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New perspectives on THCA decarboxylation and accurate GC–MS quantitation of Total THC in *Cannabis* using analyte protectants

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ABSTRACT

Forensic and commercial laboratories rely on well-characterized analytical methods to accurately determine total tetrahydrocannabinol (total THC), which is the sum of decarboxylated tetrahydrocannabinolic acid (THCA) and delta-9-tetrahydrocannabinol (Δ^9-THC) in *Cannabis sativa* samples. The Agriculture Improvement Act of 2018 restricts the level of total THC allowed in *Cannabis* for classification as hemp for commercial purposes. Gas chromatography with mass spectrometry detection (GC–MS) is frequently employed for *Cannabis* measurements due to its simplicity and speed of analysis. However, several issues complicate the determination of total THC by GC–MS, which are well-documented but unresolved. In the current study, the origins of potential GC–MS method biases are investigated, and novel approaches are presented to mitigate interferences. The behavior of THCA and Δ^9-THC during GC–MS analyses was studied extensively using test solutions containing their isotopically labeled analogs. A plant matrix effect was identified that significantly increased THCA and Δ^9-THC responses for *Cannabis* extracts compared with calibrant solutions. A mechanism is proposed based on the interaction of THCA and Δ^9-THC with silanols present on heated inlet surfaces. The use of active site blocking agents, known as analyte protectants, reduced these interactions and achieved suitable conditions for quantitation of total THC by GC–MS. When *Cannabis* plant extracts and calibrants were processed under the recommended conditions, the results were comparable to liquid chromatography with photodiode array (LC-PDA) analysis. The experimental findings ultimately provide evidence to explain the behavior of cannabinoids in the GC–MS system and offer new options for improving the accuracy of total THC measurements.

1. Introduction

Cannabis sativa is the most commonly used recreational drug in the United States (U.S.) [1], and its increasing prevalence significantly impacts public health, medicine, criminal justice, industry, and forensics [2]. According to a 2022 survey, about 22 % of people (61.9 million) over the age of 12 in the U.S. reported using *Cannabis* in the past year [3]. *Cannabis* and its primary psychoactive constituent, delta-9-tetrahydrocannabinol (Δ^9-THC), have been classified as illicit controlled substances in the U.S. since the 1970s [4]. However, legislative changes over time resulted in the decriminalization or legalization of *Cannabis* for medicinal and recreational purposes in many states [5]. Furthermore, the 2018 Agricultural Improvement Act (Farm Bill) defined hemp as *Cannabis* containing a mass fraction of less than or equal to 0.3 % total THC on a dry mass basis and removed hemp from the controlled substances list [6]. Total THC refers to the sum of decarboxylated delta-9-tetrahydrocannabinolic acid (THCA) and Δ^9-THC (see Eq. 1). Consequently, as the legal landscape changed, an emerging *Cannabis* industry concurrently expanded with the marketing of cannabinoid-containing products, such as edibles, extracts, oils, and vapes [7].

$$\text{Total THC} = (0.877 \times \text{THCA}) + \Delta^9 - \text{THC} \quad (1)$$

Accurate and reliable methods for total THC analysis are indispensable to forensic, commercial, and compliance testing laboratories. Forensic laboratories are responsible for providing scientific evidence to support findings regarding the legality of seized evidence containing *Cannabis* plant material. Historically, qualitative test schemes, such as macro- and microscopic identification of plant features and colorimetric testing, were employed for this purpose [8,9]. After the Farm Bill was established in 2018, forensic laboratories adapted their procedures to measure total THC and determine whether the *Cannabis* sample is marijuana (a federally controlled substance) or hemp (a legal commodity) according to the 0.3 % total THC threshold [10]. Furthermore,

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manufacturers of cannabinoid-containing products created for consumers must provide accurate labeling for consumer safety and transparency [7]. Independent testing laboratories offer analytical services to evaluate their clients' products to ensure compliance with state and federal regulations. Therefore, establishing dependable methods for total THC analysis is paramount as the market for *Cannabis*-derived products grows.

The quantitative analysis of other drugs of abuse is commonplace in forensic chemistry, and the accumulated knowledge applies to *Cannabis* measurements as well. Liquid chromatography (LC) and gas chromatography (GC) coupled to mass spectrometry (MS) are perceived as "gold standard" methods for quantitation in forensic chemistry [11]. Both chromatographic techniques provide identification through retention time matching and quantitation using integrated peak areas. Mass spectrometry detection offers a high level of selectivity and specificity through the structural information in the mass spectra from the mass-to-charge (m/z) ratios of the fragment ions [11]. Seized drug sections within forensic labs generally prefer GC over LC due to the shorter chromatographic separation times and use of compressed gases instead of costlier solvents for the mobile phase [12]. Because of its sensitivity, robustness, familiarity, simplicity, and speed [13], a reliable GC–MS method for total THC analysis would undoubtedly provide value to forensic laboratories seeking updated quantitative procedures.

Unfortunately, several factors complicate GC-based measurements of total THC. Gas chromatography inherently requires high temperatures for sample introduction and elution. Under these conditions, THCA decarboxylates to Δ^9-THC through the loss of CO_2, accounted for by the conversion factor in Eq. 1 (see Fig. 1) [14]. At first glance, the conversion appears to simplify the analysis since a single chromatographic peak would determine the total THC (i.e., Δ^9-THC). However, it is widely reported that THCA is incompletely converted to Δ^9-THC by GC methods [15]. Dussy et al. reported 70 % conversion efficiency using a packed glass wool injector, with the extent of conversion varying with the injector temperature [16]. These observations naturally lead to the following questions: what happens to the remaining THCA if it's not fully converted to Δ^9-THC? Since the liner material and inlet temperature are known to affect the decarboxylation rate [17], by what mechanisms does the liner interact with THCA? Given these issues, what instrumental conditions and analytical method approaches are needed to consistently produce accurate quantitation? Unless the decarboxylation process is fully understood, and all species are quantitatively accounted for, the potential for measurement bias cannot be eliminated.

In the present study, a GC–MS method was first developed to separate Δ^9-THC from eight other cannabinoids in under ten minutes. Next, isotopically labeled analogs of THCA and Δ^9-THC were utilized to study the decarboxylation of THCA under different instrumental conditions. During these experiments, a plant matrix effect for both compounds was discovered that was causing biases affecting quantitation. A new hypothesis was proposed that THCA and Δ^9-THC adsorb to active sites in the GC system, which was mitigated by the presence of the *Cannabis* plant matrix. Further studies using analyte protectant compounds to block the active sites provided additional evidence supporting the proposed theory. Finally, a proof of concept was demonstrated for solving the challenges encountered in GC–MS measurements of total THC by utilizing analyte protectants and accurately quantifying total THC in three *Cannabis* plant sample extracts.

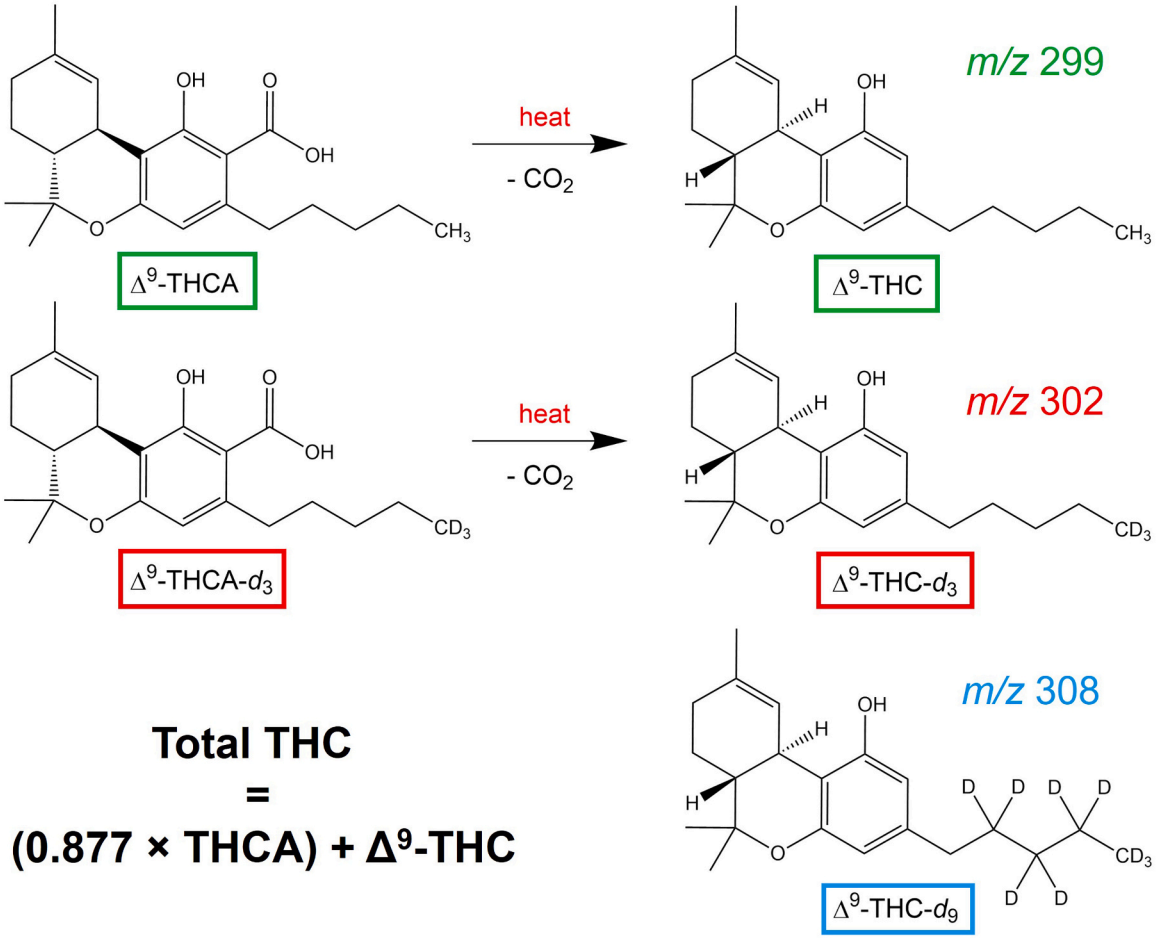

Fig. 1. Diagram showing the structures of the standards used in this study for THCA and Δ^9-THC, including their heat-induced decarboxylation. The most abundant ion (m/z) in their mass spectra was used for differentiation in the SIM method.

2. Experimental

2.1. Chemicals and materials

Ampouled calibration stock solutions of cannabidivarin (CBDV), cannabicyclol (CBL), cannabidiol (CBD), tetrahydrocannabivarin (THCV), cannabichromene (CBC), delta-8-tetrahydrocannabinol (Δ^8-THC), delta-9-tetrahydrocannabinol (Δ^9-THC), cannabigerol (CBG), and cannabinol (CBN) were obtained from Cerilliant (Round Rock, TX, USA) for method development. Cannabinoid stock solutions (1 mg/mL) of THCA in acetonitrile (ACN), THCA-d_3 in ACN, Δ^9-THC in methanol (MeOH), and Δ^9-THC-d_3 in MeOH were obtained from Cerilliant. A stock solution (1 mg/mL) of Δ^9-THC-d_9 in MeOH was obtained from Cayman Chemical (Ann Arbor, MI, USA). The structures of the cannabinoids are presented in Fig. 1 and their mass spectra in Figs. S1-S5. HPLC grade ACN and MeOH for preparing calibration solutions and extracting *Cannabis* samples were obtained from Fisher Scientific (Pittsburgh, PA, USA). Three *Cannabis* plant samples (A, B, and C) were analyzed in this study and stored in the dark at −80 °C in a freezer. Sample A was obtained from a University of Kentucky Proficiency Testing Program [18] (HM20SEP-2). Samples B and C were obtained from a commercial online source.

Different molecules and matrices were tested as silanol blocking reagents to evaluate aspects of the plant matrix effect. Standard Reference Material (SRM) 1570a, Trace Elements in Spinach Leaves, was acquired in-house as a plant matrix surrogate. A hemp blank matrix was obtained from Emerald Scientific (San Luis Obispo, CA, USA). Several chemicals were obtained from commercial sources for evaluation as analyte protectants. L(−)-Menthol and decanoic acid were obtained from Thermo Scientific (Fair Lawn, NJ, USA). 2,7-Naphthalenediol was obtained from Eastman Organic (Rochester, NY, USA). Polyethylene glycol (PEG-300) was obtained from TCI America (Portland, OR, USA). D-Sorbitol, 3-ethoxy-1,2-propanediol, gluconolactone, shikimic acid, and ethanolamine were acquired from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of calibrants and silanol-blocking mixtures

Seven calibration solutions were prepared in MeOH using the Δ^9-THC stock solution. Calibration solutions were spiked with Δ^9-THC-d_3 at a target mass concentration of 3.75 mg/L, to match total THC levels in *Cannabis* extracts at the 0.3 % threshold. Two silanol-blocking mixtures were evaluated as analyte protectants (APs). The first mixture (AP Mixture 1) was prepared by dissolving 3-ethoxy-1,2-propanediol, sorbitol, gluconolactone, and shikimic acid in acetonitrile. A second mixture (AP Mixture 2) was prepared by dissolving decanoic acid, menthol, PEG-300, ethanolamine, and 2,7-naphthalenediol in MeOH. The mixtures were stored at −20 °C when not in use. Chromatograms from injection of these two mixtures are shown in Figs. S6 and S7.

2.3. Sample preparation

The *Cannabis* plant samples were extracted following the guidelines from an official AOAC method [19] modified by using methanol instead of ethanol (Fig. S8) [20]. Previous studies were performed to thoroughly evaluate important parameters of the extraction method, such as sample grinding, sample mass, solvent choice, solvent volume, and number of extraction cycles [20]. The optimized extraction method utilized herein achieved accurate quantitation when used to measure total THC by LC with photodiode array detection (PDA) in well-characterized reference materials and samples [20]. Three replicates of the *Cannabis* samples were weighed at approximately 0.50 g ± 0.05 *g* into 50 mL polypropylene centrifuge tubes. MeOH (20 mL) was added gravimetrically to each sample, and the mixture was vortex-mixed for 10 s, shaken mechanically for 30 mins, and centrifuged for 5 mins at 104.72 rad/s (1000 rpm). The MeOH layer was decanted into a new centrifuge tube, and 20 mL of fresh MeOH was added to the remaining *Cannabis* sample for a second extraction by repeating the process. Sample extracts were combined (40 mL) and filtered with a 0.45 µm polytetrafluoroethylene (PTFE) membrane filter. All samples and extracts were stored in a −80 °C freezer in the dark. The same set of sample extracts was used to prepare LC-PDA and GC–MS samples for comparison. For LC-PDA measurements, the filtered sample extracts were diluted with MeOH to achieve a tenfold dilution. For GC–MS measurements, the filtered sample extracts and calibrants were spiked with the Δ^9-THC-d_3 internal standard working solution to achieve a tenfold dilution of the original sample extract.

2.4. Liquid chromatography – photodiode array detection

LC-PDA measurements were performed on a Shimadzu *Cannabis* Analyzer instrument (Prominence-i C-2030C 3D Plus) equipped with a photodiode array detector (PDA), binary pump, degasser, autosampler, and column compartment (Shimadzu Scientific, Columbia, MD, USA). The instrument was controlled by the accompanying LabSolutions software. Analyses were performed on a NexLeaf CBX for Potency C_{18} column with a corresponding guard column (15.0 cm length, 4.6 mm diameter, 2.7 µm average particle diameter) from Shimadzu. The method parameters were previously optimized by Shimadzu [21] as a "high sensitivity method" (Table S1) and utilized in prior analyses [22] of hemp products.

2.5. Gas Chromatography-Mass Spectrometry (GC–MS)

Analyses were performed on an HP 6890N gas chromatograph (Agilent, Wilmington, DE, USA) coupled to a 5973 series single quadrupole mass spectrometer with electron impact (EI) ionization. The GC–MS method parameters were developed and optimized in-house and summarized in Table 1. All analyses were performed on 15 m × 0.25 mm × 0.25 µm capillary GC columns containing a 35 % phenyl stationary phase (DB-35 ms UI from Agilent and Rxi-35Sil MS from Restek). Blank MeOH injections were run between each sample and calibrant to ensure no carryover. The system was tuned as necessary before analyses using the atune.u function. Raw data was acquired and processed with ChemStation software (version D.01.02.16) and the NIST Mass Spectral Search Tool (version 2.0 d).

Table 1
Optimized GC–MS parameters used for the analysis of *Cannabis* plant extracts.

GC–MS			
GC parameters			
Inlet	Split/splitless with 4 mm ID precision Split wool liner		
Injection volume	1 µL using 5 µL syringe		
Inlet temperature	280 °C		
Column	DB-35 MS UI or Rxi-35Sil MS 15 m length × 0.25 mm i.d. × 0.25 µm film thickness		
Carrier gas flow	1.2 mL/min of helium		
Oven program	Temp. (°C)	Ramp (°C/min)	Hold time (min.)
	205 °C	Initial	0 min
	230 °C	25 °C/min	0 min
	235 °C	5 °C/min	0 min
	240 °C	2.5 °C/min	2 min
	250 °C	10 °C/min	0 min
	300 °C	40 °C/min	0.75 min
MS parameters			
Transfer line	280 °C		
MS temperatures	Source: 230 °C quadrupoles: 150 °C		
Solvent delay	2 min		
Full scan range	*m/z* 50 – *m/z* 350		
SIM mode			
Quantitative	*m/z* 299 and *m/z* 302		
Qualitative	*m/z* 231, *m/z* 271, and *m/z* 314		
Dwell time	50 ms for each ion		

3. Results and discussion

3.1. GC–MS method development

This research was undertaken to support the development of an accurate and rapid GC–MS method to quantify Δ^9-THC in forensic laboratories requiring high throughput analyses. Priority was given to the separation of Δ^9-THC from eight other commonly encountered neutral cannabinoids at time of study, including CBDV, THCV, CBL, CBC, CBD, Δ^8-THC, CBG, and CBN [14,23]. A stock mixture containing equal quantities of each cannabinoid (20 µg/mL) was prepared in MeOH for method development. For cannabinoid analyses, 5 % phenyl or 35 % phenyl stationary phases are most commonly used, with column lengths typically 15 m or 30 m [23,24]. An initial evaluation indicated an improved separation of Δ^9-THC from the other cannabinoids was possible with the 35 % phenyl stationary phase. Elution time was decreased by utilizing a 15 m column and optimizing the temperature program. Column temperature was briefly ramped to 300 °C at the end of each run to remove any retained compounds before the next run. After adjusting the temperature program and flow rate parameters, a full-scan GC–MS method for separating Δ^9-THC from other compounds was established (Fig. 2A). Selected ion monitoring (SIM) of multiple ions was employed throughout to improve sensitivity for quantitation [25] using the most abundant ions (*m/z* 299, *m/z* 314, *m/z* 271, and *m/z* 231) for Δ^9-THC (see Figs. S1-S2). Solutions of Δ^9-THC dissolved in MeOH were prepared to determine the limits of detection (LOD) and quantitation (LOQ) using the built-in signal-to-noise ratio (SNR) tool in Chemstation. The LOD (SNR = 3) of the SIM method was 0.1 µg/mL, and the LOQ (SNR = 10) was 0.25 µg/mL. An example SIM chromatogram of a hemp plant extract is shown in Fig. 2B.

An isotope dilution (ID) approach was used to facilitate the study of solute-surface interactions and to identify potential sources of biases. With ID-GC–MS, an isotopically labeled analog of the analyte is used as an internal standard. For simplicity, an equal quantity of the internal standard is spiked into the calibrants and samples. Extracted ion chromatograms (EIC) are obtained separately for the calibrants and samples, and the ratio of the peak areas for the analyte and internal standard (A/IS) are determined. Analyte levels are determined from a response relationship (i.e., a calibration curve or response function) normalized with the internal standard. ID-MS measurements are less susceptible to certain types of interferences and biases originating from ion suppression or enhancement, injection volume variations, and matrix interferences. In this study, Δ^9-THC-d_3 was selected as the internal standard since it is easily distinguished from Δ^9-THC by MS. Although labeled and unlabeled standards typically coelute (see Fig. S9), the responses for Δ^9-THC and Δ^9-THC-d_3 are obtained separately by extracting ions at *m/z* 299 for Δ^9-THC and *m/z* 302 for Δ^9-THC-d_3. The most abundant ion for the internal standard was utilized in the SIM method.

3.2. Evaluation of decarboxylation efficiency

The inlet temperature is a critical parameter reported to affect the heat-induced decarboxylation of THCA to Δ^9-THC [26]. The effect of inlet temperature on decarboxylation efficiency (DE) was studied here using a solution containing equal molar quantities of THCA-d_3 and Δ^9-THC-d_9 dissolved in MeOH. Upon injection, THCA-d_3 is converted to Δ^9-THC-d_3 by decarboxylation, and Δ^9-THC-d_9 remains unchanged. These species coelute but are individually detected at *m/z* 302 and *m/z* 308, respectively. The ratio of the responses for Δ^9-THC-d_3 and Δ^9-THC-d_9 provide a direct measure of DE that can range from 0 % to 100 % (Eq. 2):

$$DE = R_{THCA}/R_{THC} \times 100\% \quad (2)$$

where R represents the signal response for the peak derived from the differentiable THCA or Δ^9-THC species in the mixture. This test mixture offers laboratories a simple and effective way to evaluate the DE under specified instrumental conditions. The mixture can evaluate DE in samples and calibrants despite the presence of native levels of Δ^9-THC (*m/z* 299) because the mass fragments are easily resolved by MS. The mixture was injected repeatedly across a range of inlet temperatures (see Fig. 3). Poor sensitivity was predictably observed at lower inlet temperatures due to the low volatility [27] of the cannabinoids, and analyte responses increased at higher inlet temperatures. Furthermore, the ratio

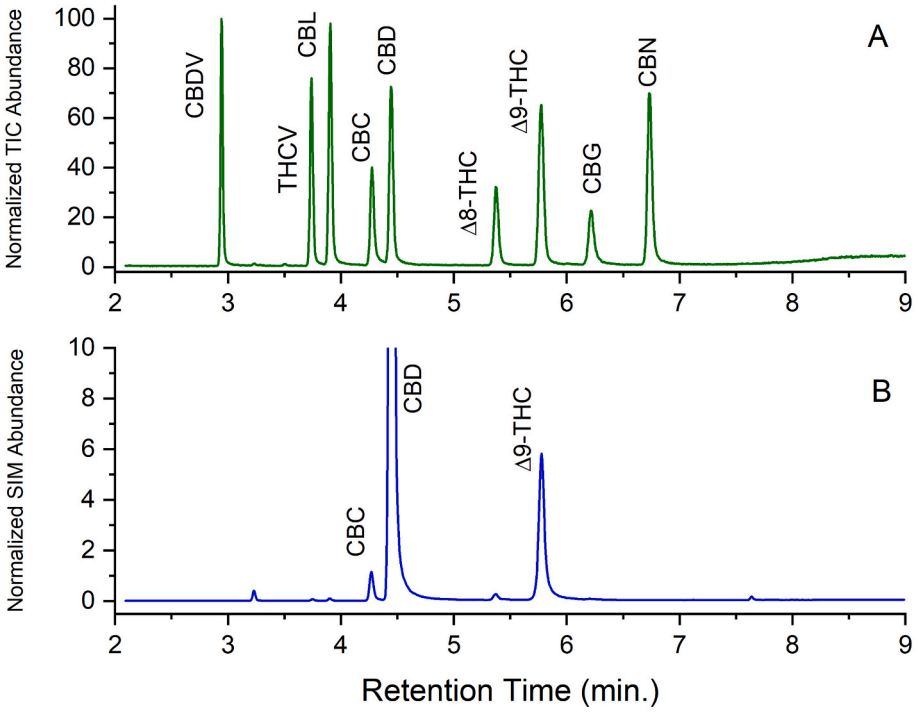

Fig. 2. GC–MS separation of nine neutral form cannabinoids with TIC detection (*m/z* 50 to *m/z* 350) (A), and analysis of a hemp plant extract with SIM detection at *m/z* 299, *m/z* 314, *m/z* 271, and *m/z* 231 (B), using the optimized GC–MS method summarized in Table 1.

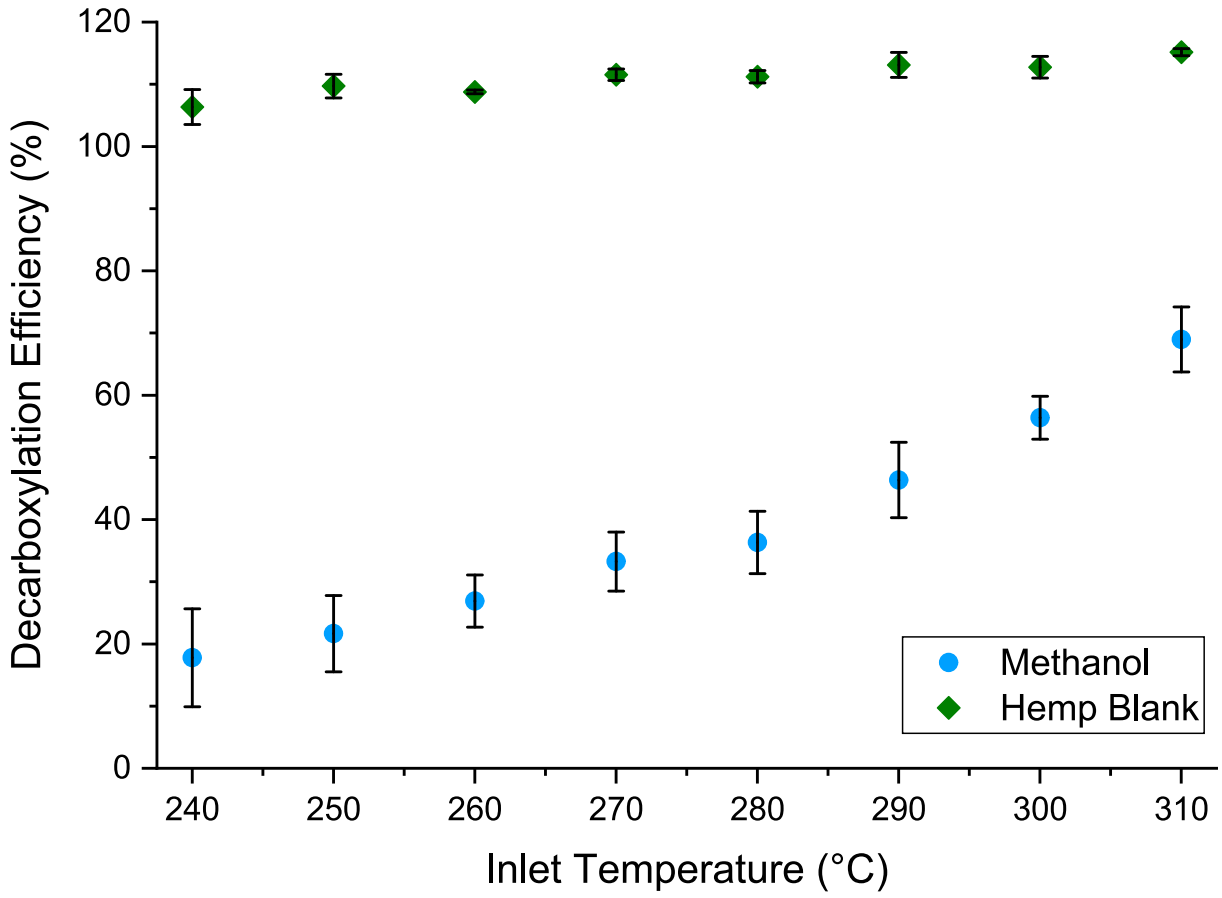

Fig. 3. Influence of inlet temperature on the decarboxylation of THCA for MeOH and hemp blank extract solutions using a test mixture containing THCA-d_3 and Δ^9-THC-d_9 (see text for details). Error bars correspond to one standard deviation from the mean value ($N = 3$).

of responses for decarboxylated THCA-d_3 and Δ^9-THC-d_9 in MeOH varied with temperature from DE ≈ 20 % at 240 °C to DE ≈ 70 % at 310 °C (see Fig. 3).

3.3. Plant matrix effect

Variability in the internal standard response became apparent during the initial SIM method development. A calibrant was prepared by dissolving Δ^9-THC in MeOH at a concentration corresponding to a *Cannabis* plant extract sample. Δ^9-THC-d_3 was then spiked equally to the calibrant and plant samples to match the concentration of total THC. Under these conditions, the signal response for Δ^9-THC-d_3 is expected to be similar in both the calibrant and plant samples. However, the response for Δ^9-THC-d_3 was significantly higher in the plant extracts. The experiment was repeated using a similar test mixture of THCA-d_3 and Δ^9-THC-d_9 (see Section 3.2) to observe both species separately (see Fig. S10). Remarkably, the responses for the labeled probes were more prominent in the plant extracts, and the extent of THCA-d_3 decarboxylation appeared to increase as well. An extracted total ion chromatogram for the unspiked plant extract confirmed the absence of interferences at *m/z* 302 and *m/z* 308. Efforts were redirected toward studying the cause of the signal increase in the plant extracts relative to calibrants.

GC–MS signal increases for analytes in plant extracts are a well-studied phenomenon referred to as "matrix-induced chromatographic response enhancement" [28]. Detector responses for certain analytes increase in a plant extract versus a neat solvent [29]. Analyte polarity is a factor that determines susceptibility to this effect. Polar functional groups, such as amines, hydroxyls, carboxylic acids, and thiols, can degrade peak shape in GC due to interactions with silanol groups [30] and result in adsorption, peak tailing, and loss of analytes [29]. The inlet liners and glass wool components are manufactured with passivated surfaces to minimize active sites; however, the deactivation becomes less effective over time [30]. Plant extracts contain high levels of biopolymers and other compounds that interact with silanols to prevent the adsorption of polar analytes [28].

The observed signal increase was further evaluated using two different plant-based matrices. SRM 1570a, which consists of powdered dried spinach leaves, was selected as a cannabinoid-free, green leaf matrix. A hemp blank matrix that did not contain Δ^9-THC served as a *Cannabis* blank. Diluted and undiluted spinach leaf and hemp extracts were spiked with approximately equimolar amounts of differentiable labeled THCA and Δ^9-THC species to assess DE. The signal response increased with the extract concentration for the spinach leaf (Fig. S11) and hemp extracts (Fig. 4). Moreover, the ratio of THCA-d_3 to Δ^9-THC-d_9 increased in the hemp blank extracts relative to the pure solvent. Complete decarboxylation (DE ≈ 100 %) of THCA-d_3 was achieved in the presence of either plant extract, but for MeOH calibrant solutions, less than complete conversion was obtained. The test solution containing THCA-d_3 and Δ^9-THC-d_9 was also prepared in ACN with similar results to MeOH. Furthermore, when the previous inlet temperature study was repeated by dissolving the labeled standards in the hemp blank matrix, the DE improved to near 100 % across all temperatures (Fig. 3).

The experiments with the plant extracts confirmed a significant observation that has important implications. Since the signal response increased for both the THCA and Δ^9-THC species, variations in analyte response cannot be solely attributed to THCA decarboxylation. Lower injection temperatures promote analyte adsorption in the inlet, whereas higher temperatures minimize adsorption. Preferential adsorption of THCA compared to Δ^9-THC may occur due to differences in polarity. The

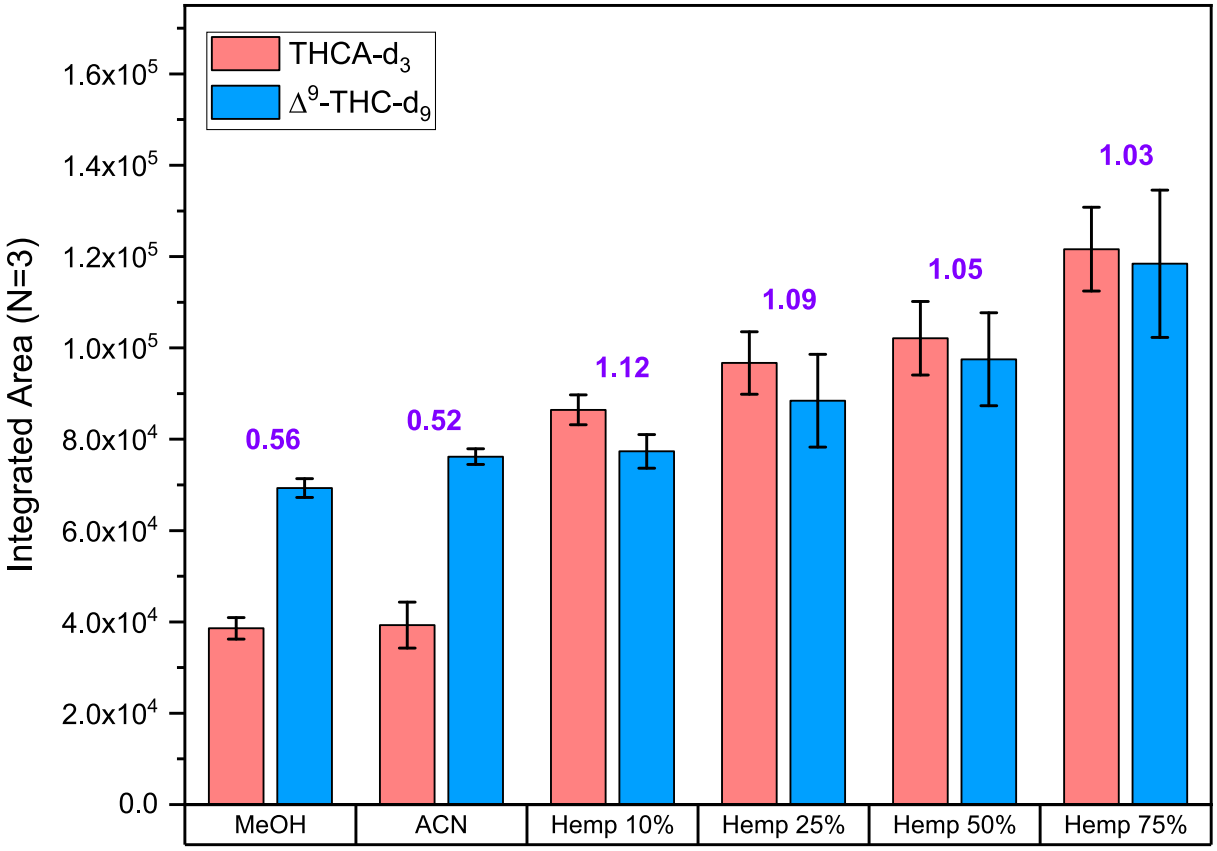

Fig. 4. Matrix-induced signal effects for THCA-d_3 and Δ^9-THC-d_9 in hemp blank extracts, methanol, and acetonitrile. Hemp blank extracts were diluted with methanol to 10 % - 75 % of the original extract. Values above the bar graphs represent the decarboxylation efficiency (DE). Error bars correspond to one standard deviation of the integrated area for THCA-d_3 or Δ^9-THC-d_9 (N = 3).

selective loss of THCA may be due to stronger adsorption rather than incomplete decarboxylation. Additionally, it is possible that constituents from the plant matrix are temporarily adsorbed at polar sites, which reduces surface activity (see graphical abstract). Increased levels of these plant constituents would compete more effectively for these sites providing higher signal response of THCA and Δ^9-THC as they are released. Comparable responses of equimolar quantities of spiked THCA-d_3 and Δ^9-THC-d_9 in plant extracts are consistent with this assumption.

The differences in response noted for plant extracts have important implications for the quantitation of total THC by GC–MS since calibrants are typically prepared in pure solvents, and samples are prepared as plant extracts. Unless corrections are made, differences in adsorption between calibrants and samples will lead to measurement biases. External standard calibration approaches are more susceptible to such biases compared to internal standard calibration. Isotopically labeled internal standards should compensate for differences in analyte adsorption for calibrants and extracts since the internal standards exhibit similar adsorption as the analytes, and the analyte/internal standard ratio would be consistent in the presence or absence of plant matrix constituents. In contrast, differences in analyte adsorption for solutions and extracts would directly affect external standard approaches. Since the response for calibrants prepared in neat solutions is suppressed by adsorption, the resulting levels determined for plant extracts would be overestimated. The extent of this bias would depend on the concentration of plant matrix constituents in sample extracts and the corresponding signal increase (Fig. 4).

The level of injection liner activity may exacerbate potential measurement biases. Although most GC injection liners are manufactured with proprietary deactivation chemistries, their surfaces degrade over time and activity increases [31]. New injection liners should initially decrease adsorption and enhance the desorption and decarboxylation of THCA, an effect that will lessen over time as reactive silanol groups form on the surfaces. To study this supposition, an equimolar solution of THCA-d_3 and Δ^9-THC-d_9 was repeatedly analyzed using a new glass wool injection liner, alternating MeOH injections between runs. In Fig. 5, the area ratio of THCA-d_3 to Δ^9-THC-d_9 decreases as the number of injections increases. Tsujikawa et al. observed that for α-pyrrolidinopentiophenone, active sites caused decomposition, which was mitigated by using a new liner [31]. Laboratories should exercise caution when assuming that complete desorption and decarboxylation has been achieved since the effect is transitory based on liner degradation over time.

3.4. Silanol blocking agents

Previous studies have shown that adding blank plant extracts to both calibrants and samples can improve the accuracy of botanical sample quantification [28]. Obtaining suitable blank matrices is not always practical and the addition of complex matrix constituents may introduce new measurement challenges, especially if the added components coelute and interfere with the targeted analytes. In addition, the introduction of plant matrix components can shorten column lifespan and contaminate the MS source. A related approach utilizes analyte protectants (APs) to act as silanol blocking agents for achieving uniform responses for neat solutions and extracts. APs are polar compounds that bind with active sites to reduce analyte-silanol interactions, permitting the analyte to reach the detector and thereby increasing signal response. The effectiveness of APs is influenced by the strength of the interactions between protectants and silanols, as well as the concentration of the protectants.

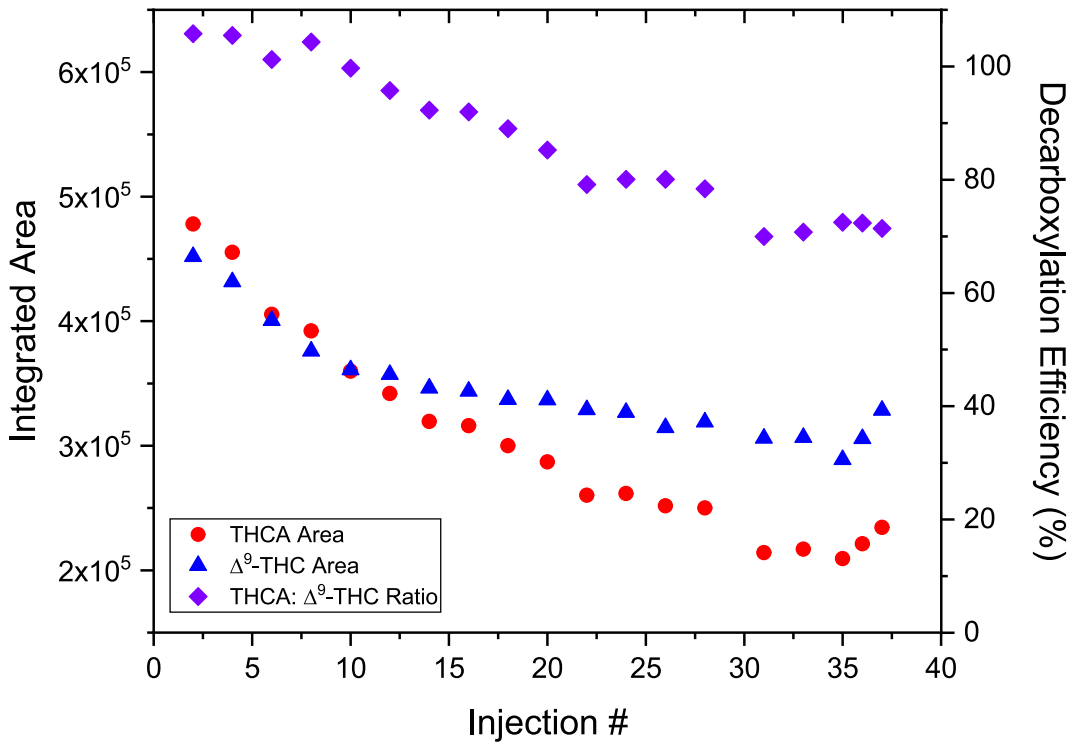

Fig. 5. Results from repeated injections of an equimolar mixture of THCA-d_3 and Δ^9-THC-d_9 in MeOH with a new glass wool liner.

A mixture of APs (AP Mixture 1, Fig. S12) described by Maštovská et al. [32] consisting of ethylglycerol, gluconolactone, sorbitol, and shikimic acid was initially evaluated here for THCA and Δ^9-THC. Solutions containing THCA or Δ^9-THC with AP Mixture 1 were analyzed, and increased responses were observed for both probe molecules (see Fig. S12). Although AP Mixture 1 initially appeared promising, the sugar derivatives were incompatible with MeOH, causing blockage of the syringe plunger over time [33]. Other reported APs include decanoic acid [34], menthol [34], and polyethylene glycol (PEG) [33], as well as ethanolamine and 2,7-naphthalenediol for their suitable molecular properties. A second mixture (AP Mixture 2) containing these five compounds was prepared for evaluation with THCA and Δ^9-THC (Fig. 6). The signal increase observed previously for plant extracts was also observed for AP Mixture 2, without blockage of the syringe. Analyte responses increased with the concentration of the protectants, and the signal increase began to approach a maximum (see Fig. S13) at

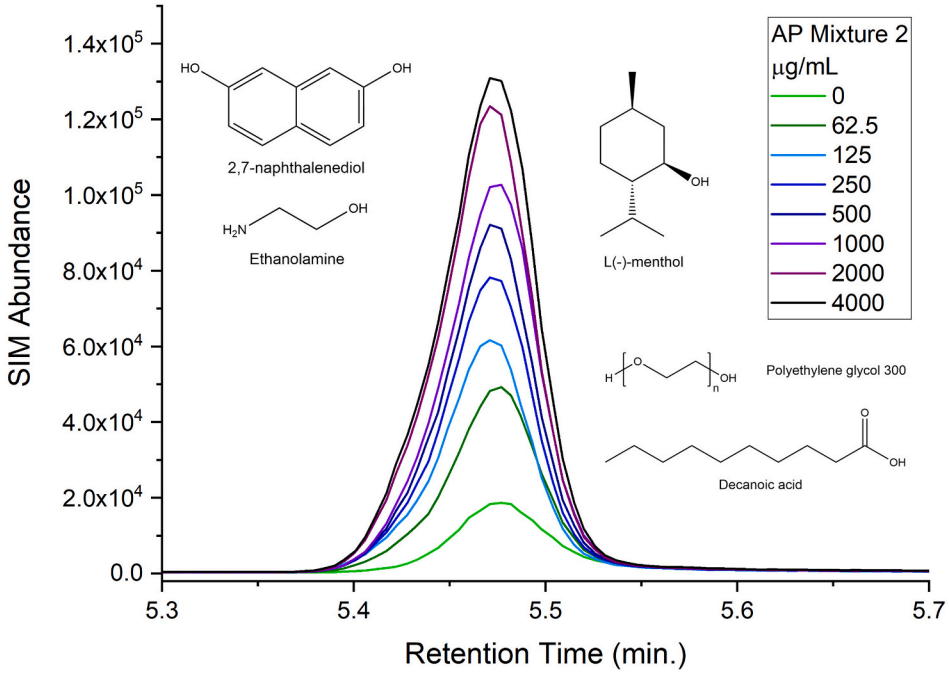

Fig. 6. Overlaid SIM chromatograms from the injection of eight MeOH solutions containing the same amount of total THC but different concentrations of AP Mixture 2.

approximately 2500 μg/mL of each compound (Fig. 6). The addition of AP Mixture 2 to a calibrant and *Cannabis* plant extract containing equimolar THCA-d_3 and Δ^9-THC-d_9 resulted in comparable signal responses and DE for both (Fig. S14). The significant improvement over neat calibrants and extracts without protectants demonstrates that adding AP Mixture 2 leads to suitable conditions for quantitation of total THC.

3.5. ID-GC-MS quantitation of plant extracts

Three *Cannabis* samples previously analyzed by LC-PDA were analyzed here by GC–MS using AP Mixture 2 (Table 2). These samples were selected because they had different ratios of THCA and Δ^9-THC with total THC levels near the 0.3 % threshold (Table S2). The same quantity of AP Mixture 2 (2500 μg/mL) was spiked into the calibrants and plant extracts. Four approaches were compared for quantifying total THC in the presence or absence of AP Mixture 2 or internal standard (see Table 2 and Fig. 7). External standard calibration significantly overestimates total THC due to the partial adsorption of calibrants at active sites (see graphical abstract). Using a deuterated internal standard minimizes measurement biases, since the A/IS ratio is similar in the calibrants and samples even though the absolute responses vary with sample type. As a result, the mass fraction levels for total THC in Table 2 and Fig. 7 have a percent error less than 10 % using Δ^9-THC-d_3 without AP Mixture 2. Similar results were obtained with the addition of AP Mixture 2 for internal standard, but the accuracy was improved for the external standard approach. Notably, the bias introduced by the external standard-based GC–MS method would result in classifying hemp samples as marijuana, whereas total THC as determined by the other methodologies indicate the samples are at the threshold limit for classification as hemp.

Further research is needed to develop an optimized GC–MS method for the analysis of cannabinoids. The analyte protectants in this research were chosen based on previous reports for proof of concept and other compounds were not investigated. Selection of specific AP(s) should include consideration of potential desirable interactions with silanols, lack of reactivity with analytes, and compatibility with the instrumentation. Although isotopically labeled internal standards are recommended, reagent costs may prevent routine use by some laboratories. Ideally, the deuterated internal standard would be spiked at the beginning of the extraction process. However, the internal standards were not commercially available at a sufficiently high concentration and the amount required would potentially be too cost-prohibitive regardless. Adding the deuterated internal standard post-extraction was therefore the best compromise as our primary goal was to study the decarboxylation efficiency in the inlet. Accurate results are facilitated by implementing APs for external standard calibrations. Although standard addition could address the observed matrix-related effects, it was not explored in this study, as literature searches indicated that external standard calibration approaches were most commonplace for total THC quantitation.

Table 2
Total THC values (mean ± standard deviation of 3 replicates) determined by LC-PDA and GC–MS for three *Cannabis* plant extracts.

% Total THC	ID-GC–MS				LC-PDA
	No AP mixture		With AP mixture 2		
	No IS	With Δ^9-THC-d_3 IS	No IS	With Δ^9-THC-d_3 IS	
Sample A	0.457 ± 0.012	0.254 ± 0.002	0.264 ± 0.002	0.264 ± 0.002	0.271 ± 0.002
Sample B	0.560 ± 0.034	0.298 ± 0.009	0.309 ± 0.009	0.312 ± 0.008	0.316 ± 0.010
Sample C	0.616 ± 0.021	0.315 ± 0.007	0.336 ± 0.011	0.337 ± 0.007	0.355 ± 0.002

This study provides a new perspective toward understanding the metrology of THCA and Δ^9-THC for GC measurements. Inconsistencies among published reports for determining total THC may be traced to surface activity within the injector or other GC surfaces that vary with the sample matrix. The apparent incomplete decarboxylation of THCA to Δ^9-THC is instead a consequence of adsorption of THCA in solution calibrants, a significant departure from previous assumptions. Based on this finding, several quantitative methods have been shown to yield accurate results, and other approaches are expected to yield accurate measurements based on fundamental principles of quantitation: (1) external standard calibration with the addition of APs to samples and calibrants, (2) external standard calibration with calibrants prepared in a botanical extract known to be free of THCA and Δ^9-THC, (3) internal standard calibration with an isotopically labeled internal standard (i.e., ID-MS), (4) internal standard calibration with addition of APs to sample and calibrants, and (5) standard addition calibration (not evaluated in this study).

4. Conclusions

The accuracy of methods for the GC–MS analysis of *Cannabis* is influenced by potential biases resulting from analyte adsorption on active surfaces and the quantitative conversion of THCA to Δ^9-THC through decarboxylation. Improved measurement accuracy is achieved by implementing measures that reduce or eliminate surface activity often attributed to silanols. A test solution containing Δ^9-THC-d_3 and Δ^9-THC-d_9 provided unique capabilities to study adsorption and decarboxylation phenomena since all relevant species were distinguished by MS. THCA is shown to be converted to Δ^9-THC at 100 % efficiency in plant extracts even at relatively low injection temperatures, and decarboxylation is not a source of measurement bias. The unexpected discovery of a plant matrix effect for Δ^9-THC in *Cannabis* accounts for measurement biases previously attributed to non-uniform decarboxylation of THCA. Plant extracts are shown to increase analyte response compared with neat solutions; this discrepancy is the source of a positive measurement bias that is particularly apparent with external standard calibration methods. The addition of APs to samples minimizes or eliminates problematic analyte adsorption. The incorporation of APs along with isotopically labeled internal standards forms the basis for the development of robust and accurate GC–MS methods for cannabinoids.

CRediT authorship contribution statement

Jerome Mulloor: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Walter B. Wilson:** Writing – review & editing, Funding acquisition. **Lane C. Sander:** Writing – review & editing, Methodology.

Disclaimer

Certain commercial equipment or materials are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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Declaration of competing interest

The authors declare that they have no known competing financial

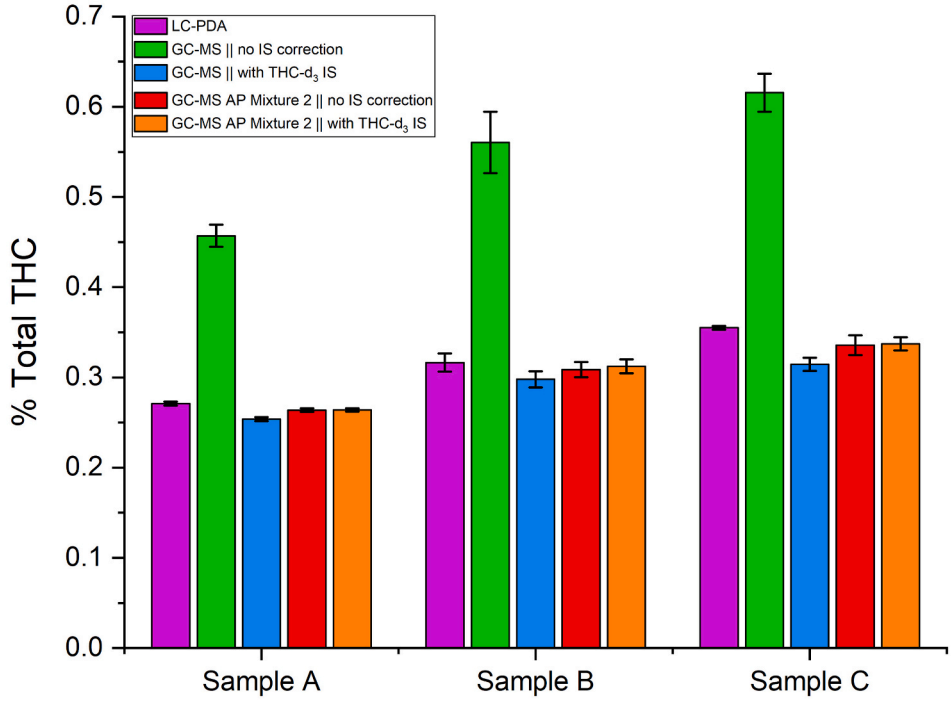

Fig. 7. Quantitation of total THC in three *Cannabis* plant samples under different conditions. The GC–MS results are compared with the benchmark LC-PDA values. Error bars represent one standard deviation (N = 3) of the mean value.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2025.100668.

Data availability

Data will be made available on request.

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