Solvent-Free Terpene & Cannabinoid Profiling of Cannabis Consumer Products using Vacuum Assisted Sorbent Extraction (VASE) Thermal Desorption-GC-MS

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Overview

Due to recent legislative changes, consumption of cannabis products are becoming accepted as a medical treatment and a mainstream recreational product worldwide including many US states and Canada.

Reliable analytical methods are critical to ensuring consistent dosing, efficacy, and safety.

Current techniques include liquid-liquid extraction followed by LC or GC-MS analyses, which generate solvent waste and require multiple laborious steps.

Vacuum Assisted Sorbent Extraction-Thermal Desorption (VASE-TD) is a solvent-free headspace extraction and sample introduction technique developed for GC-MS analyses.

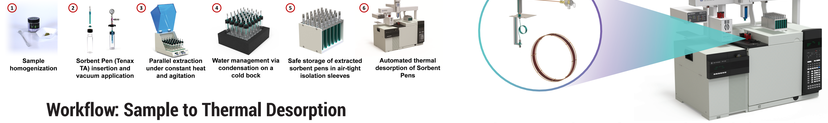
Results demonstrate that VASE-TD-GC-MS offers a quick and sensitive approach for both qualitative and quantitative determination of terpene and cannabinoid profiles of cannabis flower and cannabis infused products. Full profiles were extracted using VASE in as little as 1 minute to distinguish different strains with cannabinoid ratios consistent with the manufacturer labels.

Introduction

Vacuum Assisted Sorbent Extraction (VASE)

- Solvent-free headspace extraction and sample introduction technique for GC-MS.
- Performs extraction of full range VOCs to SVOCs (boiling point range (-50°C to over 500°C) from liquid, solid, or gas samples.
- In-vial sampling convenience of SPME with larger sorbent phase and durability of thermal desorption tubes.
- Sample extracts much faster under vacuum than while at atmospheric pressure, enhancing recovery of low volatility compounds.
- Not in contact with matrix, fewer artifacts created and longer sorbent life time.
- Cost effective - Sorbent Pens are re-usable throughtout the life of the sorbent.
- Desorptions can be performed manually or automatically.

Figure 1 - View of the Headspace Sorbent Pen (HSP) inserted into a vial for extraction and a schematic of the internal sorbent bed, which can be packed with a variety of sorbents including multi-component beds of varying physical properties.



Workflow: Sample to Thermal Desorption

1. A sample is placed inside a glass vial containing a Silonite coated stainless steel tube packed with an sorbent (e.g. Tenax TA) called a Sorbent Pen.
2. The vial containing the sample is placed under vacuum and analytes collect on the sorbent bed with or without the addition of heat and/or agitation (minutes – 24 hours).
3. After the analytes are collected on the sorbent, the Sorbent Pen is removed from the vial and placed into an isolation sleeve for up to one week.
4. Following VASE, the Sorbent Pens are subjected to TD-GC-MS using a custom designed injection port called the Sorbent Pen Thermal Desorption Unit (SPDU).

Sorbent Pen Thermal Desorption Unit (SPDU)

- The SPDU is integrated into the GC, allowing analytes to travel directly from the Sorbent Pen onto the column minimizing loss and/or carryover.
- A dual column set-up in the GC oven accommodates split or split/ splitless injection.
- VASE is conducted off-line while the transfer and return of Sorbent Pens from their isolation sleeves in the tray to SPDU is automated by the SPR (Sample Preparation Rail).

Dual-Column Set-Up for Split VOC to SVOC and Trace SVOC Analysis

Split Procedure: VOCs to SVOCs

Figure 2 - Hardware configuration for split injection of VOCs to SVOCs.
Column 1: 0.6m x 1mm ID (Silonite coated) expansion loop
Column 2: 30-60m x 0.25mm ID, 0.25 otO0.5µm

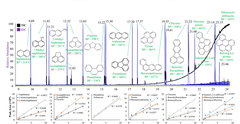
Trace Procedure: Splitless SVOC Analysis

Figure 3 - Hardware configuration for split injection of VOCs to SVOCs.
Column 1: 2-7m x 0.53mm ID, 0.25µm
Column 2: 30-60m x 0.25mm ID, 0.25 to 0.5µm

1. **Preheat –** Compounds concentrated on the sorbent bed are prepared for desorption by elevating the temperature to just below boiling point (BP) of the most volatile target analyte under no flow.
2. **Desorption –** Analytes are desorbed onto Column 1 (filmless expansion loop) by elevating the temperature and allowing the GC carrier gas to flow through the Sorbent Pen. Desorption temperatures are set above or near the BP for least volatile compound i (< sorbent max temp). Two stage desorption can be used to accommodate multi-analyte desorptions where certain analytes may be thermally liable while others require elevated temperatures become released from the sorbent. For the **Trace Procedure configuration**, a thin filmed column is installed as Column 1 which traps SVOCs during desorption as VOCs flow through unretained and become split before entering Column 2 (analytical column).
3. **Bake-out –** Cleans the sorbent by elevating the temperature with flow through the Sorbent Pen and out Split 1. This step allows the Sorbent Pen to ready for the next extraction directly following the GC run without additional conditioning. The Bake-out temperature is usually set ~10-20°C above desorption temperature (< sorbent max temp). For the **Trace Procedure configuration**, the valve configuration is different in that a valve remains closed to ensure SVOCs can travel splitlessly from Column 1 to Column 2 as the oven temperature ramps up.

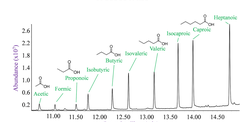
VASE: A Robust Headspace Extraction Approach for a Wide Range of Applications

Environmental



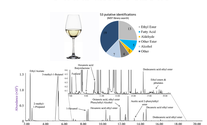
Sample: 0.5 – 10 ppb PAH mix in 10 mL 5% Na2SO4
VASE: Tenax TA (35/60) HSP, 16 h, 55°C, 150 rpm
TD: 60 sec 200°C Preheat, 3 min 325°C Desorb, 18 min 300°C Bake-out
GC-MS: DB-5MS (30m×0.25mm×0.5µm), Thermo TRACE 1310 ISQ

Clinical



Sample: 1mL water sample with a 10µM VFA mix (AccuStandard FAMQ-004)
VASE: Tenax TA (35/60) HSP, 16 h, 35°C, 250 rpm
TD: 120 sec 200°C Preheat, 3 min 260°C Desorb, 10 min 275°C Bake-out
GC-MS: DB-WAX (60m0.32mm0.5µm), 20:1 Split, Agilent 7890A/5975C

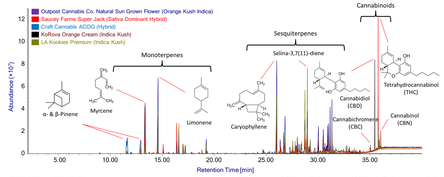
Food and Flavor



Sample: 500 µL sample, 4.5 mL 3% NaCl
VASE: Tenax TA (35/60) HSP, 16 h, 30°C, 200 rpm
TD: 60 sec 260°C Preheat, 0.5 min 300°C Desorb, 10 min 275°C Bake-out
GC-MS: DB-5MS (30m×0.25mm×0.5µm), Agilent 7890B/5977A

Method Development

Chemical Profiling of Cannabis Consumer Products



Samples: 6-8 mg flower
VASE: Tenax TA (35/60) HSP, 16 h, 70°C, 120 rpm
TD: 60 sec 175°C Preheat, 3 min 225°C Desorb, 30 min 300°C Bake-out
GC-MS: DB-5MS (30m×0.25mm×0.5µm), Agilent 7890B/5977A

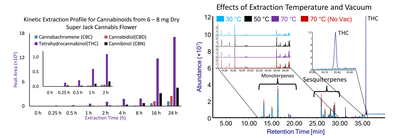
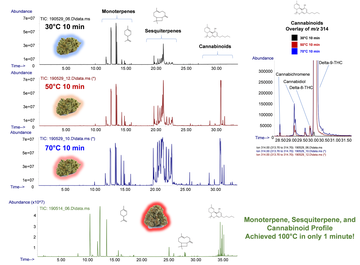


Figure 4 - Effects of extraction temperature, vacuum, and extraction time on terpene and cannabinoid profiles of dry cannabis flower. Shown are replicate measurements performed on ~6-8 mg of Saucey Farms Super Jack, which is a Sativa dominant hybrid. Both high temperatures (70°C) and vacuum are necessary for reliable extraction of cannabinoids, while terpenes are extractable under a wide range of conditions. Some terpenes (including carene) are visible immediately after vacuum application. All visible cannabinoids – including THC, CBD, cannabinol (CBN), and cannabachromene (CBC) – continue to increase in abundance for the full duration of the experiment (24 h).

- Both terpenes and cannabinoids can be analyzed from 6-8 mg of dry cannabis flower in a single experiment.
- The California Bureau of Cannabis Control dictates that a minimum 0.5 g of the representative sample needs to be analyzed to determine the terpenoid and cannabinoid sample profiles (Bureau of Cannabis Control).
- For a 16 h extraction, even small sample quantities of ~6-8 mg require a 200:1 split to avoid column saturation.
- Balancing the high sensitivity of VASE and the rich chemical makeup of cannabis is done by increasing the sample amount towards the target of 0.5 g and reducing the duration of the extraction.

Larger Sample Size Results in Full Terpene to Cannabinoid Profile in Minutes

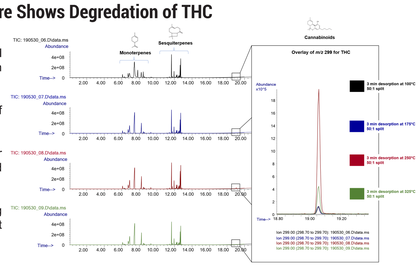


- Dry cannabis flower was homogenized and ~0.1 g was placed into each vial
- Tenax TA (35/60) Sorbent Pens were placed into the vials and subjected to vacuum pressure and placed on a hot plate for 10 minutes.
- Sorbent Pens were desorbed using the Trace Procedure for splitless analysis of SVOCs.
- Trace SVOC analysis resulted in detection of cannabinoids extracted in 10 minutes at 30, 50, and 70°C.
- Higher extraction temperatures resulted in a higher number and abundance of monoterpenes, sesquiterpenes, and cannabionoids.
- The combination of using a larger quantity of sample (0.1 g vs. 6-8 mg), a hot plate to provide heat (instead of convectional heating and agitation), and split/splitless injection of cannabinoids resulted in both terpene and cannabinoids extraction in minutes rather than hours.

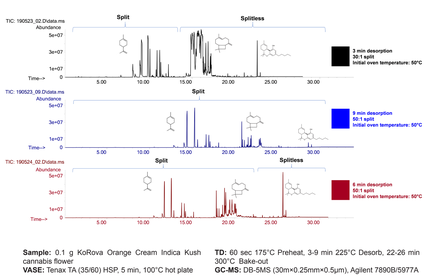
Monoterpene, Sesquiterpene, and Cannabinoid Profile Achieved 100°C in only 1 minute!

High Desorption Temperature Shows Degredation of THC

- In addition to the traditional GC-MS method parameters, thermal desorption requires method optimization conditions such as the duration and temperature of the pre-heat, desorption, and bake-out.
- In this study, 4 different temperatures were selected for desorption of both terpenes and cannabinoids.
- The lowest desorption temperature of 100°C showed the greatest number and abundance of monoterpenes, whereas higher temperatures resulted in similar terpene profiles.
- A 225°C desorption resulted in the highest abundance of THC, suggesting that 100°C and 175°C may not have been sufficient, whereas the highest temperature of 325°C may have caused thermal degradation.



Optimization of Trace Procedure Results in Splitless Analysis for Cannabinoids



Sample: 0.1 g KoRova Orange Cream Indica Kush cannabis flower
VASE: Tenax TA (35/60) HSP, 5 min, 100°C hot plate
TD: 60 sec 175°C Preheat, 3-9 min 225°C Desorb, 22-26 min 300°C Bake-out
GC-MS: DB-5MS (30m×0.25mm×0.5µm), Agilent 7890B/5977A

The aim of using the Trace Procedure for cannabis analysis is to split highly abundant volatile terpenes while allowing the cannabinoids to become trapped on Column 1 to enhance detection of these lower volatility compounds after a short extraction.

The point at which desorbed analytes become splitless is dependent upon the following factors:

1. Length of Column 1 (longer columns are more retentive).
2. Initial oven temperature during desorption (lower temperatures are more retentive).
3. Split ratio (lower flow rates are more retentive).

With an intial oven temperatures of 50°C, a 3 min desorption with a 30:1 split resulted in a profile of split monoterpenes and splitless sesquiterpenes and cannabinoids, while a 9 min desorption at a 50:1 split resulted in a split analysis of all three groups.

By reducing desorption time from 9 minutes to 6 minutes, split analysis was achieved for all terpenes, while the cannabinoids remained splitless.

Results

Terpene and Cannabinoid Profiling in minutes using Trace SVOC Procedure

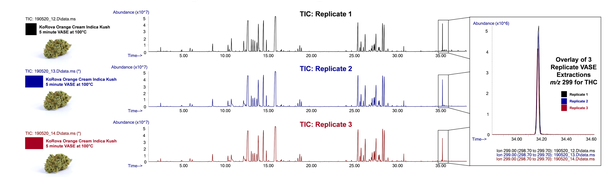


Figure 5 - Replicate sample preparations of ~ 0.1 g of KoRova Orange Cream Indica Kush cannabis flower extracted 100°C VASE for 5 minutes using unique Sorbent Pens. Similar monoterpene, sesquiterpene, and cannabinoid profiles are observed in the total ion chromatogram (TIC). A *m/z* 299 extracted ion chromatogram overlay of the 3 replicates shows similar amounts of THC were extracted in each preparation.

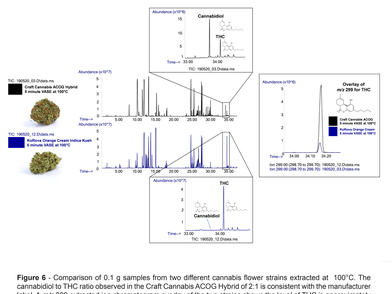


Figure 6 - Comparison of 0.1 g samples from two different cannabis flower strains extracted at 100°C. The cannabidiol to THC ratio observed in the Craft Cannabis ACOG Hybrid of 2:1 is consistent with the manufacturer label. A *m/z* 299 extracted ion chromatogram overlay of the two strains shows the level of THC is approximately 5 times higher in KoRova Orange Cream, which is also consistent with the manufacturer labels.

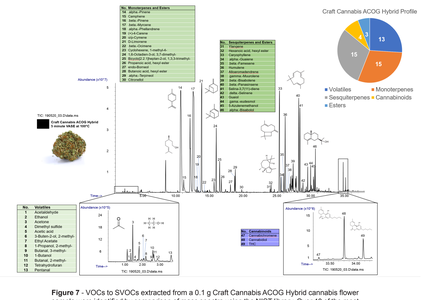
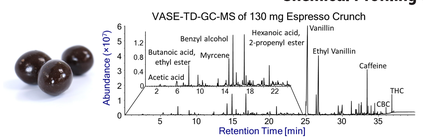
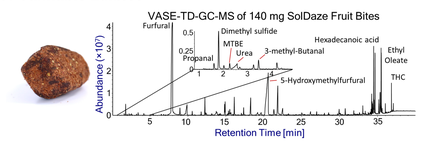


Figure 7 - VOCs to SVOCs extracted from a 0.1 g Craft Cannabis ACOG Hybrid cannabis flower sample were identified by comparison of mass spectra using the NIST library. Over 40 of the most abundant compounds were identified, including residual solvents, terpenes, and cannabinoids.

Chemical Profiling of Cannabis Edibles



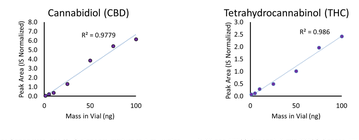
Sample: 130 mg espresso crunch chunks
VASE: Tenax TA (35/60) HSP, 16 h, 70°C, 120 rpm
TD: 60 sec 175°C Preheat, 3 min 225°C Desorb, 30 min 300°C Bake-out
GC-MS: DB-5MS (30m×0.25mm×0.5µm), Agilent 7890B/5977A



Sample: 140 mg fruit bite smudge
VASE: Tenax TA (35/60) HSP, 16 h, 70°C, 120 rpm
TD: 60 sec 175°C Preheat, 3 min 225°C Desorb, 30 min 300°C Bake-out
GC-MS: DB-5MS (30m×0.25mm×0.5µm), Agilent 7890B/5977A

Qualitative profiling of several cannabis infused edibles (e.g. fruit chews, chocolates, and beverages) demonstrates that VASE can be easily applied to detect short chain fatty acids (e.g. acetic acid), ethyl esters, residual solvents (e.g. MTBE), terpenes, cannabinoids, and flavor additives (e.g. vanillin and ethyl vanillin) in a single experiment. Our collective results demonstrate that VASE TD-GC-MS offers a simple and robust measurement approach for both qualitative and quantitative chemical profiling of cannabis and cannabis derived consumer products.

Quantitation of Cannabinoids



Sample: Cannabidiol and THC standard mixture in 1 mL water
VASE: Tenax TA (35/60) HSP, 16 h, 70°C, 120 rpm
TD: 60 sec 175°C Preheat, 3 min 225°C Desorb, 30 min 300°C Bake-out
GC-MS: DB-5MS (30m×0.25mm×0.5µm), Agilent 7890B/5977A

- VASE-TD-GC-MS calibration curves for THC and CBD extracted from water using a deuterated THC internal standard.
- Future work will involve developing a quantitative method for VASE-TD-GC-MS of cannabinoids directly from dry sample.

Conclusions and Future Work

A simple, solvent-free, and efficient method for profiling terpenes and cannabinoids in consumer products, including multiple strains of flower and several cannabis-infused edibles has been developed.

Our results show that VASE-TD-GC-MS can be used to determine terpene and cannabinoid compositions from different strains in a single extraction in under an hour total, with extraction lasting only minutes and a subsequent TC-GC-MS run of 37 minutes using the Trace Procedure for split/splitless SVOC analysis.

Future work will include developing methods using 0.5 g of cannabis to meet the California Bureau of Cannabis representative sampling requirement and a quantitative method for VASE-TD-GC-MS of cannabinoids directly from dry flower samples.

References

Article 5, Laboratory Testing and Reporting. Bureau of Cannabis Control Order of Adoption, Bureau of Cannabis Control Text of Regulations, California Code of Regulations Title 16, Division 42, (2018) 104-117.

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