# Vacuum-assisted headspace SPME with GC-MS for reliable and simultaneous determination of terpenoids and cannabinoids in *Cannabis sativa* L. inflorescence.

C. Cagliero, University of Turin, Italy

## ABSTRACT

*Cannabis sativa* L. has been exploited since ancient times for recreational, medical, textile and food purposes. The plant's most promising bioactive constituents belong to the terpenoid and cannabinoid classes. Their chemical characterization is crucial to guarantee the safe and efficient use of the plant. Vacuum assisted HS-SPME can be combined to fast GC-MS analysis to comprehensively characterize terpenoid and cannabinoid profiles in a single analysis. Vacuum in the headspace should be preferred over atmospheric pressure conditions as it ensures the fast recovery of cannabinoid markers at relatively lower sampling temperatures that do not discriminate the most volatile fraction nor cause the formation of artefacts.

## INTRODUCTION

*Cannabis sativa* L. is a very complex matrix, as it contains several classes of specialized metabolites, including more than 100 cannabinoids, 120 terpenoids and several flavonoids. Cannabinoids and terpenoids are mainly found in the inflorescences of the plant. Whether the plant is intended for recreational purposes, fiber production (hemp) or medical use, it depends on the content of two major cannabinoids: the psychoactive (-)-trans $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and the non-psychotropic cannabidiol (CBD). Varying pharmaceutical properties between different *Cannabis* varieties can be attributed to synergistic interactions between cannabinoids and terpenes. A comprehensive qualitative characterization of both the cannabinoid and terpene profiles of the plant raw material is therefore of utmost importance not only to define its rational use but also to guarantee the efficacy and safety of its potential pharmaceutical application.

The most common method for extraction of cannabinoids is solid-liquid extraction using organic solvents coupled to HPLC or GC combined with mass spectrometry. Organic solvents can also be used for the simultaneous extraction of terpenoids of the two classes of compounds, but the environmental impact of the resulting method can be large. The isolation mono- and sesquiterpenes, from plant raw materials can be performed by headspace solid-phase microextraction (HS-SPME) online combined to GC-MS analysis. Recovery of the semi-volatile cannabinoids from solid matrices by HS-SPME is also possible, but requires long sampling times due to their low volatility and low tendency to escape into the headspace. The use of high temperatures during sampling, especially when combined with relatively long extraction times, can also lead to decomposition of cannabinoids and the formation of artifacts. An alternative way to improve HS-SPME extraction efficiencies of semi-volatile compounds at mild temperatures is to sample the headspace under reduced pressure conditions using the vacuum-assisted HS-SPME (Vac-HS-SPME) approach.

In this application, a sensitive and reliable Vac-HS-SPME method is proposed for the simultaneous determination of terpenoids and cannabinoids in *Cannabis sativa* L. inflorescence. A comparative study between Vac-HS-SPME and regular HS-SPME was carried out to demonstrate the benefits of adopting the vacuum approach. Finally, the greenness and overall performance of the method was assessed using AGREEprep and RGB metric tools.

## EXPERIMENTAL

Table 1 and Table 2 describe the final optimized Vac-HS-SPME and standard HS-SPME methods, respectively. Table 3 gives details on the GC-MS method. Optimization was performed on dried fiber-type *Cannabis* (hemp) inflorescences.

*Table 1. Optimized Vac-HS-SPME method.*

| | |
|---|---|
| **Sample:** | 10 mg sample in 20 mL crimp top vial;<br>ExtraTECH Vac-closure (PN: 20-101) |
| **Air- evacuation** | 1 min before sample introduction,<br>pumping unit with 7 mbar ultimate vacuum |
| **SPME Fiber:** | PDMS/DVB 65 $\mu$m coating + 10 $\mu$m overcoating |
| **Incubation:** | 5 min, 90 °C, agitation |
| **Extraction:** | 5 min, 90 °C, agitation |

*Table 2. Optimized regular HS-SPME method.*

| | |
|---|---|
| **Sample:** | 10 mg sample in 20 mL crimp vial |
| **Fiber:** | PDMS/DVB 65 $\mu$m coating + 10 $\mu$m overcoating |
| **Incubation:** | 5min, 150 °C, agitation |
| **Extraction:** | 5 min, 150 °C, agitation |

*Table 3. GC-MS method.*

| | |
|---|---|
| **Column:** | MEGA-5 15 m × 0.18 mm $d_c$, 0.18 µm $d_f$ |
| **Oven:** | 50°C (30 s) //7.2°C/min// 250°C (2 min) |
| **Carrier Gas:** | Helium, 0.7mL / min constant flow |
| **Injection:** | Split, split ratio 1:20 |
| **Desorption:** | 10 min at 250 °C (injector temperature) |

## RESULTS

The two HS-SPME procedures necessitated the optimization of sampling temperature and time. Results showed that poor recovery of cannabinoids is observed when using Reg-HS-SPME at 90 °C. When sampling at higher temperature (150 °C), it is possible to observe a strong discrimination against the recovery of the more volatile markers (i.e., terpenoids). Moreover, a decomposition of cannabinoids and the formation of other components or artifacts is observed. In fact, by submitting a CBD standard HS-SPME for only 5 minutes at 150 °C, it is possible to observe that the compound is degraded forming cannabinoids, including cannabichromene (CBC), $\Delta^9$-THC and $\Delta^8$-THC. Thus, the Reg-HS-SPME method can provide unreliable and misleading results. Higher extraction efficiencies for semi-volatile compounds can be achieved in shorter sampling times and at milder extraction temperatures in Vac-HS-SPME. Indeed, it can be observed that when sampling at mild temperature (90 °C), regardless of the sampling time, the amount of CBD extracted with vacuum is several times higher than the amount obtained under regular conditions (Figure 1). In only 5 minutes, a sufficient amount of CBD could be extracted with vacuum to achieve an acceptable instrument sensitivity. Moreover, no degradation of CBD can be observed at this temperature.

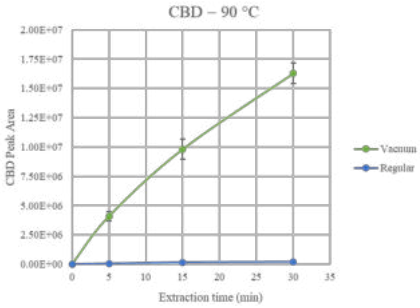

*Figure 1. Extraction time profiles for CBD obtained under Vac-HS-SPME (green profile) and Reg-HS-SPME (blue profile) conditions at 90 °C.*

### Analysis of *C. sativa* L. inflorescences

The analysis of fiber-type *Cannabis* inflorescences under vacuum conditions at 90°C for 5 minutes is reported in Figure 2. The GC-MS profile shows that it is possible to simultaneously recover both the volatile (i.e., mono and sesquiterpenes) and semi-volatile markers (i.e., cannabinoids) enabling to obtain a reliable picture of the sample in a very short sampling time.

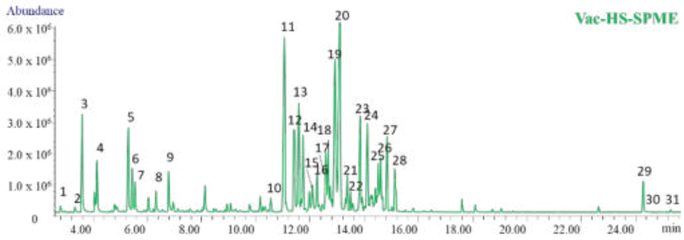

*Figure 2. GC-MS of fiber-type Cannabis sativa* L. *inflorescence after Vac-HS-SPME sampling. Legend: (1) α-Pinene, (2) β-Pinene, (3) β-Myrcene, (4) Limonene, (5) Linalool, (6) Fenchol, (7) cis-Pinene hydrate, (8) Borneol, (9) α-Terpineol, (10) β-Patchoulene, (11) trans- β-Caryophyllene, (12) trans-α-Bergamotene, (13) α-Humulene, (14) trans- β-Farnesene (15) β-Selinene, 16) α-Selinene17) α-Farnesene, (18-19) Sesquiterpenes (MW 204), 20) Selina-3,7(11)-diene, (21) trans-Nerolidol, (22) Caryophyllene oxide (23) Guaiol, (24) 10-epi- γ-Eudesmol, (25) β-Eudesmol, (26) α-Eudesmol, (27) Bulnesol, (28) α-Bisabolol, (29) Cannabidiol, (30) Cannabichromene, (31) Cannabinoid 2 (Supposed Δ9-THC).*

### Greenness and overall performance of the methods

The methods can be evaluated in terms of their greenness and overall performance. Among the available tools, the RGB model was chosen. In this model, the red color represents the analytical performance of the method, the green color represents its safety and greenness, and the blue color represents its productivity and practical effectiveness. An overall method score (called method brilliance) is calculated by combining the results of the three attributes. The comparison of the RGB results of the two HS-SPME methods and the reference solvent extraction method is shown in Figure 3 and highlights that the analytical performance of the Vac-HS-SPME method is comparable to the conventional method, while strong improvements are obtained in terms of greenness (measured with the AGREEprep metric tool) and practical efficiency, indicating that the method is the most reliable for a reliable characterization of *C. sativa* inflorescences.

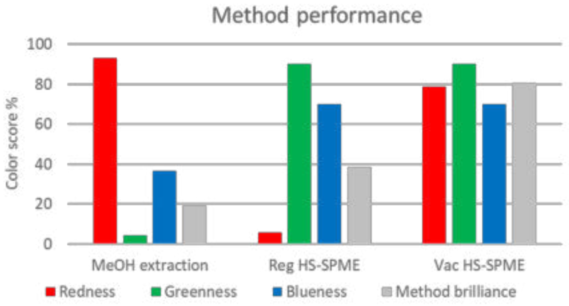

*Figure 3. Results of the overall performance of the method for the reference method consisting in methanol extraction, and Reg-HS-SPME and Vac-HS-SPME methods developed for the determination of terpenoids and cannabinoids from C. sativa* L. *inflorescences. Legend: red- analytical performance, green- greenness of the method determined with AGREEprep metric tool, blue – practical efficiency, method brilliance – overall performance.*

## CONCLUSIONS

The results proved that, compared to Reg-HS-SPME, vacuum conditions in the HS ensure the fast recovery of cannabinoid markers at considerably lower sampling temperature (i.e., 90°C) that do not discriminate the most volatile fraction nor cause the formation of artefacts when the sampling time is minimized. Overall, since the method is fast, totally automatable and solvent-free, the combination of Vac-HS-SPME and fast GC-MS should be considered as a green alternative analytical approach for the characterization of *Cannabis sativa* inflorescences.

**REFERENCE:** F. Capetti et al., Adv. Sample Prep. 2 (2022) 100014 (https://doi.org/10.1016/j.sampre.2022.100014)