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HPLC Method for Better Separation of THC Isomers to Ensure Safety and Compliance in the Hemp Market

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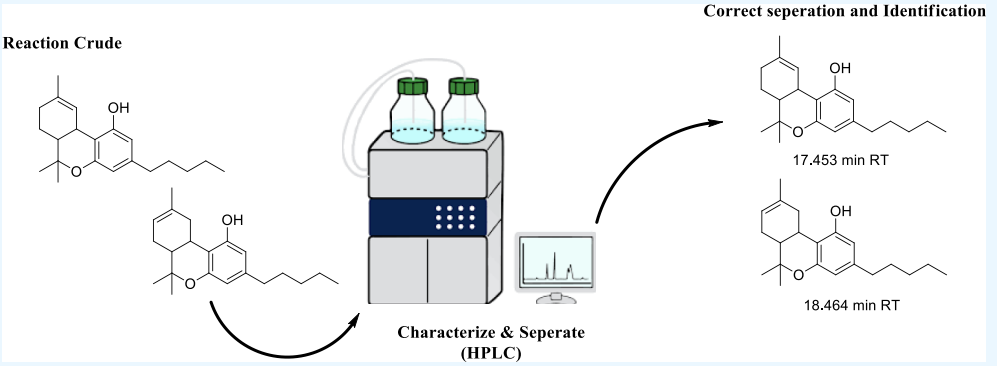

ABSTRACT: The 2018 Farm Bill dictates that delta-9-tetrahydrocannabinol (Δ^9-THC) concentrations must not exceed 0.3% in hemp and hemp-derived products in order to be "compliant." This narrow margin of error necessitates very precise testing methods throughout every facet of the hemp industry. Though gas chromatography has become the industry's gold standard, many hemp laboratories still use high-performance liquid chromatography (HPLC) to quantify cannabinoids, and thus there exists a need for HPLC methods that can separate delta-8-tetrahydrocannabinol (Δ^8-THC) and Δ^9-THC—a notoriously difficult task. This article details one such method, while simultaneously acknowledging the inevitable limits of using HPLC to separate cannabinoids. The method was also used to test Δ^8-THC samples that were marketed as compliant, and it was found that all of the samples contained well over 0.3% Δ^9-THC. The use of refined testing methodologies is crucial for hemp companies to ensure compliance, prevent adverse health effects, and provide consumers with accurate cannabinoid profiles of the products that they purchase.

1. INTRODUCTION

Tetrahydrocannabinol (THC), the primary psychoactive component of *Cannabis sativa*,[1] is accompanied in most consumer-ready products by a slew of minor cannabinoids, most notably CBD, which is nonpsychoactive. In recent years, the synthetic production of THC variants,[2−9] such as Δ^8-THC and Δ^9-THC, has become a focal point within the hemp industry, presenting both opportunities and challenges. While the potential therapeutic benefits of cannabinoids are widely acknowledged, a recent surge in popularity and availability of these products raises concerns about their quality, safety, and legality.

Hemp-derived products and synthetic production go hand-in-hand. The synthesis of Δ^8-THC and Δ^9-THC often begins with CBD isolate, allowing the finished products to fall under hemp regulations thanks to the argument that the starting material and the compounds that result from its conversion are hemp-derived. CBD is transformed through various techniques, from batch and flow chemistry to semi- and even fully synthetic routes. Each transformative technique has the potential for unique contaminants and accidental byproducts, some of which have been identified, such as THC's 30 isomers and enantiomers, and some of which have not.[10] The diversity of Δ^8-THC production methods and their contaminants have created an influx of products into the market that may not adhere to established regulations. Couple that with a lack of standardized testing procedures, and the result is an alarming prevalence of contaminated Δ^8-THC products in various retail outlets—especially in those outside the confines of regulated cannabis spaces - such as gas stations, smoke shops, and convenience stores. The consequences of consuming such products can be severe, with potential health risks stemming from contaminants, impurities, and, notably, illegal concentrations of Δ^9-THC—a compound restricted in hemp and hemp-based products to concentrations <0.3%.[11,12]

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Table 1. HPLC Method for the Separation of Δ^9-THC from Δ^8-THC[a]

time (min)	solvent A (%)	solvent B (%)	flow (mL/min)
0.00	45	55	1.5
5.50	43	57	1.5
6.51	40	60	1.5
27.00	40	60	1.5

[a]Solvent A: H_2O + 0.1% H_3PO_4. Solvent B: ACN + 0.1% H_3PO_4.

Table 2. Calibration Parameters for Target Analytes

	Δ^9-THC	Δ^8-THC
regression equation	7.67x + (−1.64)	6.29x + (−2.32)
R^2	1.00000	1.00000
retention time (min)	17.453	18.464
LOD (ppm)	0.25	0.27
LOQ (ppm)	1.55	1.98

As the science behind the chemical analysis of cannabis expands, gas chromatography (GC) has become the industry's gold standard thanks to its ability to separate cannabinoids that tend to coelute on high-performance liquid chromatography (HPLC)—namely, Δ^8-THC with Δ^9-THC, $\Delta^{4(8)}$-*iso*-THC with Δ^9-THC, and Δ^8-*iso*-THC with Δ^8-THC.[13] Despite this, many hemp companies and the third-party testing laboratories that they rely on for their products' certificates of analysis (COAs) continue to use HPLC to quantify cannabinoids. The reasons for this vary. HPLC cannabinoid methods tend to be faster and more efficient than GC cannabinoid methods, companies are often simultaneously looking for acidic cannabinoids—which are decarboxylated or experience thermal breakdown under heat—or perhaps they simply do not have the budget for more instruments. Whatever the reason, the continued use of HPLC to quantify cannabinoids presents a few problems for the hemp industry, and at the top of the list is the notorious separation of Δ^8-THC and Δ^9-THC.[14] Although there are many published methods for quantifying cannabinoids using HPLC,[15−21] quantification of Δ^9-THC at <0.3% requires precision beyond what typical methods offer, as most do not achieve baseline resolution of Δ^8-THC and Δ^9-THC. Advancements in cannabinoid-based methodology are crucial not only for regulatory compliance, but also to ensure consumer safety, and to prevent adverse health effects associated with contaminated or mislabeled cannabinoid products.[22] In this context, the development of more precise testing methods stands as a critical step toward maintaining industry integrity and safeguarding public health.

2. MATERIALS AND METHODS

2.1. Developed HPLC Method for the Separation of THC Isomers. The HPLC used to develop the method was the Agilent 1100 series with diode array detector, equipped with a Restek Raptor C18 2.7 μm column (150 × 4.6 mm). Solvent A was water (H_2O) buffered with 0.1% phosphoric acid (H_3PO_4). Solvent B was acetonitrile (ACN) buffered with 0.1% H_3PO_4. Solvents were purchased from Sigma-Aldrich (Burlington, MA). Sample injection size was 5 μL, column temperature was 45 °C, flow rate was 1.5 mL/min, and all data was collected at a wavelength of 220 nm. The gradient is available in Table 1. A runtime of 27 min allows for quantification of later-eluding cannabinoids such as cannabichromene and tetrahydrocannabinolic acid, but because Δ^9-THC and Δ^8-THC elude at roughly 17 and 18 min respectively, this method may be shortened depending on the analytes of interest. Certified reference materials (CRMs) were purchased from Cayman Chemical Company, (Ann Arbor, MI) and were diluted upon arrival to nine different concentrations for the development of calibration curves and method validation.

2.2. Method Validation. The concentration and area of each peak was used to create a calibration curve for both Δ^8-THC and Δ^9-THC. R^2 values for the calibration curves of both analytes were 1.00000. Limit of detection (LOD) was calculated based on signal/noise ratio (S/N) = 3, and limit of quantitation (LOQ) was calculated based on S/N = 10. LOD for Δ^9-THC was found to be 0.25 ppm, and LOQ was found to be 1.55 ppm. LOD for Δ^8-THC was found to be 0.27 ppm, and LOQ was found to be 1.98 ppm (Table 2). Accuracy and precision were calculated based on triplicate runs of two batches of standards, diluted to three different concentration levels. Percent relative standard deviation (% RSD) ranged from 0.23 to 1.93 across the two quantified cannabinoids, and percent recovery ranged from 100.38 to 112.90 (Table 3). Therefore, this method is considered both accurate and precise, and future runs will fall within the acceptance limits of 80−120%.

3. RESULTS AND DISCUSSION

The developed method was able to completely resolve Δ^9-THC (17.453 min) and Δ^8-THC (18.464 min) (Figure 1) with just over 1 min between apexes in a 500 ppm, eight-cannabinoid standard (Figure 2). Because this method was developed for the sole purpose of quantifying Δ^8-THC and Δ^9-THC, peak shape

Table 3. Accuracy and Precision Parameters for Target Analytes

			mean	standard deviation	% RSD	% recovery
Δ^9-THC	batch 1	10 ppm	10.04	0.04	0.39	100.38
		50 ppm	50.31	0.41	0.82	100.61
		1000 ppm	1097.09	2.99	0.27	109.71
	batch 2	10 ppm	10.45	0.18	1.76	104.50
		50 ppm	51.58	0.14	0.27	103.16
		1000 ppm	1129.89	2.80	0.25	112.90
Δ^8-THC	batch 1	10 ppm	10.11	0.11	1.11	101.08
		50 ppm	50.29	0.27	0.55	100.58
		1000 ppm	1093.86	2.28	0.21	109.39
	batch 2	10 ppm	10.48	0.20	1.93	104.75
		50 ppm	51.82	0.12	0.23	103.64
		1000 ppm	1119.96	2.84	0.25	112.00

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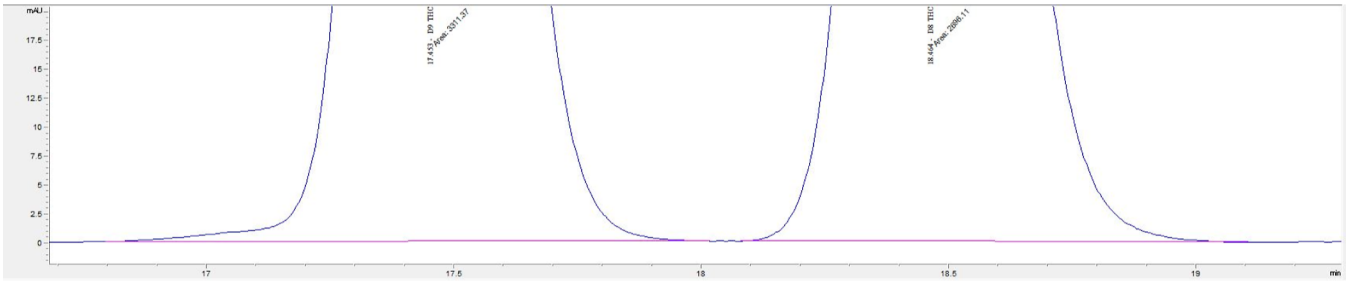

Figure 1. HPLC chromatogram that shows baseline resolution of the Δ^9-THC and Δ^8-THC isomers in a 500 ppm of CRM standard run using the developed methodology.

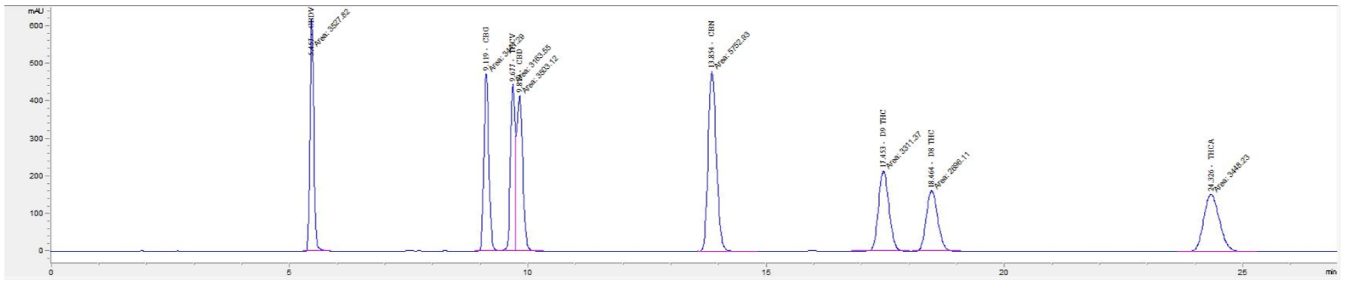

Figure 2. HPLC chromatogram of an eight-cannabinoid, 500 ppm of CRM standard run using the developed methodology.

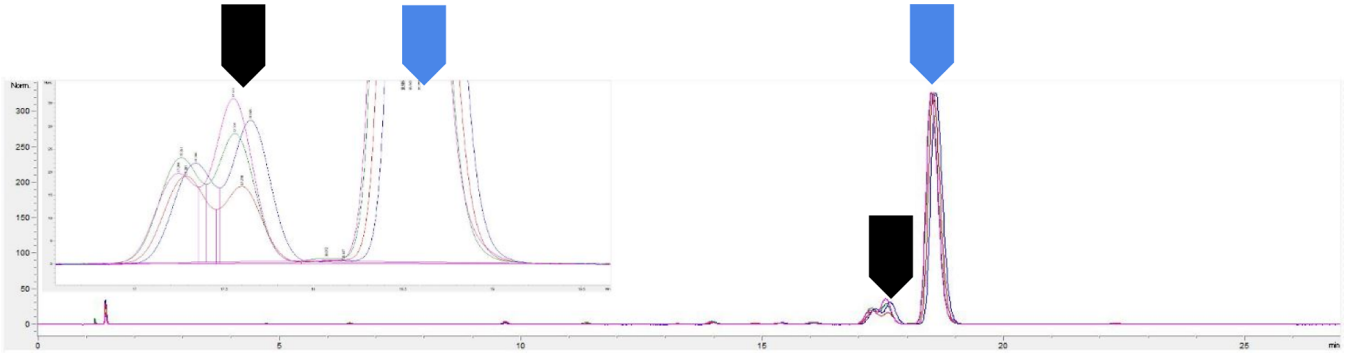

Figure 3. Overlaid HPLC chromatograms of the four supposedly complaint distillate samples. The black arrows denote Δ^9-THC, and the blue arrows denote Δ^8-THC.

and method length were sacrificed in the name of better separation. Of note, this method did not provide adequate separation of tetrahydrocannabivarin and CBD. And while HPLCs traditionally cannot separate Δ^8-THC and Δ^9-THC from all of their many isomers and enantiomers, analysts can rest assured that if their Δ^9-THC peak reads <0.3%, that their product is compliant.

The developed method was then used to test four distillate Δ^8-THC products from two different vendors that were marketed as compliant. All four of the products contained >0.3% Δ^9-THC (Figure 3). In fact, they ranged from 3.3 to 7.1% Δ^9-THC, with a mean of 5.525 ± 1.577% Δ^9-THC (CI = 95%). GC analysis revealed that the peaks were almost entirely Δ^9-THC, as $\Delta^{4(8)}$-*iso*-THC was found only in trace amounts in each of the samples. This is expected, because although $\Delta^{4(8)}$-*iso*-THC is a common byproduct of CBD to THC conversion,[10] it is rarely if ever found in large concentrations. Δ^8-THC results between the four samples were more consistent, with a range of 81.5−83.3% Δ^8-THC, and a mean of 82.625 ± 0.814% Δ^8-THC (CI = 95%) (Table 4). Impurities, including the Δ^9-THC, accounted for a mean of 15.8% of the total chromatographic area between the four samples.

Table 4. Potencies of Supposedly-Compliant Δ^8-THC Distillate Products from Different Vendors

vendor	product	Δ^9-THC (%)	Δ^8-THC (%)
1	1	5.6	83.2
	2	7.1	82.5
2	3	6.1	81.5
	4	3.3	83.3

Willfully falsifying analytical results is a problem across the hemp industry. Companies have been known to make a batch of compliant Δ^8-THC, obtain a third party COA that marks their product as compliant, and then make noncompliant Δ^8-THC and sell it using the compliant COA. This is because compliant Δ^8-THC takes significantly longer and is much more expensive to produce than noncompliant Δ^8-THC. Understandably, compliant Δ^8-THC's price point is also higher. Because the Δ^8-THC will often be mixed into a blend of other minor cannabinoids, the end product, if tested again, may still receive a complaint COA. Regardless, cannabinoid manufacturers have an ethical obligation to ensure that anything marketed as compliant tests as such.

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Using the developed method, companies selling compliant Δ^8-THC can characterize their product batch-by-batch and ensure that their Δ^9-THC content is <0.3%. In a market where demand for such products is ever-growing, it is of the utmost importance that consumers can rely on producers to disclose their true product makeup.[23−25]

4. CONCLUSION

The proliferation of the cannabinoid market, particularly products that consist of hemp-derived Δ^8-THC, underscores the urgent need for robust regulation, accurate testing methodologies, and stringent oversight. The developed method achieves baseline separation of Δ^8-THC from Δ^9-THC, offering a promising avenue toward ensuring accurate results in laboratories that use HPLC to quantify cannabinoids. This separation is especially important in hemp-derived products that are marketed as compliant. Addressing the discrepancies in claimed Δ^9-THC content among commercial products highlights the importance of reliable testing to safeguard consumer health and maintain industry integrity. Moving forward, the industry must prioritize standardized testing protocols to ensure consumer safety and confidence, and to guarantee that regulatory compliance is met.

■ ASSOCIATED CONTENT

Data Availability Statement

All data can be found in the Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03897.

(PDF)

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Author Contributions

M.K.P. and G.A.R. Both authors contributed equally. All authors have read and approved this manuscript for submission. Conceptualization: GAR, WC, TTT. Methodology: MKP, GAR, TTT, WC. Formal Analysis: GAR, WC. Writing Original Draft: GAR, MKP, WC. Writing Review & Editing: MKP, GAR, WC. Supervision: KPR, WC. Project Administration: KPR, WC.

Notes

The authors declare the following competing financial interest(s): GAR, MKP, and TTT are employees of Colorado Chromatography Labs. WC and KPR are founders of Colorado Chromatography Labs.

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■ ABBREVIATIONS

ACN: acetonitrile; CBD: cannabidiol; COA: certificate of analysis; CRM: certified reference material; Δ^8-THC: delta-8-tetrahydrocannabinol; Δ^9-THC: delta-9-tetrahydrocannabinol; GC: gas chromatography; H_2O: water; H_3PO_4: phosphoric acid; HPLC: high performance liquid chromatography; LOD: limit of detection; LOQ: limit of quantitation; % RSD: percent relative standard deviation; *S*/*N*: signal/noise ratio; THC: tetrahydrocannabinol

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