, only the peak for THC was analyzed. In a previous study it was already shown that THC does not degra de during evaporation with the Volcano (Hazekamp 2006a).

Data for each cannabinoid analyzed was evaluated in the following ways:

- 1) calculating the *overall* mean and standard deviation of all measurements over 14 days (70 data points). See **Figure 1**
- 2) calculating the *daily* mean and standard deviation for each day seperately (5 data points). See **Figure 2**
- 3) calculating the difference between daily mean and full mean. This will show the deviation (in %) of *daily* mean from the *overall* mean. See **Figure 3**

Results

Representative UPLC chromatograms obtained during study of herbal cannabis and pure THC are shown in appendix 1. Full integration data is shown in appendix 2. All original data (chromatograms and integration data) is available from Prisma BV.

Figure 1: Overall mean and standard deviation of all measurements, per cannabinoid analyzed (70 datapoints over 14 days).

Cannabinoid	Mean peak area (UPLC 228nm)	RSD(%)
<i>Herbal Cannabis in the Volcano</i>		
CBG	197473	11.6
THCV	72173	17.4
CBN	132791	11.3
THC	817561	7.6
CBC	125463	8.4
THCA	208406	12.6
<i>THC in the Volcano</i>		
THC	704329	13.5

Figure 2: Daily mean and standard deviation, per cannabinoid analyzed (5 data points per day)

day	mean	179984	63794	136411	786827	130112	181597
	stdev	41186	13134	15114	56191	11363	31168
	RSD	22.9	20.6	11.1	7.1	8.7	17.2
2	mean	199611	83883	135504	755718	129817	188638
	stdev	23317	16443	10756	72932	9820	41343
	RSD	11.7	19.6	7.9	9.7	7.6	21.9
3	mean	206548	82945	131835	816142	131101	200564
	stdev	10877	18484	16979	56678	7579	6594
	RSD	5.3	22.3	12.9	6.9	5.8	3.3
4	mean	181790	67865	128719	731164	117314	175603
	stdev	15669	7096	16622	70501	9328	28271
	RSD	8.6	10.5	12.9	9.6	8.0	16.1
5	mean	195308	73825	136416	809869	128821	205624
	stdev	18957	17191	10682	47294	14658	11870
	RSD	9.7	23.3	7.8	5.8	11.4	5.8
6	mean	193336	71367	136686	810185	125731	199168
	stdev	16304	5199	9014	40061	9183	7259
	RSD	8.4	7.3	6.6	4.9	7.3	3.6
7	mean	198807	77534	155630	832000	124846	210555
	stdev	8505	12026	13371	32865	9746	11720
	RSD	4.3	15.5	8.6	4.0	7.8	5.6
		CBG	THCV	CBN	THC	CBC	THCA

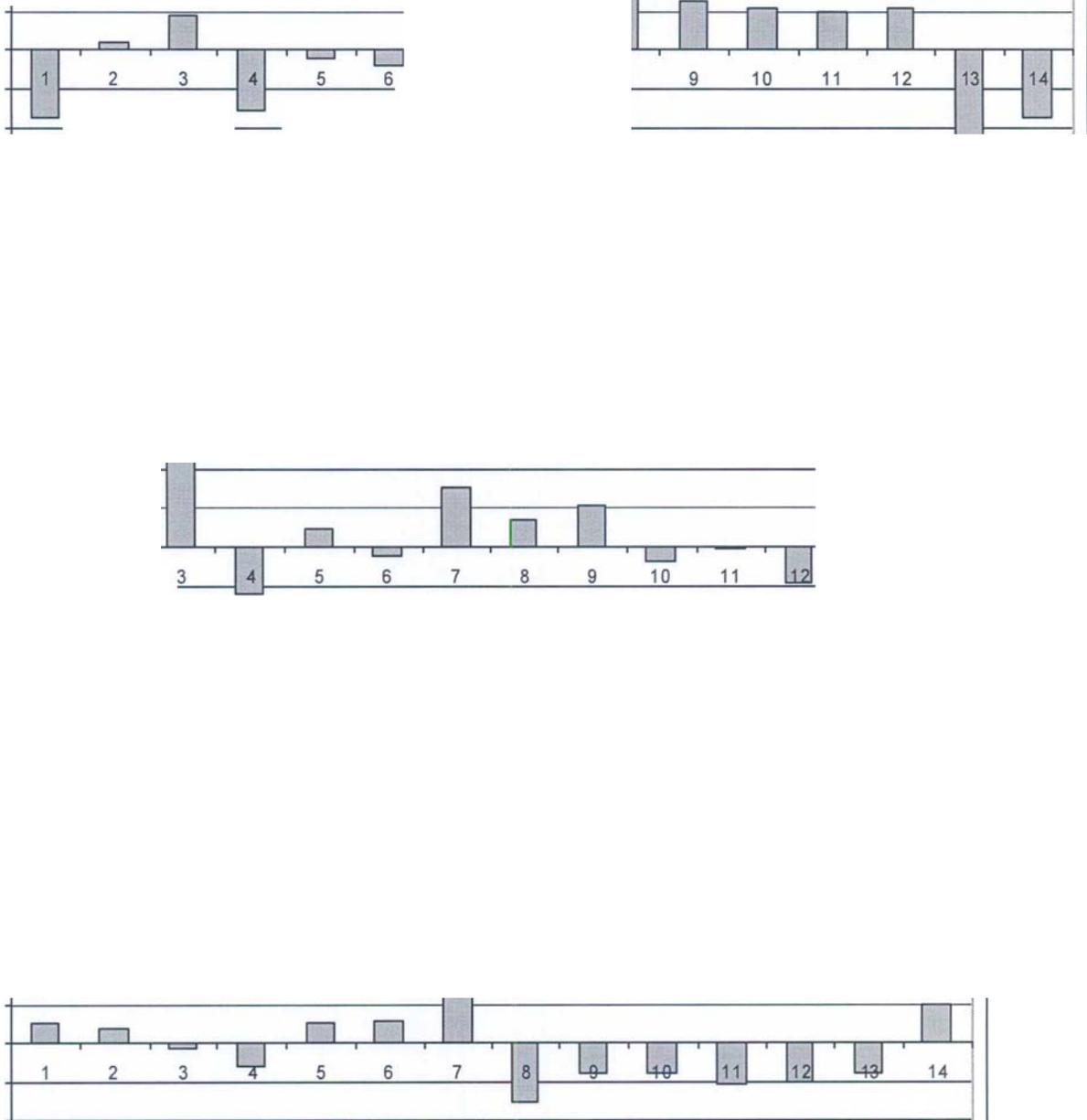
day	8	mean	218963	74700	122570	849749	131395	219171
		stdev	20419	12584	5430	54650	9132	22172
		RSD	9.3	16.8	4.4	6.4	7.0	10.1
9	mean	210007	75996	127593	831169	125817	232945	
	stdev	16523	5262	12677	46695	5619	5881	
	RSD	7.9	6.9	9.9	5.6	4.5	2.5	
10	mean	208133	70867	127691	827149	120099	206603	
	stdev	24596	9108	24078	75889	13841	36780	
	RSD	11.8	12.9	18.9	9.2	11.5	17.8	
11	mean	207317	71979	125800	871495	128436	224299	
	stdev	23767	14106	15496	67698	12941	18779	
	RSD	11.5	19.6	12.3	7.8	10.1	8.4	
12	mean	208003	68850	126035	879232	127475	236885	
	stdev	14411	8889	9638	21600	9365	8100	
	RSD	6.9	12.9	7.6	2.5	7.3	3.4	
13	mean	171650	62774	127795	826110	119654	220217	
	stdev	17700	7038	16290	19354	6092	5832	
	RSD	10.3	11.2	12.7	2.3	5.1	2.6	
14	mean	179995	60490	139392	820751	114704	218175	
	stdev	14875	7375	9653	39468	7400	14170	
	RSD	8.3	12.2	6.9	4.8	6.5	6.5	

CBG	THCV	CBN	THC	CBC	THCA
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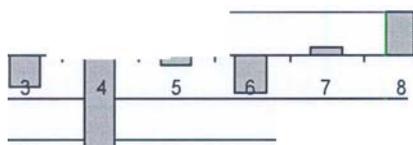
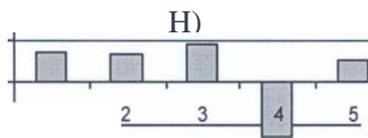
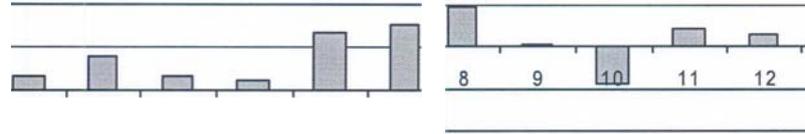
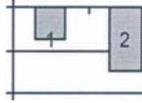
Figure 2: continued*2b: The in the Volcano*

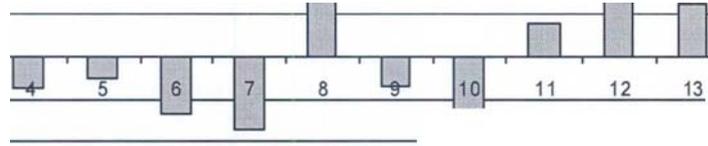
day		THC	day		THC
	mean	756368	8	mean	768188
	stdev	96097		stdev	95593
	RSD	12.7		RSD	12.4
2	mean	704472	9	mean	680706
	stdev	82186		stdev	38260
	RSD	11.7		RSD	5.6
3	mean	712575	10	mean	623547
	stdev	125741		stdev	43279
	RSD	17.6		RSD	6.9
4	mean	678271	11	mean	732098
	stdev	96593		stdev	130814
	RSD	14.2		RSD	17.9
5	mean	686708	12	mean	770340
	stdev	109137		stdev	104555
	RSD	15.9		RSD	13.6
6	mean	656928	13	mean	746866
	stdev	51902		stdev	85675
	RSD	7.9		RSD	11.5
7	mean	643870	14	mean	699667
	stdev	90928		stdev	111028
	RSD	14.1		RSD	15.9

Figure 3: plots showing deviation (in%) of *daily* mean (5 measurements in a single day) from *overall* mean (70 measurements over 14 days)



G)





Discussion & Conclusion

This study was performed to answer the question if it is possible to keep re-using a Volcano balloon for an extended period of time, without causing a significant change in the composition of the vapor present inside the balloon. A single balloon was filled for 14 days, with 5 fillings each day, resulting in a total number of 70 fillings. The experiment was performed with herbal cannabis (medicinal grade) as well as with purified delta-9-tetrahydrocannabinol (THC), the main biologically active component of cannabis.

The conditions for the analysis of cannabinoids in the vapor of the Volcano balloon have been optimized in previous studies (Hazekamp 2006a, Zuurman 2008). It was shown that cannabinoids are fully retained by the glass fiber filter used. During extraction of the filter the recovery of cannabinoids is 95 -100%. The vapor extract is therefore fully representative of the vapor originally present in the balloon. In other words: the composition of vapor in the balloon and of vapor extract can be considered equal.

Previous study on the vaporizing of pure THC has indicated that the average variability (relative standard deviation, RSD) of the THC concentration in the balloon is about 15%. Those results were obtained with a fresh balloon for each measurement (Hazekamp 2006a, Zuurman 2008). In the current study, the maximum RSD found for THC was 7.6% (cannabis in Volcano) and 13.5% (THC in Volcano), as can be seen in figure 1. It seems that the use of a 'dirty' balloon does not influence the variability of the concentration in the balloon of the major cannabinoid THC.

The other cannabinoids show a variability ranging from 8.4% (CBC) to 17.4% (THCV). It should be noted that THCV was the smallest UPLC peak analyzed in this study, explaining the relatively larger RSD.

Another way to evaluate the data is to determine the difference between daily values and overall mean obtained for the whole study. In this way it can be visualized what the deviation is each day from the long term (14 days) average. Figure 3 shows that the maximum deviation from the long-term average ranges from -11.5% (day 10) to +9.4% (day 12) when pure THC is used, and from -16.2% (THCV, day 14) to + 17.2 (CBN, day 7) when herbal cannabis was used.

N.B.: Results in this report are *qualitative* only, based on the peak areas of UPLC analysis. The data has not been converted into *quantitative* data, to express the values in absolute values such as milligrams. However, the proper controls and standards have been analyzed to convert data if needed.

References

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OMC: Office of Medicinal Cannabis, the Netherlands (2009) Official website: www.cannabisbureau.nl. Website visited 26 March 2009

Zuurman L, Roy C, Schoemaker R, Hazekamp A, den Hartigh J, Bender JCME, Verpoorte R, Piquier JL, Cohen AF, van Gerven JMA (2008) Effect of intrapulmonary THC administration in humans. *J. Psychopharmacol.* 22(7): 707-716



SAMPLE

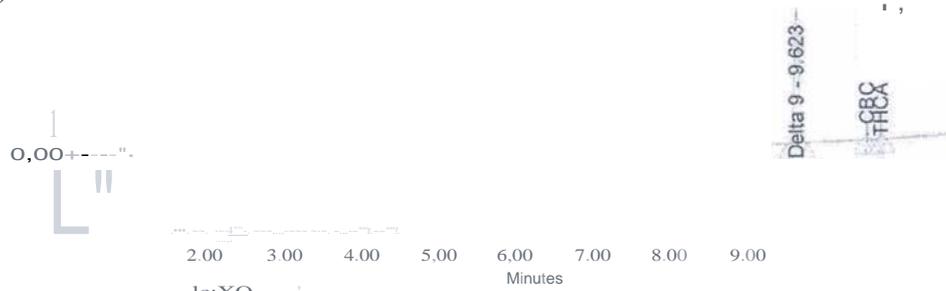
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Sample Set Name: Cannabinoids full loop Bed ropuur
Acq. Method Set: Acids
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Channel Name: PDA 228.0nm
Proc. Cnnl. Oeser.:

week1-1.2
Unknown
1:A,5
1
Sample Name: 20.00 ul
Sample Type: 12,5 Minutes
Vial:
Injection #:
Injection Volume:
Run lime:

Sytze

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Date Processed: 2/11/2009 12:06:22 PM CET

L)



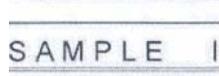
M)

Peak Name	RT	Area	Height	Amount	Units
5	THCV	6.289	60170	6907	
6	THCVA (?)	8.194			
7	CBN	8.630	145876	23243	
8	Delta 9	9.623	3290242	788722	
9	Delta 8	9.708			
10	CBNA	9.772			
11	Can. Acid. unk 1	10.228			
12	CBC	10.525	132561	42539	
13	THCA	10.708	177851	66801	
14	CBCA	11.099			

Report Method 10 9844
Page: 1 of 1

T4~75;

2 CeGA 5.054
3 CeG 5.369



Acquired By:
 Sample Set Name:
 Acq. Method Sel:
 Processing Method:
 Channel Name:
 Proc. ChnL Oescr.:

01

N)

week1-1.1
 Unknown
 1:A,4
 1
 5.00 ul
 12.5 Minutes

Sample Name:
 Sample Type:
 Vial:
 Injection #:
 Injection Volume:
 Run Time:

Sytze
 Amo Volcano test The week1
 Cannabinoids 290806 Bedropuur
 Acids
 228 nm
 DA 228.0 nm

0

Date Acquired: 3/11/2009 3:49:06 PM GET
 Date Processed: 3/24/2009 2:14:42 PM CET

6.00 700
 Minutes

O)

Name	RT	Area
1	9.833	748189

Peak Results
 Height, Amount Units 233227

Day	repeat	CBG	THCV	CBN	THC	CBC	THCA
1	1	107956	*	129364	698437	117080	131640
	2	194357	60170	145876	803358	132561	177851
	3	185480	48820	113723	770593	126861	184490
	4	206208	65773	141339	816737	126205	200809
	5	205918	80414	151754	845011	147854	213196
2	1	212363	89836	135961	769393	131891	197648
	2	199910	87177	134491	767991	132868	204455
	3	213517	78850	144562	821843	130859	207476
	4	213108	104147	144356	788191	140001	217782
	5	159158	59404	118150	631173	113467	115827
3	1	197852	93906	123726	834652	132279	200180
	2	210583	100585	131936	798573	134530	200826
	3	198032	93791	138224	745994	117756	195639
	4	223671	58764	155506	900157	135050	211356
	5	202601	67680	109785	801335	135892	194819
4	1	203316	78490	125468	774767	122472	192618
	2	188731	59618	154859	753368	113308	197510
	3	162645	68465	108980	674778	120005	128961
	4	171629	63572	124253	642004	103334	168919
	5	182631	69181	130034	810905	127450	190006
5	1	212422	90056	133809	842068	132031	214304
	2	162838	66302	122534	740409	109453	192854
	3	202691	94539	135434	862201	149880	217122
	4	197878	58790	152339	813129	123324	192821
	5	200711	59438	137964	791539	129416	211018
6	1	185642	64188	138358	801018	126888	195699
	2	174867	70980	147010	815963	130497	203402
	3	218940	68984	139542	874879	136722	209592
	4	191718	75542	122289	768629	122242	191391
	5	195512	77139	136230	790438	112304	195755
7	1	211635	66215	139261	851734	137211	200164
	2	198712	75067	172465	833806	122282	204413
	3	192999	75975	161581	834691	130272	223252
	4	201138	72477	159762	862470	123359	223318
	5	189553	97937	145081	777299	111105	201630

Day	repeat	CBG	THCV	CBN	THC	CBC	THCA
8	1	231749	86554	128158	867547	124797	228030
	2	231958	83137	127154	884514	135974	224269
	3	183786	55127	121450	753877	119101	180263
	4	218475	69960	121470	859323	141336	227357
	5	228847	78723	114618	883482	135767	235934
9	1	197880	69892	128510	840184	124891	226471
	2	233998	79220	120905	900178	128312	233803
	3	197056	83230	110579	818275	117255	228879
	4	220577	74615	143897	826808	132588	241803
	5	200522	73022	134074	770400	126041	233771
10	1	191456	68068	114758	855783	128161	222572
	2	231463	72291	108310	857796	135445	227805
	3	202811	67209	151113	808509	108119	215962
	4	235471	61219	156509	907090	125720	225389
	5	179466	85548	107764	706569	103048	141287
11	1	243356	89447	147642	976961	146638	245545
	2	186147	71749	113716	811777	117314	229651
	3	187890	51019	108314	826556	120528	208403
	4	201701	78554	130602	899576	137569	236565
	5	217490	69125	128727	842604	120133	201332
12	1	202188	81194	120816	892276	131034	226008
	2	203267	70679	132343	871813	139951	232573
	3	216958	72016	132470	900556	129859	242362
	4	190186	60091	111438	845470	120156	236820
	5	227414	60271	133110	886043	116373	246660
13	1	*	*	*	*	*	*
	2	161309	69940	119240	828330	121504	226168
	3	152042	54987	115871	825083	114626	215802
	4	186621	67352	124412	801880	115014	214656
	5	186627	58817	151655	849146	127471	224241
14	1	179990	54529	132403	821579	109384	221610
	2	167364	60421	135644	826441	119111	224014
	3	166928	54440	130096	776969	104594	197701
	4	203440	60510	152781	881730	122255	235509
	5	182252	72549	146036	797035	118178	212042

Dav	repeat	THC
1	1	748189
	2	851651
	3	607106
	4	745129
	5	829766
2	1	760750
	2	688054
	3	595126
	4	807076
	5	671352
3	1	677989
	2	632987
	3	889365
	4	575207
	5	787328
4	1	710786
	2	590830
	3	691514
	4	581695
	5	816529
5	1	823883
	2	729890
	3	727663
	4	556117
	5	595986
6	1	582180
	2	628444
	3	673774
	4	713563
	5	686680
7	1	631475
	2	622762
	3	794034
	4	625028
	5	546053

Day	repeat	THC
8	1	773416
	2	633603
	3	890494
	4	813112
	5	730315
9	1	637987
	2	676200
	3	677189
	4	742891
	5	669265
10	1	672495
	2	601775
	3	642287
	4	560483
	5	640695
11	1	729848
	2	811091
	3	860259
	4	740971
	5	518321
12	1	690956
	2	711909
	3	681185
	4	896172
	5	871477
13	1	653810
	2	780067
	3	832425
	4	656722
	5	811307
14	1	584609
	2	867324
	3	658247
	4	749682
	5	638474