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Application Note

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Cannabis Testing: In-House Prepared Water as an Alternative to Bottled HPLC-Grade Water

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Abstract

In many testing laboratories, bottled HPLC-grade water is the standard when performing quantitative potency analysis for cannabinoids like THC (Tetrahydrocannabinol) or CBD (Cannabidiol). In-house produced water, on the other hand, may be qualitatively equivalent and also a more cost-effective alternative.

The objective of this study was to determine if aqueous mobile phase preparations using in-house purified Type 1 water (prepared with Sartorius's arium® Water Purification System) yielded qualitatively comparable results to those from purchased HPLC-grade bottled water. We analyzed cannabis samples from mobile phase preparations using each source of. Additionally, we analyzed a blank matrix specimen using homogenized nettle leaf (*Urtica dioica*), both with and without cannabinoid spikes, using designated mobile phases for each water type, to assess any specimen effects from the water.

The results indicate that water types and sources did not affect specimen quantitation.

Introduction

Laboratory-grade water is used for preparing the aqueous mobile phase in both HPLC (High Pressure Liquid Chromatography) and UHPLC (Ultra High Performance Liquid Chromatography), and is essential for maintaining sensitivity on analytical instruments. However, purchasing lab-grade bottled water is an ongoing expense when compared to generating lab-grade ultrapure water in-house (Figure 1).

We prepared aqueous mobile phases fortified with 5 mM ammonium formate and 0.1% formic acid using water from both Sartorius's arium® mini-plus water purification system (Type 1 water) and commercially purchased HPLC-grade water. We compared composite samples with the mobile phases prepared using the two sources of water for several cannabis specimens run on HPLC columns.

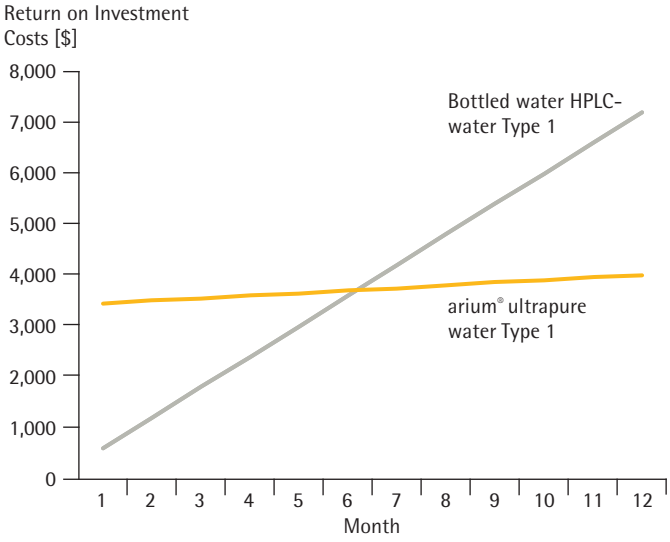

Figure 1: Comparison of expenses between In-house produced arium® water Type 1 (ultrapure water) and bottled water (HPLC grade). Calculation based on following assumptions: two liters water consumption per working day, 20 working days per month, arium® mini system list price = $3,400, annual arium® mini consumables list price = $600 and costs bottled water HPLC grade = $15/liter.

Materials and Methods

Individual cannabinoid reference standards, as well as the internal standard (ISTD) phencyclidine (PCP), were purchased from Cerilliant (Round Rock, TX) as single substances. Ricca ACS Reagent Grade, HPLC-grade water was purchased from Quartzy. Ultrapure arium® water ASTM Type 1 was produced using the arium® mini plus on the same day it was to be used. HPLC measurements were done with a Shimadzu Nexera-i LC2040L 3D Plus analyzer, fitted with a Restek Rapture ARC-18 (931421E) 100 mm x 3.0 mm ID x 1.80 µm column and coupled with a Restek UltraShield pre-column filter, 0.2 µm frit (25809) at 30° C. The HPLC operated in isocratic mode with 25% aqueous (Water, 5 mM ammonium formate, 0.1% formic acid) and 75% organic (Acetonitrile, 0.1% formic acid) phase at a flow rate of 1 mL/min. A sample volume of 1 µL was injected with photodiode array detection at 190-400 nm wavelength.

A stock solution in methanol containing 17 cannabinoids and the internal standard (ISTD) at the concentration of 100 µg/mL was prepared. This stock solution was serially diluted and used as a calibrator. A second stock solution in methanol containing the same 17 cannabinoids was prepared and diluted for use as a positive control. A 4-constituent control prepared from an alternate reference standard source was included as a high control. A matrix methanol solution was prepared with 20 mg of ground stinging nettle leaf (*Urtica dioica*) with 20 mLs methanol, sonicate and vortex, then centrifuge, filter and store for preparation of calibrators, controls, and blanks.

Cannabis samples were prepared in the following way: one to two grams of cannabis was placed in a 50 mL centrifuge tube, a grinding ball added, and the tube frozen for 1 hour. This centrifuge tube was then placed on a Geno Grinder for 1 minute at 1600 rpms. 0.2 grams ground cannabis was removed and placed in separate 50 mL centrifuge tube with 20 mLs methanol. Extraction was performed by cycling three times between vortexing for 1 minute and sonication for 5 minutes. A 1 mL aliquot was removed and filtered, then ultra-centrifuged for 5 minutes at 14000 rpms. After filtering, 100 µL of undiluted and diluted samples were added to a 150 µL auto-sampler vial, internal standard added, and analyzed on the HPLC.

A calibration curve, blanks, and controls were prepared to determine acceptance and tolerance limits. The cannabis specimens were evaluated against the linear dynamic range of the calibration curve and reported in µg/mL.

A total of 60 samples were tested. For each water type, a blank, a positive control, and a cannabis sample were analyzed quantitatively, with 10-fold repetition on a set of 17 cannabinoids (see Table 1).

Mobile Phase	Samples	Replicates
Bottled water	Blank	10
Bottled water	Positive Control	10
Bottled water	Cannabis Sample	10
arium® water	Blank	10
arium® water	Positive Control	10
arium® water	Cannabis Sample	10

Table 1: Summary of analytical runs. Bottled water = bottled water HPLC-grade, arium® water = arium® ultrapure water type 1.

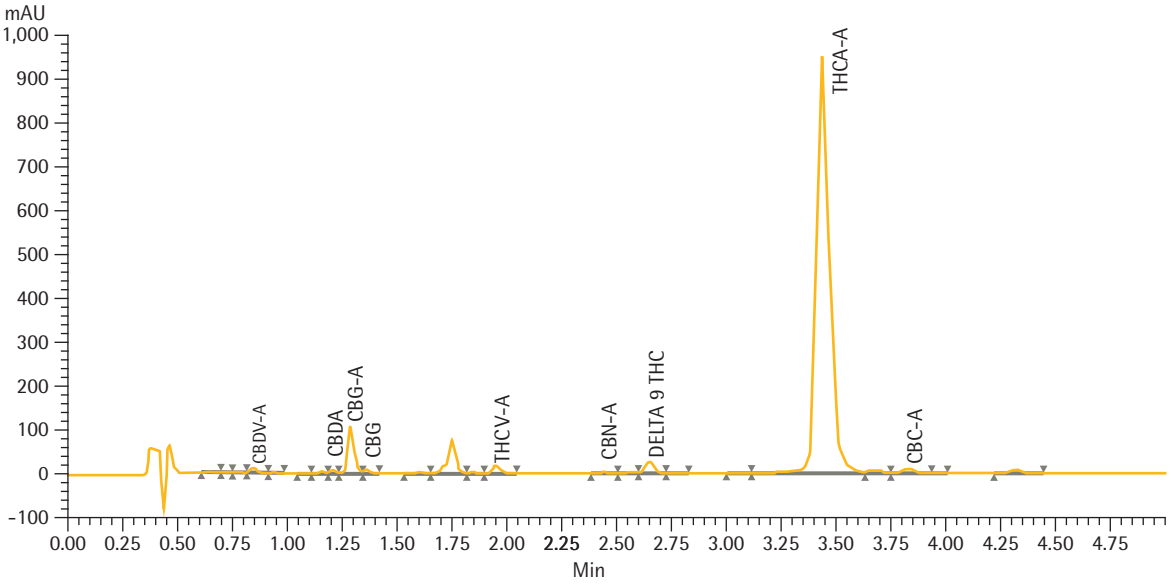

Figure 2: Cannabis samples were quantitatively analyzed using a set of 17 different cannabinoids. Only seven analytes (CBDA-V, CBG-A, CBG, THCV-A, DELTA 9 THC, THCA-A, CBC-A) were above the LOQ and were subsequently compared.

Results and Discussion

In this study, we compared the purity of water from two different sources for cannabis potency testing using HPLC: (1) HPLC-grade bottled water and (2) arium® water ASTM Type 1. Figure 3 shows the results from both positive controls and blanks. Figure 3A shows that all positive controls fell within our expected range. Most of the controls were injected at a concentration 10 µg/mL; however, CBDA and CBD were injected at 50 µg/mL and Delta 9 THC and Delta 8 THC at 75 µm/mL. Figure 3B shows that neither water source contained interfering compounds.

In a second step, both water types were compared to determine if the mobile phase affected the total amount of cannabinoids detected in a real sample. Figure 4 shows that no significant effect on the cannabinoid titer resulting from the use of either water. For this comparison only quantifiable analytes were evaluated – values below the LOQ were not considered.

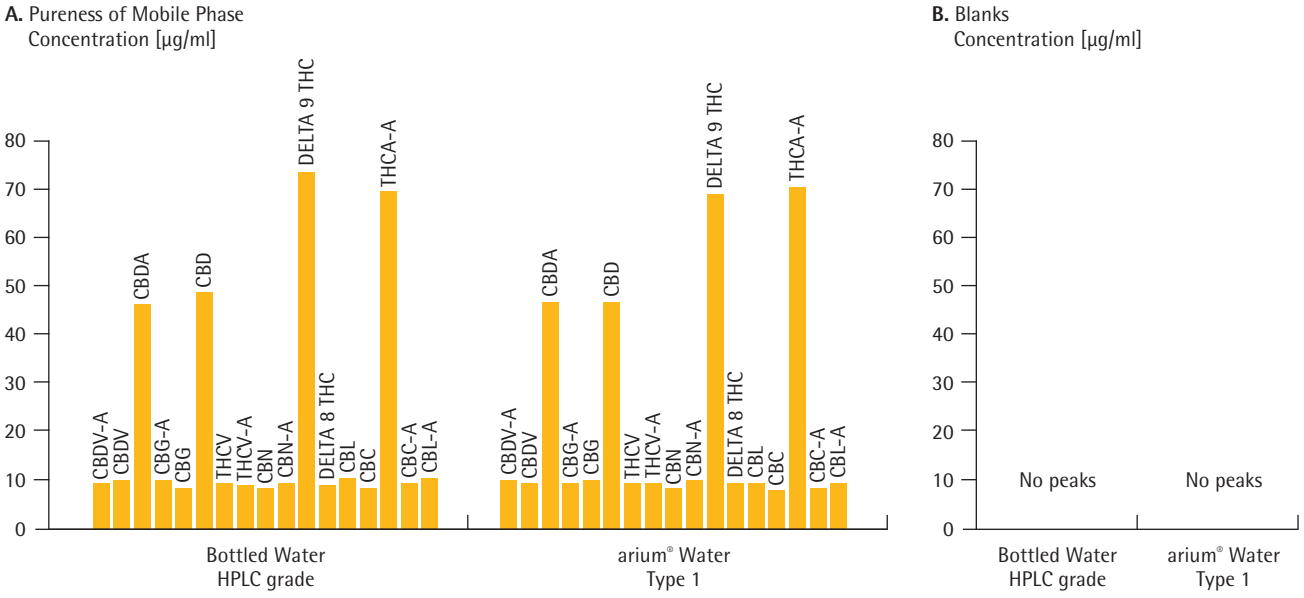

Figure 3A: All positive controls quantify within the expected range. Figure 3B: No interfering compounds were detected. We used two different water sources 'HPLC grade bottled water' and 'ultra-pure arium® water Type 1' to prepare the mobile phase (isocratic: 25% water, 75% acetonitrile, 0.1% formic acid) for cannabinoid analysis using HPLC. Both water sources are pure and suitable for HPLC in cannabis analytics.

Conclusion

Herein we compared the effect of two different water sources, freshly-produced ultrapure water (produced using the arium® mini plus water filtration system) and HPLC-grade bottled water, to prepare the mobile phase during the quantitative determination of cannabinoids by HPLC.

We conclude from our study that quantitative determination using freshly produced ultrapure water show comparable results with bottled water. Both water types used as mobile phase support an accurate quantification of cannabis analytes by HPLC.

An example analysis of the expected costs over a year shows an advantage to using in-house filtered water; the purchase of a device amortizes approx. after 6 months with a daily requirement of 2 L per day and leads to a substantial cost saving within the course of one year.

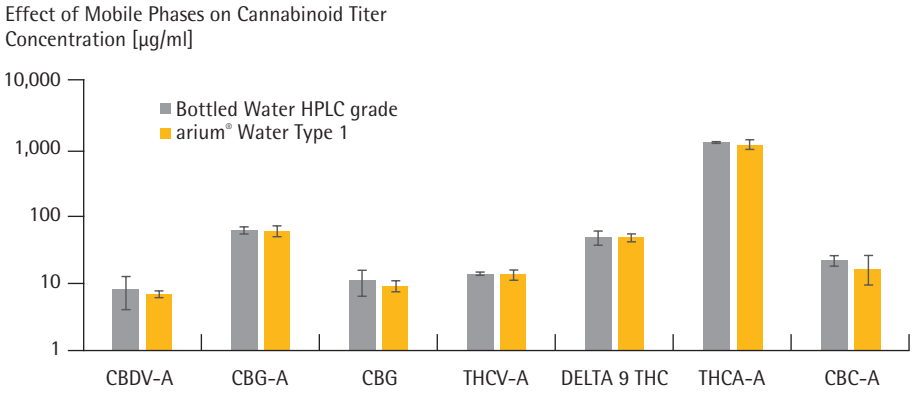

Figure 4: No significant differences in cannabis quantities were found by using two different water sources for mobile phase. The bars show concentration of seven cannabinoids analyzed by HPLC using arium water Type 1 (yellow bars) as mobile phase compared to HPLC-grade bottled water (gray bars)

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