



A COMPLETE SOLUTION FOR THC REMEDIATION AND QUANTITATION OF CANNABINOIDS FROM DISTILLED HEMP EXTRACTS

APPLICATION NOTE

HPLC-UV: AVANT

Flash: Interchim ITM-5015

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In this application note, both the purification and analytical processes for THC remediation using the Interchim ITM-5015 and Advion AVANT™ HPLC-UV systems are shown to form a complete solution for THC remediation in the hemp industry.

INTRODUCTION

Hemp contains hundreds of cannabinoids with Cannabidiol (CBD) being the most prevalent in the plant and Δ^9 -Tetrahydrocannabinol (THC) being the active ingredient causing psychotropic effects. However, many more compounds are formed by the hemp plant and have been investigated for their medical effects.

This limit often requires THC remediation of the distilled hemp extract (starting material) and can be achieved using preparative scale chromatography such as the Interchim ITM-5015 system (Figure 1).

HPLC analysis of the starting material (third pass distillate), fractions collected during the remediation process, and the finished product can be performed using the Advion AVANT HPLC-UV analytical system (Figure 2). Both the purification and analytical processes are shown in this application note to form a complete solution for THC remediation in the hemp industry.

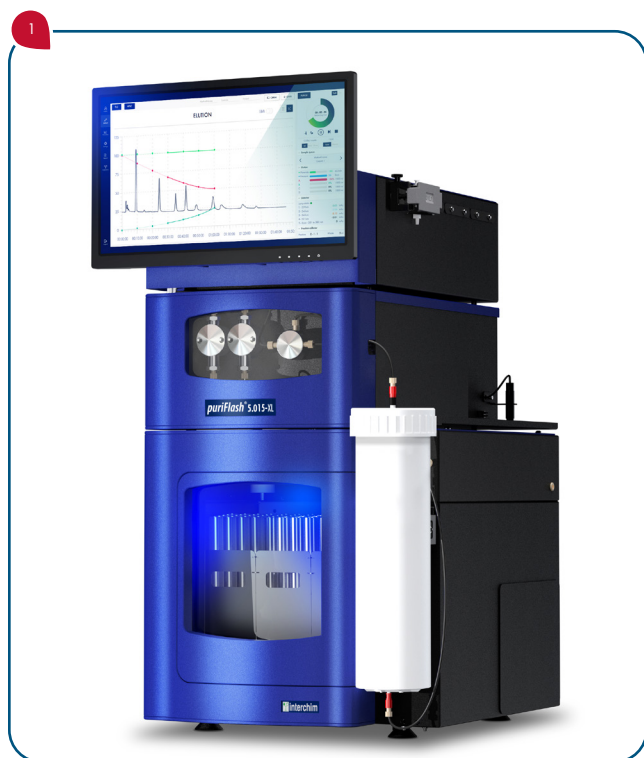


Figure 1: Interchim ITM-5015 flash chromatography system.



Figure 2: Advion AVANT HPLC-UV system.

HPLC-UV METHOD

The HPLC baseline separation of 11 cannabinoids is presented in Figure 3. All 11 cannabinoids elute within 10 minutes whilst achieving baseline resolution. Note that Δ^9 -THC (peak 7) and Δ^8 -THC (peak 8) separation is also possible with the current method.

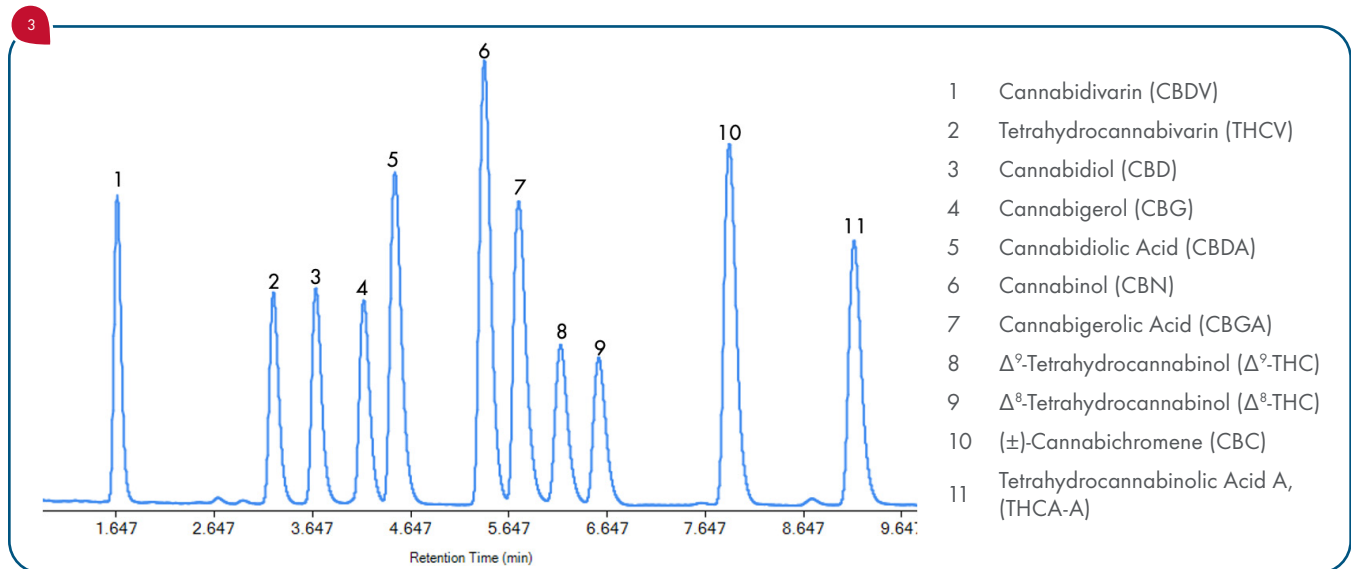


Figure 3: HPLC-UV chromatogram of an 11 Phytocannabinoid standard.

To simplify CBD and Δ^9 -THC analysis during the purification process, a 5 phytocannabinoid standard was used to generate calibration curves for CBD and Δ^9 -THC. The HPLC-UV chromatogram with baseline separation of the 5 phytocannabinoids is shown in Figure 4.

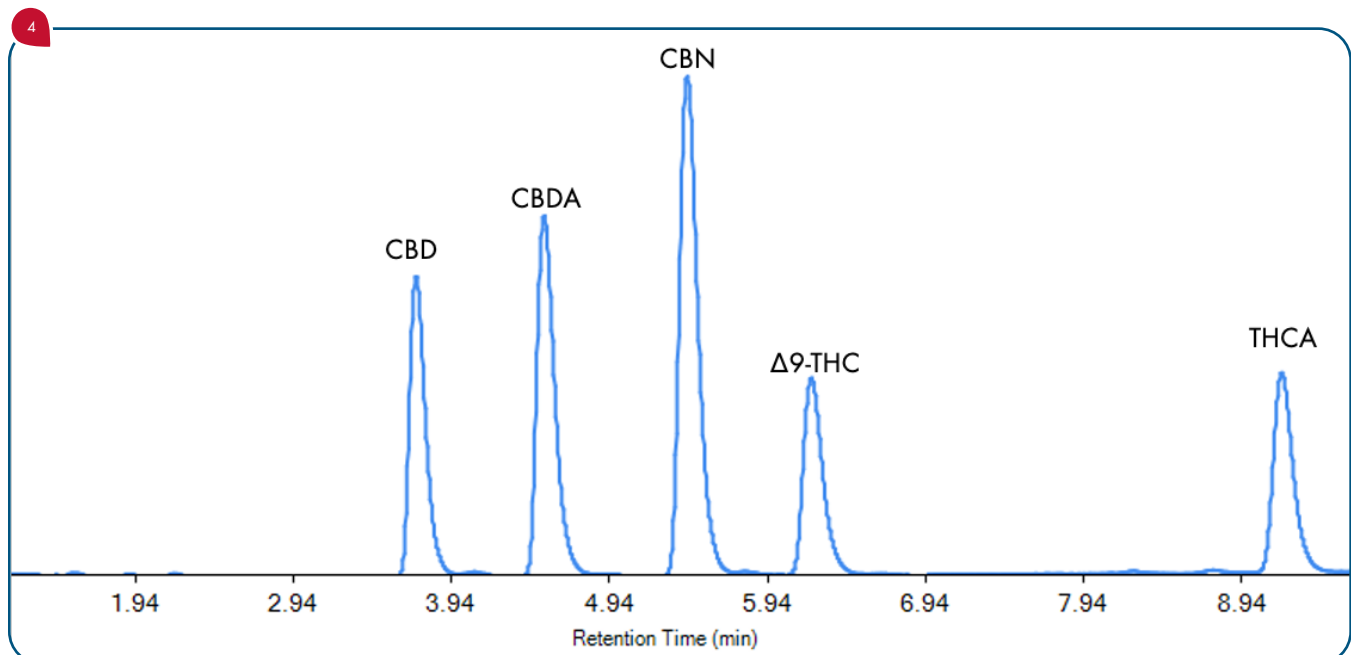
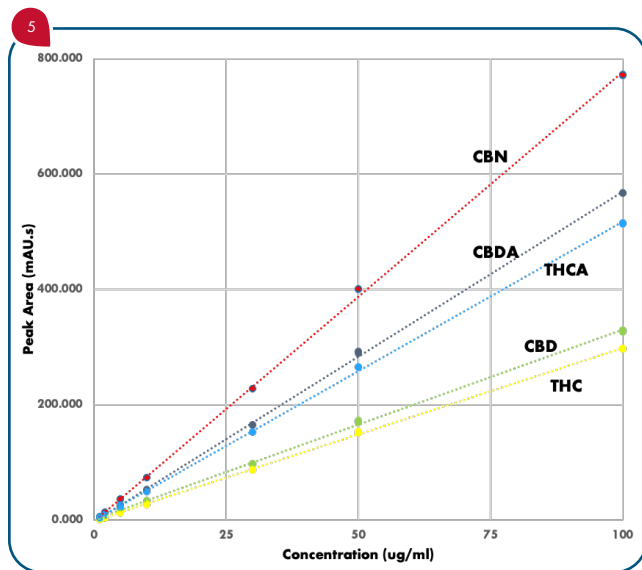


Figure 4: HPLC-UV chromatogram of a 5 Phytocannabinoid standard.

In Figure 5, the calibration curves of the five phytocannabinoids are shown from 1 to 100 $\mu\text{g}/\text{mL}$ with linear regression analysis showing an R^2 factor of greater than 0.9995 (details listed in Table 1).



	CBD	CBDA	CBN	THC	THCA
Slope	3.291	5.725	7.797	3.009	5.189
Intercept	0.765	-2.730	-2.183	-2.117	-1.325
Error Slope	2.712	3.610	5.876	1.856	3.336
LOQ	$\approx 2 \mu\text{g}/\text{mL}$				
LOD	$\approx 1 \mu\text{g}/\text{mL}$				

Figure 5: HPLC-UV calibration curves of 5 phytocannabinoids.

HEMP DISTILLATE ANALYSIS

Before running the third pass distillate (starting material), a small portion of the material was diluted 500 times in methanol and then analyzed by HPLC to determine the amount of CBD and Δ^9 -THC. A typical chromatogram example of hemp distillate analysis is shown in Figure 6.

The CBD concentration in the distillate was calculated to be 238.8 mg/mL and Δ^9 -THC was calculated to be 9.22 mg/mL (3.86% Δ^9 -THC (calculated as a % of CBD in the distillate solution)) - requiring remediation.

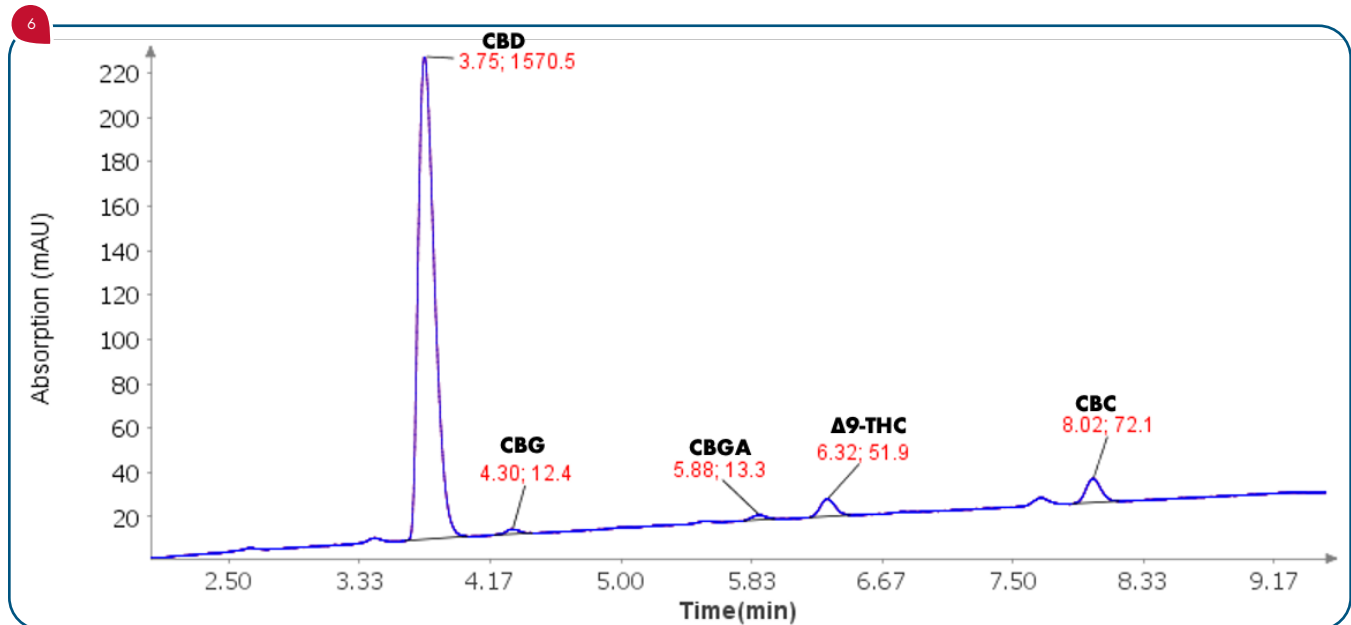


Figure 6: HPLC-UV chromatogram of the diluted 500x hemp distillate (starting material).

THC REMEDIATION

The THC remediation is performed on an Interchim ITM-5015 system with a C18 column ID of 20 cm (MJRP 40x20 cm). An example of a typical UV trace during the purification run is shown in Figure 7.

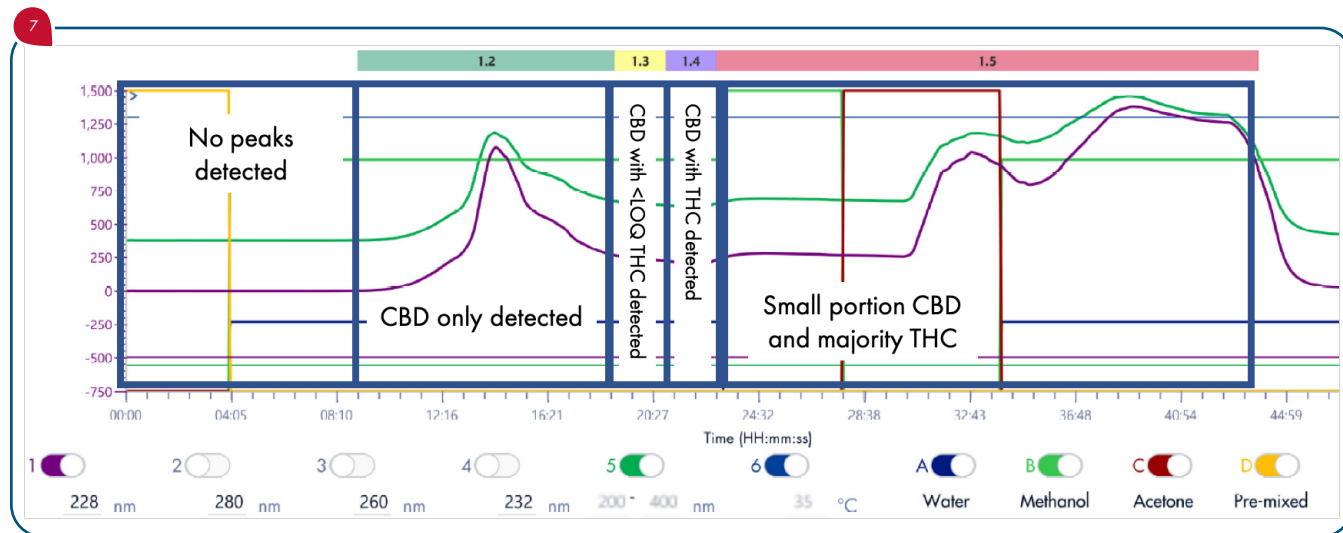


Figure 7: The chromatogram of THC Remediation by Interchim ITM-5015.

To monitor the purification process, fractions were taken at multiple time points during the run and analyzed using the Advion AVANT HPLC method described above. In the fractions collected in the first 9 minutes, no CBD or Δ^9 -THC is detected. These fractions correspond to the void volume of the system and the start of the sample purification without analyte present (see Figure 8A).

CBD is detected starting at the 9 min fraction. Until the 19 min fraction, the CBD collected shows no Δ^9 -THC content (see Figure 8B). From 19 to 23 min, large amounts of CBD and only small amounts of Δ^9 -THC are detected (see Figure 8C). These safety fractions 1.3 and 1.4 indicate the start of the elution of THC from the column. The fractions collected up to 21 min, including safety fraction 1.3 can be used for legal product, whereas the later fraction 1.4 is discarded. Fractions collected beyond 23 minutes contain large amounts of THC and are directed to waste.

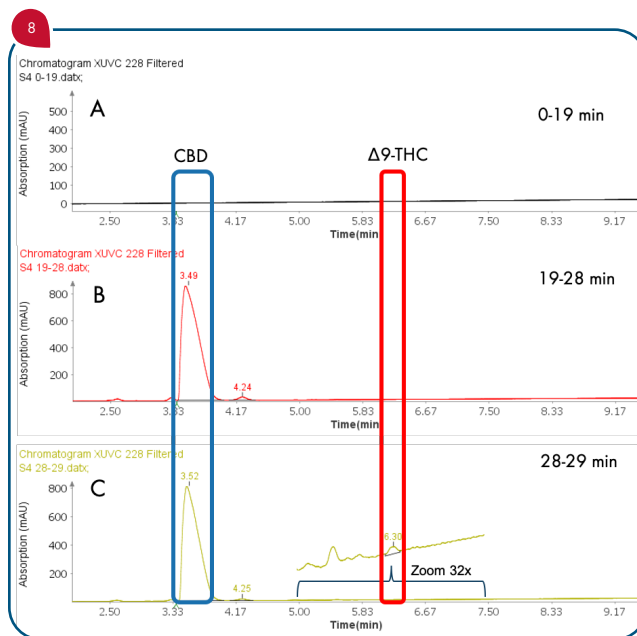


Figure 8: Chromatograms of CBD collection in three time segments.

SUMMARY

A combination of Interchim ITM-5015 large scale THC remediation and Advion AVANT HPLC-UV systems provides a total solution for THC remediation/cannabinoid purification and analytical quantitation quality control of cannabinoids in hemp products from starting material to finished product.

With the demonstrated methods, distilled hemp extract can be purified to provide broad spectrum oil with complete confidence of meeting allowable requirements for Δ^9 -THC concentrations of less than 0.3%.