The Volcano® Medic cannabis vaporizer: Effect of reported hygienic reprocessing of the mouthpiece

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Introduction:

The Volcano® vaporizer by Storz & Bickel GmbH (Tuttlinge, 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 application 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 an extract.

For the specific use in combination with medicinally used cannabis, the Volcano Medic is currently under development by Storz & Bickel. For medicinal use by patients with an infectious disease, a mouthpiece was developed with a one-way valve. This mouthpiece allows air to be drawn out of the balloon (inhalation of the vapor), but prevents the blowing of air into the balloon. In this way, no contaminated (microbes) air can contaminate the inside of the balloon, allowing the re-use of the same balloon for at least 2 weeks. Used mouthpieces can be cleaned and disinfected by means of a process called hygienic reprocessing, which includes a cleaning, disinfection, and sterilizing step. In this report, this treatment will be shortly referred to a *sterilizing*. In this way a single mouthpiece can be re-used many times, reducing the costs of the use of the Volcano Medic.

Objective of this study:

The content of the balloon (vapor) has to pass through the mouthpiece of the balloon when it is inhaled by the user. However, the repeated sterilizing treatment of the mouthpiece potentially may after the physical properties of the materials the mouthpiece is made of. Consequently the question may arise whether the mouthpiece after repeated sterilizing has a different interaction with the vapor. If this is the case, the profile of inhaled components (cannabinoids) may change over time.

The objective of this study was to compare the vapor extracted from a series of balloons fitted with untreated mouthpieces, versus similar balloons fitted with several stressed (50x sterilized) mouthpieces. It is expected that no significant differences can be observed in the cannabinoids profiles of the resulting vapor extracts.

Experimental

Chemicals and Solvents

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

Cannabis plant material

Cannabis plant material (female flowertops, variety 'Bedrocan®) was medical grade and obtained from Bedrocan BV (Veendam, The Netherlands). Batch number was (AI) 01.87.011208 and harvest date was March 2, 2009.
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, supercised by the Office of Medicinal Cannabis (OMC). After harvest, the plant material was air-dried in the dark under constant temperature and humidity for I 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, 6 grams of cannabis was grinded (in small portions) with the Storz & Bickel Grinder, as delivered with the Volcano device, and homogenized by mixing with a spoon. Material was used immediately without storage.

Treatment of the mouthpieces

Three original mouthpieces with one-way valve were used without any treatment (controls, mouthpiece #1-3).
Three other mouthpieces were submitted to repeated sterilizing by means of hygienic reprocessing (cleaning, disinfection, sterilizing) according to hospital standards. This procedure was repeated 50 times in order to severely stress the mouthpieces, representing a worst-case scenario for the repeated use of a single mouthpiece (treated, mouthpiece #4-6).

Filling of the balloons

In total 18 balloons were filled with vapor and extracted to analyze the vapor composition (see below). For each experiment 250 mg grinded material was accurately (+/-2 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 according to the instructions of the manufacturer. The balloon was then removed from the filling chamber and the vapor was extracted within two minutes.
For each experiment, fresh cannabis was used, and the filling chamber was cleaned with ethanol. Filling chambers were allowed to dry before starting the next experiment. The same balloon was used throughout the whole study. Previous results have shown that the same balloon can be used at least 70 times without significant effect on the cannabinoid composition of the vapor.

Preparation of vapor extracts

Each of the mouthpieces was used to empty three separate balloons in order to determine a mean value based in three measurements. Consequently, 18 balloons were extracted:

- 3 control mouthpieces (#1-3) x 3 replicates = 9 control extract
- 3 treated mouthpieces (#4-6) x 3 replicated = 9 treated 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 vacuumpump, the vapor was aspired 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 50 ml. These final vapor extracts were stored at -20°c until analysis.

The conditions mentioned above for the analysis of cannabinoids in the vapor of the Volcano balloon have been optimized in precious studies (Hazekamp 2006a, Zuurman 2008). It was shown that the profile of cannabinoids analyzed in the vapor extract is identical to the composition of the vapor present in the balloon (accuracy ca. 95%).

Cannabinoid analysis by UPLC

Quantitative analysis was performed with a Waters Acuity 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 ethanolic samples (vapor extracts) were diluted 4 times in the mobile phase of the UPLC method (acetonitrile/water 70:30, + 0.1% formic acid), and analyzed by UPLC to determine the cannabinoid composition. 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 449). Equipment control, data acquisition and integration were performed with Water Empower 2 software. Chromatographic separation was achieved using a Waters C_{18} analytical column (1.7 µm, 2.1 x 150 mm) protected by a Waters C_{18} 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 200-400 nm. 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 228 nm for each cannabinoid analyzed. The following cannabinoids were determined:

CBG	cannabigerol
CBN	cannabinol
THC	delta-9-tetrahydrocannabinol
D8-THC	delta-8-tetrahydrocannabol
CBC	cannabichromene
THCA	tetrahydrocannabinolic acid

Other cannabinoids were below the treshhold of detection. In a previous study it was already shown that THC dose not degrade during evaporation with the Volcano (Hazekamp 2006a). Indeed, no CBN or delta-8-THC 9 degradation products of THC) were detected in any of the samples.

A representative UPLC chromatogram of one of the vapor extracts analyzed in this study is shown in appendix I.

Results

The exact weight of the cannabis samples (target weight: 250 mg) in each balloon filling is shown in figure 1. UPLC integration data, corrected for variation sin sample weight is shown in figure 2.

All original data (chromatograms and integration data) is available form LabAssistent.

Figure 1: exact weight of cannabis used in each experiment

#1-3: control mouthpieces
#4-6: repeatedly sterilized mouthpieces

mouthpiece #	replicate	mg cannabis weighed
1	a	249
	b	248
	c	250
2	a	250
	b	249
	c	251
3	a	249
	b	250
	c	252
4	a	248
	b	252
	c	250
5	a	250
	b	251
	c	251
6	a	248
	b	250
	c	251

Figure 2: UPLC peak integration data after correction for variation in cannabis sample weight (figure 1). The cannabinoids CBN and delta-8-THC were not detected in any of the samples. Numbers indicate peak area at 228 nm, and have no dimension.

#1-3: control mouthpieces
#4-6: repeatedly sterilized mouthpieces

Cannabinoid analyzed

mouthpiece #	replicate	CBG	THC	CBC	THCA
	a	88138	1580500	50878	142139
	b	88656	1476765	50338	102275
1	c	70303	1155810	40341	90366
	a	81928	1693413	53463	183153
	b	85234	1364817	36224	191550
2	c	93509	1565044	51905	131538
	a	86136	1655024	52169	166741
	b	93400	1583823	53249	144228
3	c	90864	1721970	53399	136648
	mean	**86463**	**1533019**	**49107**	**143182**
	stdev	7142	178857	6317	33793
	RSD (%)	8.3	11.7	12.9	23.6
	a	74547	1456484	56900	149671
	b	67075	1448829	54813	154006
4	c	76560	1520999	49425	164420
	a	79482	1595517	49718	177606
	b	57360	979695	29437	112870
5	c	91978	1581159	58393	172696
	a	88505	1487735	43143	136836
	b	92366	1706611	48113	159367
6	c	85435	1770616	59505	177648
	mean	**79257**	**1505294**	**49939**	**156124**
	stdev	11804	225610	9390	21118
	RSD (%)	14.9	15.0	18.8	13.5

Figure 3: bar-diagram presentation of the data in figure 2. Errorbars indicate standard error (n=9).

DIAGRAMM!

Discussion & Conclusion

This study was performed to determine whether repeated (50x) sterilizing of the newly developed Volcano Medic mouthpiece with one-way valve has an effect on the profile of cannabinoids in the inhaled vapor. Nine balloons were empties through non-treated mouthpieces, while 9 other balloons were empties through severely stressed mouthpieces. Finally, the co9mposition of the resulting vapor extracts were compared by UPLC analysis of the major cannabinoids present.

The results clearly indicate there is no significant difference in vapor cannabinoid profile between untreated and treated mouthpieces. It can be concluded that the Volcano Medic mouthpiece with one-way valve can be cleaned for re-use by hygienic reprocessing treatment at least 50 times without consequences.

It is interesting to note that previous study on the vaporizing of pure THC and herbal cannabis has indicated that the average variability (relative standard deviation, RSD) of the THC concentration in the vapor is about 15 %. In this current study, the RSD of the THC content in the vapor was again found to be in the same range (11.7 – 15.0 %), further showing the consistent performance of the Volcano Vaporizer when used in combination with medicinal cannabis.

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.

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APPENDICES!!