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Factors influencing the recovery of Δ^9 -tetrahydrocannabinol, cannabidiol, and cannabinol from a breath sampling device

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E-mail: kavita.jeerage@nist.gov**Keywords:** cannabinoid extraction, drug recovery, impaction filter deviceSupplementary material for this article is available [online](#)

Abstract

Cannabinoids can be captured from breath after cannabis use, but sample processing varies between studies, even when using the same sampling device. The BreathExplor impaction filter device has been used to capture Δ^9 -tetrahydrocannabinol (THC) in breath after cannabis use in multiple studies, but processing differed in solvent type, keeper addition, and concentration method. In this study we test the effect that container material, vacuum concentration, keeper, elution solvent, cannabinoid mass, agitation, and elution time have on the recovery of THC, cannabidiol, and cannabinol from spiked breath samples. Only container material and keeper had a significant impact on recovery, although limiting the use of plastic vials, one of the two container materials studied, did have some mitigating effects. Despite these two factors having a significant effect, the recovery of cannabinoids remained relatively low. Further investigation showed that our process for the preparation of spiked breath samples, specifically the complete evaporation of a small volume of spike solvent, leads to large cannabinoid losses. As recently published high-accuracy vapor pressure data indicates that these cannabinoids should be considered semi-volatile, a microelution process was additionally explored as it requires less solvent and therefore no concentration is necessary during sample processing. The microelution process resulted in similar or higher recoveries of cannabinoids as compared to the large volume elution process, suggesting that it is a superior processing method in terms of recovery and processing time.

1. Introduction

Breath sampling is being investigated as a means to determine recent cannabis use based on the success, over many decades, of fieldable devices for the determination of alcohol impairment. While a fully fieldable solution would have many advantages, roadside sampling with subsequent forensic laboratory analysis is also under consideration. Published work in this field thus far has used liquid chromatography with tandem mass spectrometry (LC-MS/MS) to sensitively and specifically identify Δ^9 -tetrahydrocannabinol (THC) and other cannabinoids from breath samples collected under a

variety of circumstances. In these studies [1–10], the breath sampling devices have been designed to capture exhaled breath aerosols where THC is expected to be found, with the notable recent demonstration that THC can be recovered from exhaled breath condensate [11].

The BreathExplor impaction filter device, first introduced in 2018 [12], consists of three polypropylene filters housed within a polypropylene casing with a wide mouthpiece. This device has been used to capture THC after known cannabis use [8, 10], and has also been used to screen for a wider panel of drugs of abuse under circumstances where drug use was unknown [13, 14] or known from reported

prescription drug use [12, 15]. In these studies, the drug molecules were recovered by the addition of an eluting solvent (methanol) in a large volume that can easily wet all the surfaces. The bulk of the solvent was then removed through evaporation, which can be done at elevated temperatures.

While these studies all used elution followed by concentration, there are variations in processing that could impact drug molecule recovery. Seferaj *et al* 2018 [12] and Feltmann *et al* 2022 [13] first add internal standard and then elute with methanol. Maalouli Schaar *et al* 2024 [15] and De Jong *et al* 2025 [14] elute with methanol containing their internal standards, while Jeerage *et al* 2023 [8] and Sinapour *et al* 2024 [16] additionally include ethylene glycol as a keeper in their methanol. Maalouli Schaar *et al* 2024 [15] concentrate analytes with nitrogen evaporation, while Seferaj *et al* 2018 [12] and Feltmann *et al* 2022 [13] do not report their concentration process. Jeerage *et al* 2023 [8], Sinapour *et al* 2024 [16], and De Jong *et al* 2025 [14] use vacuum concentration but for varying time periods and temperatures. Different processing options would be expected to impact recovery of the studied drug molecules, and, when comparisons have been made, the drugs with the lowest recoveries in these studies were cannabinoids. De Jong *et al* 2025 [14] found that THC and cannabidiol (CBD) have recoveries from 20% to 40% for both a manual and robot-based procedure. Although the BreathExplor impaction filter device is the focus here, the older SensAbues electrostatic interception filter device also requires a similar elution plus concentration process when studying breath from cannabis users [1–3, 5–7, 9]. Studies with this device similarly show THC recoveries below 40% when reported [1, 17].

Recently published low uncertainty vapor pressure data for THC, CBD, and cannabinol (CBN) call into question the use of evaporation and elevated temperature [18]. The accuracy of the values measured at temperatures of 60 °C and higher enable the use of thermodynamic correlations to calculate vapor pressures at room and breath temperatures. Absorptive partitioning theory was then used to estimate the fraction of cannabinoids in the vapor phase, which is substantial. This suggests that some fraction of the cannabinoids could be lost to the vapor phase during sample processing. Herein, we examine the large volume elution process in detail and identify factors that influence the recovery of THC, CBD, and CBN. In this paper we use a replicated factorial design to test eight factors that could influence cannabinoid recovery after a large volume elution plus concentration process. We identify two important processing factors, but importantly, we also identify that our process for the preparation of spiked breath samples confounds the experimental design and compare recovery from an efficient microelution process.

2. Materials & methods

2.1. Chemicals and materials

Certified reference materials for THC, CBD, and CBN (three-component mixture) and individual deuterated internal standards (denoted by -d3); LCMS-grade solvents; octanol; and ethylene glycol were used as received. BreathExplor components including devices, filter transfer tools, and plastic elution vials were made from medical-grade polypropylene and were provided in kind by Munkplast AB, Inc.

2.2. Preparation of spiked filters

A known non-cannabis user added blank breath matrix to the filters by exhaling deeply 20 times through each device. Individual filters were pushed into their own container and any condensed water from exhaled breath was allowed to evaporate at room temperature for 16 h (overnight). Individual filters were spiked with 20 μ l aliquots (measured gravimetrically) of ethanol containing THC, CBD, and CBN and the solvent was allowed to evaporate at room temperature for 3 h. Complete evaporation of the condensed water and ethanol within the allotted time was confirmed by gravimetric measurements.

2.3. Elution of spiked filters

Filters were eluted by adding 1.5 ml of solvent (measured gravimetrically) to each filter, with a keeper molecule (nominally 0.2%) if required by the study design. If agitation was required, the filters soaking in elution solvent were agitated in a sonicator for 10 min. Afterwards, the solvent was evaporated in a vacuum concentrator and the residue was reconstituted in 100 μ l of mobile phase (measured gravimetrically) containing nominally 10 ng g⁻¹ of THC-d3, CBD-d3, and CBN-d3 at the gradient starting conditions. In traditional studies, internal standards are added early in the elution process to account for losses across processing. As this study aims to identify loss pathways, internal standards were added as late as possible in the elution process so that no internal standard loss is expected.

Table 1 lists the different processing factors tested in this study. Factor A considers container material, which is either silanized glass or plastic. Cannabinoids are lipophilic and are known to ‘stick’ to plastic containers, so glass containers might seem like the ideal choice to avoid losses. But the manufacturer-supplied vials, designed to exactly fit the filters, are plastic and plastic containers are both more economical and less likely to break. For the glass setting, silanized tubes (ThermoFisher Scientific) were used for elution and silanized screw cap vials (Sigma Aldrich) were used for vacuum concentration. For the plastic setting, the manufacturer-supplied vial (Munkplast) was used for elution and microfuge tubes (Eppendorf Protein LoBind) were used for vacuum concentration. All samples were transferred to silanized glass

Table 1. Experimental factors considered in the three experiments. E1/E2/E3 denote experiment 1/experiment 2/experiment 3. 'X' indicates that a factor (denoted by row) was included in the experiment in the corresponding column.

Factor	Explanation	Low value (–)	High value (+)	E1	E2	E3
A	Container material	Glass	Plastic	X		X
B	Concentrator temperature	35 °C	50 °C	X		X
C	Concentrator	Unit 'a'	Unit 'b'	X		
D1	Keeper added to elution solvent	No	Yes	X	X	
D2	Keeper molecule	Octanol	Ethylene glycol	X	X	
E	Elution solvent	Ethanol	Methanol	X	X	
F	Cannabinoid mass	5 ng	50 ng	X		
G	Agitation	No	Yes	X	X	
H	Time	1.4 h	2.1 h			X

inserts in autosampler vials for LC-MS/MS analysis. Factor *B* considers the temperature during vacuum concentration, which was set to either 35 °C or 50 °C. Temperature impacts the time required for concentration, as well as cannabinoid evaporation during this time. Factor *C* considers two different vacuum concentrator units (same manufacturer and model) available in our laboratory.

Factor *D* considers whether a keeper molecule should be added to the elution solvent. Keepers must have lower volatility than the solvent that is being evaporated; they solvate the molecules of interest during evaporation and minimize interaction with the container. Factor *D* is a three-level factor that compares no keeper, octanol, and ethylene glycol (recommended by the device manufacturer). While the nominal keeper concentration was 0.2% in the elution solvent, the measured concentration was $0.16\% \pm 0.02\%$ across all experiments. Keeper is recommended by the manufacturer but could cause ion suppression during LC-MS/MS analysis, so both the manufacturer recommended ethylene glycol and octanol were explored. Octanol has one less hydroxyl group than ethylene glycol, so theoretically it may cause less ion suppression than ethylene glycol while still acting as a good keeper. Ideally, a keeper would not be necessary which is why no keeper is explored.

Factor *E* considers elution solvent, either methanol or ethanol. While both solvents are suitable for cannabinoids, methanol evaporates more readily. Factor *F* considers the cannabinoid mass in the spike solution, which was nominally 5 ng or 50 ng, to investigate if the recovery is independent of concentration. Factor *G* considers agitation, which occurs while the filter is soaking in elution solvent, to explore whether sonication helps solvate cannabinoids. Finally, Factor *H* considers how long the filters soaked in elution solvent. While this is hard to control when multiple devices were analyzed sequentially as done in this study, in practice the times were similar within each group.

2.4. Experiment designs

An experiment design may be summarized using the notation (n, k^p) , where n denotes the number of

experimental runs, k the number of levels, and p the number of factors. In the case of a mixed design, such as including both two and three-level factors, the multiple levels and their respective number of factors are noted sequentially, e.g. $(n, k_1^{p_1} k_2^{p_2})$.

2.4.1. Experiment 1

This experiment was conducted on six two-level factors (*A–C* and *E–G*) and one three-level factor. The three-level factor (*D*) related to keeper, where experimental samples were treated with one of three options: no keeper, octanol, or ethylene glycol. The effect of keeper was estimated through two effect estimates: *D1*, which compared cannabinoid recovery when either ethylene glycol or octanol were used as keeper, to recovery when no keeper was used; and *D2*, which compared cannabinoid recovery when ethylene glycol was used to recovery when octanol was used.

In this experiment, constraints on factors related to vacuum concentration affected the construction of the design. Specifically, a concentrator cannot simultaneously run samples in different containers (due to the rotor dimensions) or at different temperatures. Additionally, vacuum concentration requires approximately 1 h–3 h in a sequential protocol that took approximately 12 h to complete and the vacuum concentrator only holds 24 vials at one time, meaning each concentrator could only be run once per day. To efficiently accommodate these constraints, the experiment was blocked on concentrator—i.e. batches of samples sharing container type and temperature were run simultaneously. To avoid confounding from any potential difference in concentrator performance, the $2^2 = 4$ unique treatment combinations of container material/temperature were performed on each concentrator, resulting in a $(8, 2^3)$ full factorial design for factors *A–C*. The full factorial design for the entire experiment would have required running the $3 \times 2^3 = 24$ treatment combinations for factors *D–G* at each of the 8 combinations for factors *A–C*. For efficiency, a nonregular $(12, 3^1 2^3)$ design on factors *D–G* was performed at each combination of factors *A–C*, resulting in a total of $12 \times 8 = 96$ experimental runs. The nonregular design matrix is given in table S1.

2.4.2. Experiment 2

This experiment was conducted on factors *D*, *E*, and *G*, with all other factors held constant: filters were spiked with 5 ng cannabinoids and processed in glass containers in the unit 'a' vacuum concentrator at 50 °C. The full factorial ($12, 3^{12^2}$) experiment was replicated four times for a total of $12 \times 4 = 48$ experimental runs.

2.4.3. Experiment 3

This experiment was conducted on factors *A*, *B*, and *H*, with all other factors held constant: filters were spiked with 5 ng cannabinoids, eluted with methanol plus ethylene glycol, not agitated, and processed in the unit 'a' vacuum concentrator. Factor *A* was modified to be a comparison between full glass and half plastic (e.g. plastic for elution and glass for vacuum concentration), based on the findings of experiment 1. Factor *H* was added to explore the effect of time that filters were soaked in the elution solvent. Time could not be explored in experiments 1 or 2 due to the large number of factors and experimental runs that made timing unfeasible. The short time for solvent elution was 83 ± 1 min (1.4 h) while the long time was 127 ± 1 min (2.1 h), resulting in a 44 min (0.7 h) difference in soaking time. The full factorial ($8, 2^3$) experiment was replicated four times for a total of $8 \times 4 = 32$ experimental runs.

2.5. Cannabinoid analysis

THC, CBD, and CBN and their respective deuterated internal standards were monitored by LC-MS/MS and quantified by multiple reaction monitoring (MRM) with the settings in table S2. Additional method information can be found in the supplemental file. Nine calibrators were prepared in the initial LC-MS/MS mobile phase from 100 ng g to 0.5 ng g⁻¹ (experiment 1) or 10 ng g⁻¹ to 0.1 ng g⁻¹ (all other experiments) with a calibration curve weighting of $1/x$ to determine cannabinoid concentration. Samples containing octanol or ethylene glycol were analyzed with matrix-matched calibrators also containing keeper. While the nominal keeper concentration in reconstituted samples was 2%, the prepared concentration in matrix-matched calibrators was $2.8\% \pm 0.8\%$ and $1.907\% \pm 0.003\%$ for ethylene glycol and octanol, respectively. Analytes were identified by their retention times compared to their respective internal standard (<0.05 min) and their quantifier-to-qualifier ratio ($\pm 20\%$). Detected concentrations (ng g) were converted to masses (ng) by multiplying by the reconstitution solvent mass. Recovery was calculated by dividing the detected mass (in ng) by the gravimetrically determined spiked cannabinoid mass (also in ng) and multiplying by 100 to get a percentage.

3. Results and discussion

3.1. Important factors in large volume elution of spike evaporated filters (experiments 1–3)

In all but the most complex of systems, it is reasonable to assume that the effects of three-term and higher interactions are negligible. Therefore, in analyzing the results from the first three experiments, we focused on determining the relative importance of main effects and two-term interactions on cannabinoid recovery. For each experiment, a separate analysis was conducted for each cannabinoid (THC, CBD, CBN). All analyses were conducted in R [19].

Experiment 1 investigated the initial factors we predicted would affect cannabinoid recovery without replication. During data collection, three experimental runs deviated from their planned experimental settings. Two runs deviated in their keeper setting and one run deviated in spiked cannabinoid mass. This minor imbalance should not affect the conclusions presented here. Experiment 1 data were analyzed using the methods of Hamada & Wu [20], which first entertains main effects and orthogonal two-term interactions using standard methods, then subsequently considers additional effect estimates using an iterative forward selection regression procedure. The partial aliasing structure imposed by the nonregular design matrix was mild, resulting in only a small subset of interaction terms (*D2E*, *D2G*, *EF*, *EG*, *FG*) being excluded during the first step of the analysis. Significant effects from the remaining subset of orthogonal effect estimates were determined by half normal probability plots and Lenth's method [21]. Figure 1 illustrates main effects by showing the change in average recovery across the factor settings. This 'main effects plot' is a visualization of the relative importance of factor effects. Important factors will exhibit steep slopes (e.g. factor *A*), indicating a large change in recovery between the two settings for that factor. Factors that are relatively unimportant will exhibit little change in recovery through a shallow slope (e.g. factor *G*).

Two main effects were identified as significantly affecting cannabinoid recovery for all three compounds: container material (*A*) and addition of a keeper (*D1*). Higher recovery was obtained for all three cannabinoids when elution was performed in glass containers and a keeper was used. The effect of keeper molecule (*D2*) was significant only for CBN, with ethylene glycol leading to higher recovery than octanol. Additionally, the effect of solvent (*E*) was significant for CBN, with higher recovery obtained using methanol.

Two significant interactions, container $\times$ concentrator (*AC*) and temperature $\times$ concentrator (*BC*), had a notable effect on cannabinoid recovery. The container $\times$ concentrator interaction, significant for

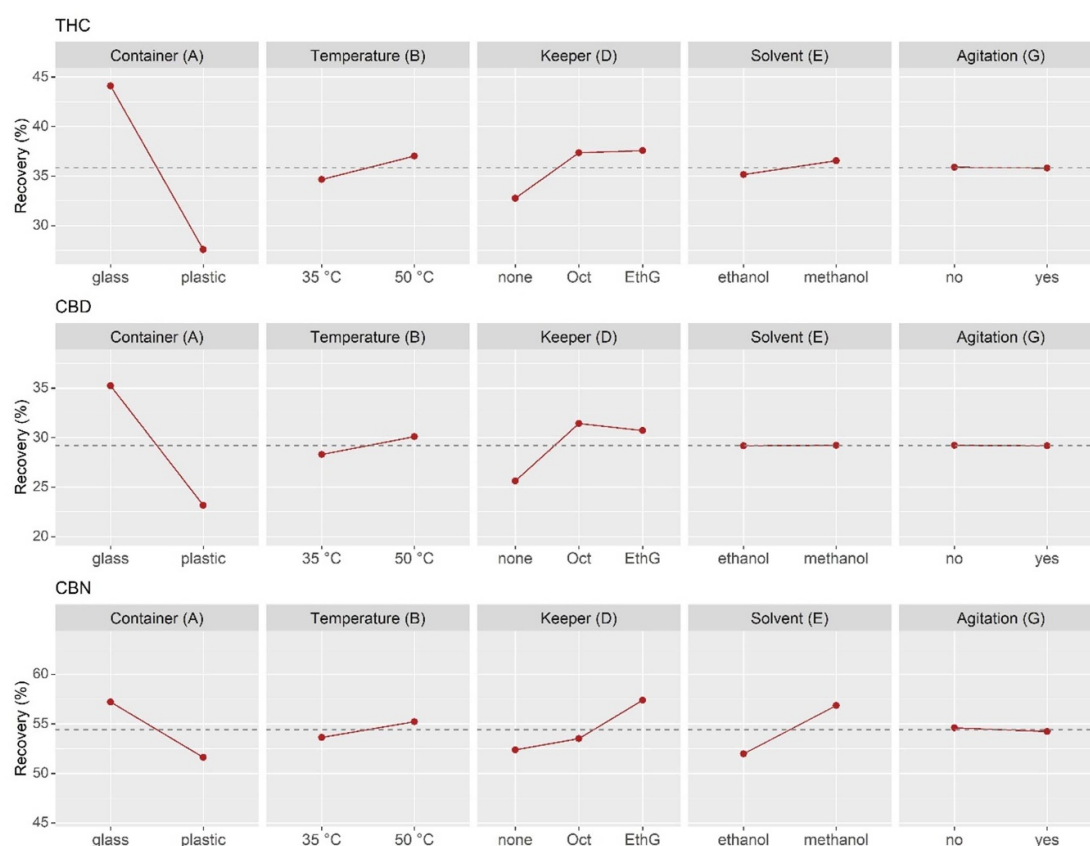


Figure 1. THC, CBD, and CBN recovery (%) from filters processed by large volume elution plus concentration. The main effects plots show the effect of container material (A), concentrator temperature (B), keeper (D1 & D2), elution solvent (E), and agitation (G) explored in experiment 1. Each panel shows the average recovery obtained for each cannabinoid at the low and high setting for that factor. The dashed line indicates overall average recovery for the respective cannabinoid.

THC and CBD recovery, indicated that while cannabinoid recovery was higher overall using glass containers, the detrimental effect of using plastic containers was augmented for one concentrator. The temperature $\times$ concentrator interaction was significant for all cannabinoids and showed that optimal recovery occurred at different temperatures for the two concentrators. In other words, unit 'a' had a higher cannabinoid recovery at 50 °C, while unit 'b' performed better at 35 °C. This result was unexpected and upon further investigation, a cracked ballast cap was discovered on the unit 'b' vacuum concentrator. While no testing was done to confirm if this caused the difference between the concentrators, these findings demonstrate that products from the same manufacturer with different histories can have differential performance that may need to be explored. Subsequent experiments were conducted using a single concentrator (unit 'a').

Experiment 2 focused on effect estimates that were partially aliased in experiment 1. Data from the replicated full factorial design of experiment 2 were collected as prescribed and analyzed using half normal probability plots, Lenth's method, and analysis of variance (ANOVA) at the 0.05 significance

threshold [21, 22]. ANOVA results are presented in table S3. Consistent with experiment 1, addition of a keeper significantly improved recovery of all three cannabinoids, though only for recovery of CBN did the choice of keeper make a difference, with ethylene glycol offering the highest recovery. The effects of solvent, agitation, and their interactions on cannabinoid recovery were negligible, supporting the results found in experiment 1.

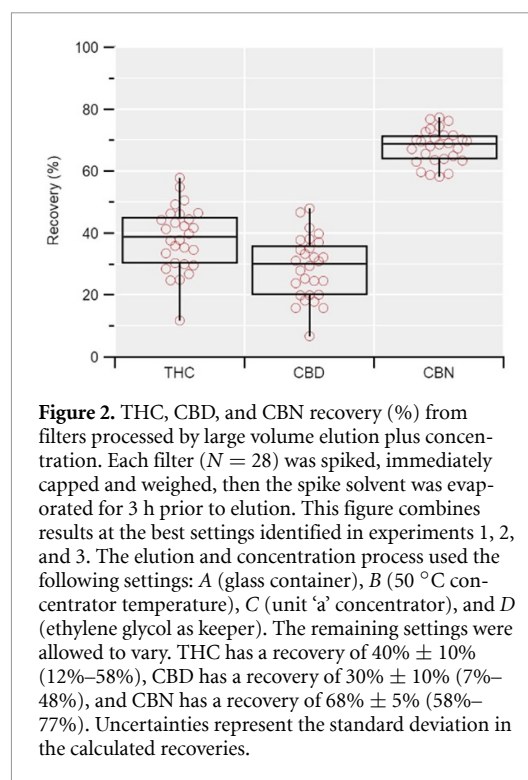
Experiment 3 reconsidered container material (A) and concentrator temperature (B) previously explored in experiment 1, as well as the effect from time that filters were exposed to elution solvent (H). In this experiment, the high setting for container material changed from full plastic to half plastic. Experiment 1 had previously determined that cannabinoid recovery was significantly improved when glass vials were used. However, glass vials are less appealing as they can break and are more expensive, so experiment 3 minimized plastic contact while still using the device manufacturer's plastic vial. For the half plastic setting, the device manufacturer's plastic vial is used for elution, but silanized glass screw top vials are used during vacuum concentration. So, container material only differs during

elution. It is possible that the loss of cannabinoids observed in experiment 1 occurred when heating the plastic containers during concentration, so this switch potentially mitigates the highest loss pathway with realistic choices. In a previous study we used the half plastic protocol and showed that it is a practical option [8].

Data from the replicated full factorial design of experiment 3 were collected as prescribed and analyzed using the same methods as experiment 2. ANOVA results are presented in table S4. Only the container $\times$ time interaction (*AH*) had a significant effect on cannabinoid recovery. This interaction shows that the two different container regimens perform better for different time frames. When elution was performed using glass containers, a higher recovery was obtained when filters had longer contact with the solvent. In contrast, when elution was performed using plastic containers, higher recovery occurred when filters were exposed to solvent for the shorter amount of time. The lower recovery for plastic containers at long time frames could be explained by having a longer amount of time for cannabinoids to be lost to plastic, although this does not explain why glass performs worse than plastic at shorter time frames.

3.2. Optimized large volume elution of spike evaporated filters (experiments 1–3)

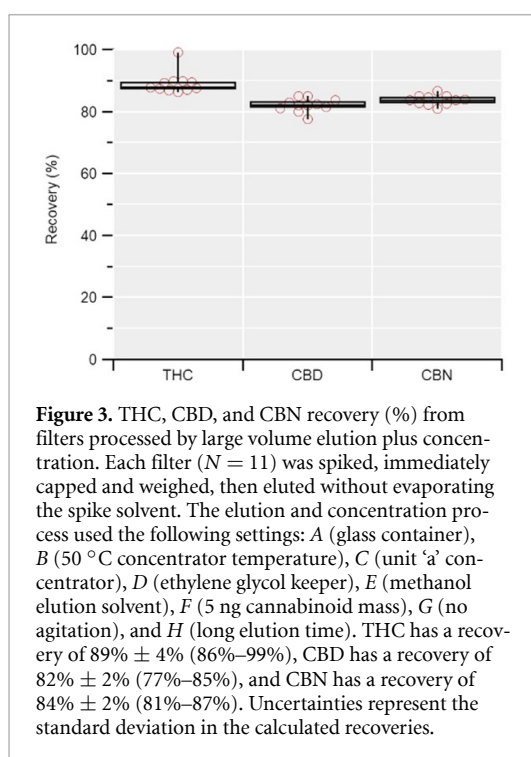
Experiments 1–3 tested eight factors, but few had a significant impact on cannabinoid recovery, meaning that recoveries will be low even with the best settings. This suggests that the factor that leads to low recovery was not explored, or that some other aspect of the experimental design is confounding the results. Figure 2 shows THC, CBD, and CBN recovery across all experimental runs with the best settings determined in experiments 1–3 ($N = 28$). THC has an average recovery of 40% here, comparable to the THC recoveries reported by De Jong *et al* 2025 [14]. THC also has a higher recovery than CBD despite the two cannabinoids having the same molecular formula. Despite its structural similarity as a THC degradation product, CBN has significantly higher recovery. One potential explanation for the results in figure 2 is vapor pressure. In a recently published paper [18], we measured the vapor pressure of CBN for the first time, along with much higher accuracy THC and CBD vapor pressures than previously reported. Lower vapor pressure would be expected to result in less evaporative loss, and indeed, CBN has the lowest vapor pressure and the highest recovery. Conversely, CBD has the highest vapor pressure and the lowest recovery. The recoveries presented in figure 2 prompted us to probe our experimental design for confounding factors, described in section 3.3, and to investigate a microelution process, described in section 3.4.



3.3. Large volume elution of filters without spike evaporation

Large volume elution plus concentration, as investigated here, includes two periods during which cannabinoids may evaporate: during spike evaporation (20°C) and during vacuum concentration (35°C or 50°C). While some form of concentration is unavoidable with a large volume elution, spike evaporation was a deliberate choice. We fully evaporated the spike solvent so that the cannabinoids could interact with the breath matrix and the filter material. We used ethanol as the spike solvent, despite its lower volatility, because we observed good wetting of the filter surfaces. While these were reasonable choices, the consequence is that spiked filters were exposed to the laboratory environment for three hours. We assumed that at the temperature experienced during spike evaporation (room temperature), cannabinoid evaporation would be negligible. Experiments 1–3, combined with vapor pressure data, call this assumption into question. Cannabinoid evaporation may also have been enhanced by placing the filters in a biological safety cabinet, which directs filtered air downward over the filters. Cannabinoids that evaporate are swept away from the filter, maximizing the driving force for evaporation. Therefore, we performed an experiment without spike evaporation, allowing us to isolate losses that occur during vacuum concentration from losses that occur during spike evaporation.

Figure 3 presents cannabinoid recovery for filters in which the spike solvent was not given time to evaporate before adding the elution solvent. Instead, the filters were spiked, immediately capped, and



weighed, providing $13 \text{ min} \pm 2 \text{ min}$ for the cannabinoids to interact with the breath matrix and filter material before elution. Therefore, the cannabinoids may be in solution instead of in direct contact with the filter material. Figure 3 shows that cannabinoid recovery was both higher and less variable. This was true for all three cannabinoids. Additionally, the trend in cannabinoid recovery did not match the vapor pressure trend. This may indicate the effectiveness of the ethylene glycol keeper. Figure 3 shows that cannabinoid recovery was more influenced by the spike evaporation process than by the vacuum concentration process. This result could be due to cannabinoid evaporation during the 3 h spike evaporation when there is no keeper molecule. However, this result could also be due to cannabinoids interacting with and adhering to the filter material. While we cannot distinguish between these causes, we are convinced that the volatility of cannabinoids must be considered when processing laboratory-generated samples and authentic breath samples.

Laboratory-generated samples are typically created to examine matrix effects (analytical method validation) or cannabinoid recovery. Yet most studies do not report cannabinoid recovery for their processing methods, and even fewer describe their methodology in detail. Of the three studies that report cannabinoid recoveries from a similar large volume elution plus concentration process [1, 6, 14], two report fortification of blank samples without further description. De Jong *et al* 2025 [14] spiked each filter with a $20 \mu\text{l}$ methanol aliquot containing the drugs of interest. De Jong *et al* 2025 also varied their spike protocol to allow different interactions with the filter material. They

spiked filters after adding breath matrix (similar to our protocol), but also spiked filters with no breath matrix, and spiked filters then added breath matrix. They allowed 30 min for spike solvent evaporation, but did not specify whether elution occurred immediately afterward. For both THC and CBD recovery, the large reported standard deviations do not allow for statistical comparisons or any conclusions about the effect of breath matrix. In our human studies with individuals who use cannabis products [8, 11, 23], authentic breath samples are stored on ice or in a freezer within minutes of collection. They are brought to room temperature before processing, but this only requires a few minutes at room temperature, plus the devices remain capped. Therefore, there is nothing comparable to the 3 h spike evaporation inside a biosafety cabinet. These differences between laboratory-generated samples and authentic breath samples highlight the inadequacy of spike procedures.

3.4. Microelution of spike evaporated filters

Large volume elution plus concentration is intuitively appealing because the solvent fully wets the filter; however, an alternative microelution process was employed by Henion *et al* 2024 [10] and Berry *et al* 2025 [23] for the analysis of authentic samples. In the microelution process, a small volume of solvent without a keeper is added to the filter. The filter plus solvent is vortexed to fully wet the filter surfaces, then centrifuged to recover the solvent, which is then transferred for analysis. The volume of solvent is small enough that no concentration is needed, and the entire process requires approximately 10 min per filter. Figure 4 presents cannabinoid recovery for filters processed by microelution in the device manufacturer's plastic vials, chosen because of their previously mentioned durability. Filters were prepared identically to the filters in experiments 1–3, except that the spiked cannabinoid mass was 0.3 ng to better match authentic breath samples. The spike solvent was fully evaporated. The filters were eluted with $150 \mu\text{l}$ of a 35% H_2O 65% methanol solution (measured gravimetrically) containing 10 ng g^{-1} (nominal) of each internal standard. The filters were vortexed horizontally for 5 min then centrifuged for 5 min. While there are fewer replicates, the recoveries in figure 4 are comparable (CBN) or higher (THC and CBD) than the recoveries in figure 2. Therefore, there is no apparent advantage to the time and labor intensive large volume elution process; microelution provides comparable or better recoveries.

The microelution process was previously validated by our analysis of spiked elution solvents in Berry *et al* 2025 [23], where recoveries were within the analytical method uncertainty (e.g. recoveries were $100\% \pm 20\%$). The microelution process was also assessed by examining the variability of cannabinoid masses recovered from the three individual filters collected as part of each authentic breath sample. While

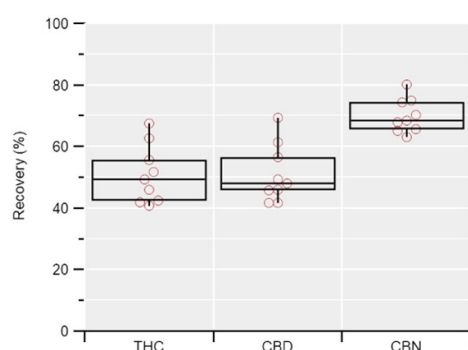


Figure 4. THC, CBD, and CBN recovery (%) from filters processed by microelution. Each filter ($N = 9$) was spiked, immediately capped and weighed, then the spike solvent was evaporated for 3 h prior to elution. This figure reports recoveries that were determined by an LC-MS/MS method described in Berry *et al.* 2025 [23]. The method monitors eight additional cannabinoids, but only THC, CBD, and CBN, and their deuterated internal standards were analyzed here. THC has a recovery of $50\% \pm 10\%$ (41%–67%), CBD has a recovery of $51\% \pm 9\%$ (42%–69%), and CBN has a recovery of $70\% \pm 6\%$ (63%–80%). Uncertainties represent the standard deviation in the calculated recoveries.

the true concentration of cannabinoids in breath cannot be verified, low variability in the recovered cannabinoid masses indicates reproducibility in the elution process. Berry *et al* 2025 [23] reported that most filters processed by microelution were within 20% of the average for the device; a few filters were near 50%. Altogether, our results suggest that microelution is an effective process that avoids evaporation of cannabinoids during processing. This suggests that microelution is a superior elution method that requires less solvent and less processing time.

4. Conclusions

Large volume elution of the impaction filters in the BreathExplor device has been the main processing method for analyzing authentic breath samples to explore drug use, particularly cannabis use. But using a large volume of solvent to elute each filter means that the analytes must be concentrated by evaporating the solvent, leading to a variety of additional processing choices. In this study, we used three sequential experiments to test eight factors that could affect THC, CBD, or CBN recovery. Only two factors were found to have a significant impact on recovery for at least one cannabinoid: container material and the addition of a keeper to the elution solvent, with glass containers and ethylene glycol as keeper resulting in higher cannabinoid recovery. While these factors did affect recovery, the implementation of best settings did not dramatically improve the THC recovery seen in this study and previous studies. Additional

experiments suggested that cannabinoids evaporated while evaporating the spike solvent. Vapor pressure could account for the losses during evaporation, as a recently published paper found that THC, CBD, and CBN should be described as semi-volatile [18]. With volatility in mind, a microelution process that does not require analyte concentration was compared to large volume elution. With the same spike evaporation time, microelution results in similar or higher recoveries. Overall, this study suggests that evaporation during handling or processing breath samples can have a large effect on cannabinoids. Microelution requires less time and solvent and yields similar or better results. We conclude that breath sampling studies should utilize processing methods that minimize the quantity of solvent used and that do not require solvent evaporation. As fieldable solutions for cannabis use are increasingly in demand, there is an increased need for breath surrogates to properly model how cannabinoids are captured by breath collection devices, as well.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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