# High-Throughput Filtration Using the [MPE]$^2$ for Cannabinoid Analysis

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## Introduction

An increasing number of jurisdictions within the United States have legalized the use of medicinal marijuana, along with several states that have also legalized it for recreational sale. As with any consumer product, quality control methods are necessary to ensure product safety; additionally, properly characterized cannabinoid profiles give consumers confidence in purchasing products with the desired dose and physiological response. While several methods are used to evaluate these compounds of interest, it is important to keep in mind that scalability and throughput are crucial factors to consider as laboratories will need to accommodate increased analytical requests. One difficulty with cannabis and cannabis infused products is the amount of manual labor in the sample preparation procedures, including separation and filtration of extraction solutions. After extracting the compounds of interest with a solvent, the mixtures are typically either centrifuged or manually separated with syringe filters or other tedious manual methods. In this study, an automated high-throughput filtration method, using the [MPE]$^2$ — Monitored Multi-flow, Positive Pressure Evaporative Extraction module — from Hamilton Robotics, was evaluated for cannabinoid analysis in multiple matrices.

## Materials and Methods

For flower and concentrate samples, two extractions of 500 mg of sample (for flower) and 100 mg of sample (for concentrate) were prepared in a conical tube with the addition of 10 mL acetone. For edible samples, two extractions of 2000 mg were prepared in a glass VOA vial with the addition of 10 mL cyclohexane. For each extraction, the mixture was homogenized and sonicated. The two mixtures of each respective sample were then subjected to two separate filtration methods (Figure 1). In the manual filtration method, the mixture was transferred to a syringe with a 0.2 µm luer lock syringe filter attached (Phenomenex®) and pushed through the filter by hand. For the automated method, the mixture was transferred to an individual well of a 0.2 µm regenerated cellulose 96-well filter plate (Chrom Tech, Inc.® P/N: 96F-RC020) then loaded into the [MPE]$^2$ (Figure 2) and pushed through the filter plate into a collection plate according to a predefined pressure and hold profile (40 psi, 30 sec hold). All filtrates were then diluted into an LC vial with the addition of an internal standard.

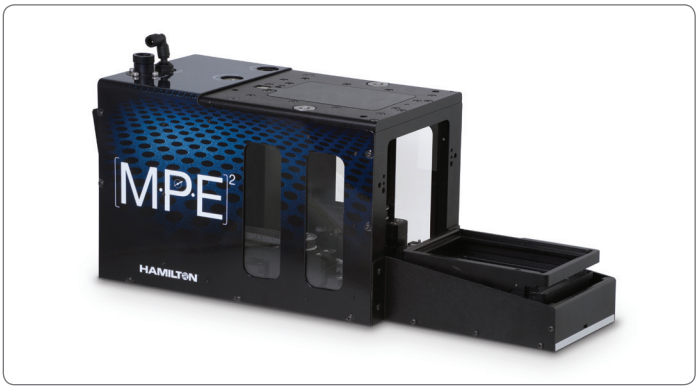


*Figure 2: [MPE]$^2$ from Hamilton Robotics is an automated positive pressure extraction module.*

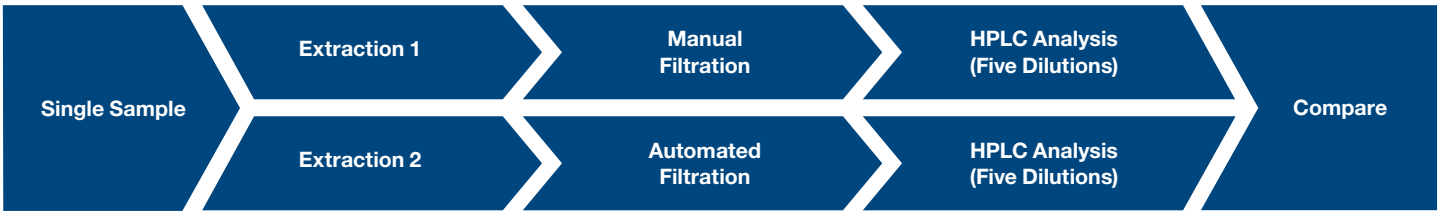


*Figure 1: Extraction, filtration, and analysis workflow.*

Samples were analyzed by LC-DAD (Agilent 1100 Series HPLC system) equipped with a Restek® Raptor ARC-18 HPLC column. Five dilutions of each sample were run for repeatability. Quantitation was performed against an 11-point calibration curve prepared from commercially available analytical grade standards for THC-A (Restek), THC (Restek), CBD-A (Cerilliant®), and CBD (Restek) at 1000 µg/mL down to 0.56 µg/mL.

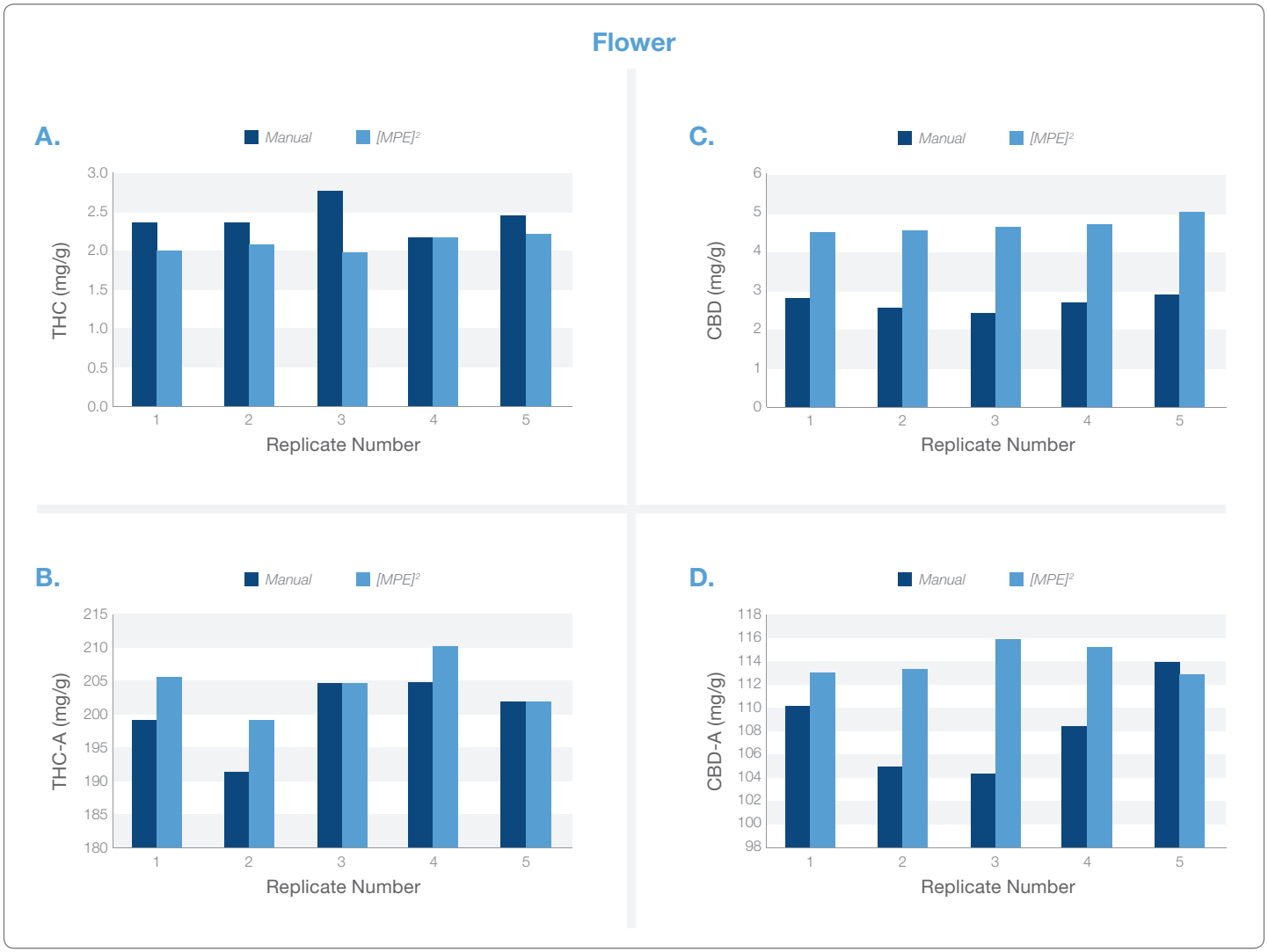


*Figure 3: Measured concentrations of A. THC, B. THC-A, C. CBD, and D. CBD-A in flower samples.*

## Results and Discussion

The efficacy of the automated filtration method can be illustrated by a direct comparison of filtrates from the manual method. As previously mentioned, five dilutions of each sample filtrate were analyzed on the LC for reproducibility. The results for THC, THC-A, CBD, and CBD-A in flower are given in Figures 3A – 3D. With the exception of THC, the mean values of all cannabinoids were higher when filtered with the [MPE]$^2$ compared to the manual method (Table 1, Page 3). The most notable difference was for CBD, where the mean value measured after filtration with the [MPE]$^2$ was 1.7 times higher than that via the manual syringe filter method. It is also interesting to note that smaller relative standard deviations were observed for all compounds in flower when using the [MPE]$^2$ and filter plate.

## Table 1: Statistical Measures of Samples Measured by Both Methods

| | | Mean (mg/g) | | Std. Dev. (mg/g) | | Std. Dev. (as % of mean) | |
|---|---|---|---|---|---|---|---|
| | | Manual | [MPE]² | Manual | [MPE]² | Manual | [MPE]² |
| Flower | THC | 2.44 | 2.11 | 0.22 | 0.11 | 8.97% | 5.10% |
| | THC-A | 200.54 | 204.51 | 5.58 | 4.24 | 2.78% | 2.07% |
| | CBD | 2.71 | 4.71 | 0.20 | 0.20 | 7.23% | 4.26% |
| | CBD-A | 108.47 | 114.19 | 3.94 | 1.39 | 3.63% | 1.22% |
| Edible | THC | 0.72 | 0.77 | 0.03 | 0.02 | 3.55% | 3.09% |
| Concentrates | Sample 1 CBD | 1003.56 | 1021.01 | 47.15 | 20.93 | 4.70% | 2.05% |
| | Sample 2 CBD | 264.20 | 270.06 | 10.57 | 5.10 | 4.00% | 1.89% |

For the measured THC values in edible, the manual filtration yielded values from 0.68 to 0.75 mg/g, while those for the automated method were between 0.74 and 0.80 mg/g (Figure 4), and the resulting average value was about 7% higher for the automated method. Similar results were observed for CBD in both concentrates, where the mean values were higher utilizing the [MPE]². There was also a notable decrease in measured standard deviations in the dilution sets of the samples filtered with the [MPE]² (Figures 5 and 6, Page 4).

When comparing the average values obtained for all sample types, measured cannabinoid concentrations were consistently higher when processed with the [MPE]² compared to the manual method (Table 1). It is unclear if this is just an artifact or a result of more efficient filtering (i.e. less analyte binding to membrane) in the case of using filter plates and the [MPE]². In terms of reproducibility, all analytes had standard deviations within 5.1% or less of the mean values (Table 1) using the [MPE]², while standard deviations of up to 9.0% of the mean were observed for the manually filtered samples. Currently, it is unclear why the standard deviations with the filtrates from the [MPE]² were smaller; however, the results were consistent across all data sets. Future work will focus on identifying the cause. It is also interesting to note that THC in flower exhibited the highest standard deviation of both methods. Further testing is necessary to elucidate whether THC itself is generally just more difficult to measure consistently in flower compared to the other cannabinoids.

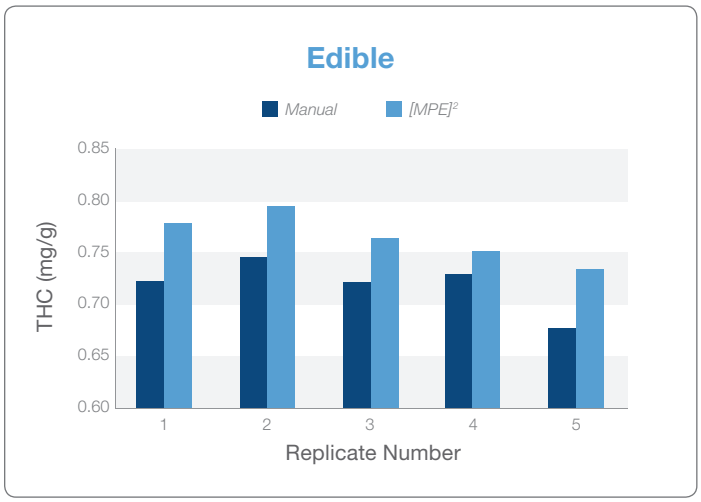


*Figure 4: Measured concentrations of THC in edible samples.*

In addition to comparing measured concentrations obtained via both methods, it is also important to note the savings in time and materials. By utilizing the 96-well plate and the [MPE]², the tedious and time consuming syringe filter method is avoided. A sample typically takes ~30 sec to filter via a syringe; therefore, labs could realize around 45 minutes of time savings filtering a full plate of samples. The automated method also reduces strain and potential injury to technicians by avoiding strenuous forcing of fluid through luer lock filters. Based on available products prices, it is estimated that the filter plate method costs up to 40% less per sample than using luer lock syringe filters, and also significantly cuts down on waste.

## Conclusion

In this study, two methods of sample filtration for cannabinoid analysis are compared. Specifically, three sample types (flower, edible, and concentrate) are filtered through both a manual syringe filter and a 96-well filter plate with an automated positive pressure extraction unit and then analyzed for cannabinoids. It was demonstrated that filtration with the [MPE]$^2$ typically gave higher yields of cannabinoids and also significantly increased reproducibility compared to the manual syringe filters. Additionally, savings in time and consumables cost make the automated method attractive for high-throughput sample handling.

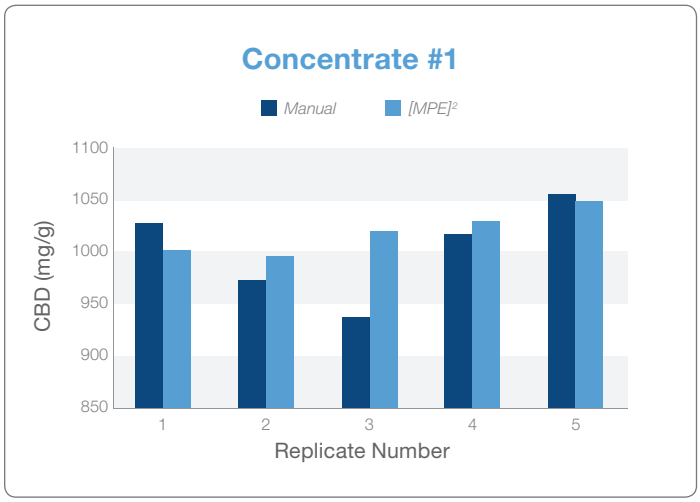


*Figure 5: Measured concentrations of CBD in concentrate #1 sample.*

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***Keywords:***
*Cannabis, Filtration, LC Sample Prep, Chrom Tech, Inc., Hamilton, [MPE]$^2$*

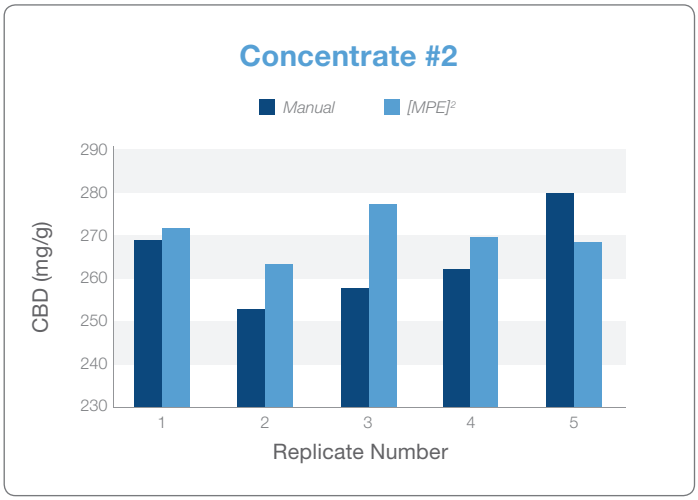


*Figure 6: Measured concentrations of CBD in concentrate #2 sample.*



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