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Quantitative analysis of cannabinoids by zone heat-assisted DART-MS with in-situ flash derivatization

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ABSTRACT

Accurate quantitation of cannabinoids, particularly Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), is essential for regulatory compliance, forensic investigations, and cannabis product development. Traditional methods, such as liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry, provide reliable results but are time-consuming and resource-intensive. This study introduces a rapid and high-throughput analytical method using zone heat-assisted direct analysis in real time mass spectrometry (DART-MS) combined with in-situ flash derivatization. The method employs trimethylphenylammonium hydroxide (TMPAH) for efficient derivatization, allowing for the differentiation of THC, CBD, and their acidic precursors, Δ9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA). A custom heated transfer zone was implemented to enhance derivatization efficiency and reduce carryover effects. The method was optimized for reagent concentration and gas stream temperature, achieving high specificity by minimizing interference from isomeric cannabinoids. Validation studies demonstrate good accuracy (relative error within ±15.9 %) and precision (relative standard deviation ≤15 %), with limits of quantitation of 7.5 μg/mL for THC/CBD and 0.5 μg/mL for THCA/CBDA. Comparative analysis of cannabis samples showed a strong correlation with reference LC/MS results, highlighting the reliability of the proposed method. DART-MS offers a significant time advantage, requiring only 10 s per analysis, making it a promising tool for high-throughput screening of cannabis samples in forensic laboratories.

Introduction

Cannabis sativa (*C. sativa*), or cannabis, has been used for medical and recreational purposes over the centuries. The accurate quantitation of cannabinoids such as Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), and their acidic precursors, Δ9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), in cannabis, is a significant focus in analytical chemistry impacting legal compliance, medical research, agricultural practice, and product development. THC is psychoactive and responsible for the 'high' associated with cannabis use, whereas CBD is non-psychoactive and is often associated with potential therapeutic benefits. The 2018 Farm Bill establishes a 0.3 % threshold of total Δ9-THC (including Δ9-THC following the post-decarboxylation conversion of Δ9-THCA) to distinguish the fiber-type cannabis (as hemp, CBD-rich) from the drug-type cannabis (as marijuana, Δ9-THC-rich) under federal law, making hemp legal while marijuana as a Schedule I controlled substance. Therefore, forensic laboratories are often required to quantify the level of Δ9-THC in seized samples to determine the legality. However, there are significant resource challenges in the US to perform a full quantitative analysis on all seized marijuana samples, given controlled substance analysis has already been the most backlogged discipline in forensic science.[1] The US Drug Enforcement Administration (DEA) adopts a decision-point approach with a 1 % threshold of Δ9-THC to differentiate legal hemp from illegal marijuana, which is not quantitative in nature but provides improved analytical efficiency.[2]

The challenge of accurately quantifying Δ9-THC lies in its structural similarity to other major cannabinoids in cannabis, especially CBD,

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which may interfere with the quantitative results. Δ9-THC/Δ9-THCA differs from its isomer CBD/CBDA by a hydroxyl recycling between the isoprenyl group on its monoterpene moiety and the hydroxyl group on the resorcinol ring. Standard test methods for quantitative analysis of cannabinoids involve time-consuming separation of these isomers through either liquid chromatography (LC) or gas chromatography (GC) coupled with a mass spectrometric detector (ASTM D8375) or flame ionization detection (ASTM D8442).[3,4] Therefore, a high-throughput and chromatography-free method for the quantitation of Δ9-THC/Δ9-THCA and CBD/CBDA is needed to differentiate between hemp and marijuana. Colorimetric assays with reagents such as the Duquenois-Levine,[5] Fast Blue B,[6] and Fast Blue BB (FBBB),[7] are used as presumptive preliminary tests for the presence of cannabinoids in the sample. Recently, Jose R. Almirall's group reported the success of using FBBB colorimetric tests to differentiate between Δ9-THC and CBD[8] and quantitatively determine the Δ9-THC in the cannabis plant extract when coupled with a portable fluorescence measurement instrument.[9] Despite the significant merit of the field testing, the colorimetric methods generally lack specificity, leading to false positives and negatives due to the interference of other substances present in the sample, and are limited in their usefulness because their interpretation is subjective and they do not usually provide quantitative results. Therefore, their use often requires follow-up testing for conclusive results for legal proceedings or regulatory compliance. Mass spectrometric (MS) methods offer high sensitivity and specificity and have been employed to differentiate between THC and CBD and even to conduct semi-quantitative analysis of the cannabinoids. Silver (Ag)-impregnated paper spray mass spectrometry was used to distinguish THC and CBD based on their fragmentation spectra (MS^2). Due to the binding affinity difference to Ag(I) ions, $[THC + Ag]^+$ and $[CBD + Ag]^+$ were dissociated into characteristic product ions at *m/z* 313 for THC and *m/z* 353 and 355 for CBD which could be used to quantify the THC/CBD ratios in commercial CBD oils.[10] It is worth mentioning that *m/z* 313 product ion was also derived from $[CBD + Ag]^+$ at lower relative abundance; therefore, the method was incapable of quantifying individual cannabinoids due to the interference. Electrospray ionization (ESI)-MS was also applied to study the Ag-ligand ion complexation of cannabinoids including Δ9-THC/Δ9-THCA and CBD/CBDA and the characteristic product ions for CBD (*m/z* 421/423) and CBDA (*m/z* 465/467) were observed which were then developed into a 1 % semi-quantitative decision-point assay for the differentiation of hemp and marijuana with a 90 % classification accuracy for 20 cannabis samples.[11] With additional differential mobility spectrometry (DMS), the ESI-DMS-MS/MS was able to distinguish five isobaric cannabinoids, including Δ9-THC, Δ8-THC, *exo*-THC, CBD, and CBC, based on the distinct fragmentation patterns for each cannabinoid-Ag complex.[12] Glen P. Jackson's group developed the Expert Algorithm for Substance Identification (EASI) to resolve the diastereomers of cocaine electron ionization (EI)-MS[13] and THC and CBD ESI-MS spectra.[14] The algorithm was supported by Rice-Ramsperger-Kassel-Marcus theory to model and predict the correlations of the relative abundances between mass spectral fragments from different chemical isomers, resulting in improved classification rates comparing the consensus-based approach. The above-mentioned chromatography-free mass spectrometry methods, although they have presented great values for THC and CBD analysis, are nevertheless insufficient for quantitative analysis of individual cannabinoids in cannabis plants.

In this study, we propose a novel analytical strategy coupled with direct analysis in real time mass spectrometry (DART-MS) with in-situ flash derivatization for the quantitative determination of cannabinoids, including Δ9-THC, Δ9-THCA, CBD, and CBDA in the cannabis plant extract. Offline derivatization of cannabinoids is commonly employed in GC based methods in order to prevent the acidic cannabinoids from decarboxylation in the GC inlet and improve the peak shapes due to the increased volatility, but this time-consuming and tedious process is considered as a main disadvantage of sample preparation.[15] Recently, Rabi A. Musah's group introduced a method to differentiate CBD and THC in food, beverage, and personal-care product matrices using offline silylation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), followed by DART-MS analysis. The approach leveraged the difference in hydroxyl (–OH) groups between THC and CBD – one in THC and two in CBD – allowing the derivatized products to be readily distinguished by DART-MS. However, the silylation process required heating in a water bath at 70 °C for 1 h, with an additional vortex step at the 30-min mark. Moreover, the study primarily focused on the identification of the presence of cannabinoids rather than their quantification.[16] As an alternative, quaternary ammonium reagents such as tetramethylammonium hydroxide (TMAH) for the flash alkylation could be used to derivatize the cannabinoids without complicated sample pretreatment steps prior to the GC analysis.[17] This thermally assisted process could occur rapidly in the hot GC inlet and methylate carboxylate and hydroxyl groups.[18] DART is an ambient ionization method in which charged molecules are formed primarily via Penning ionization and proton transfer and a hot inert gas (typically helium, He) is commonly employed in the ionization process.[19] Derivatization reactions could occur in the hot gas stream of the DART ion source region, and their product compounds could then be ionized and detected by mass spectrometry.[20] The derivatization of cannabinoids also increases the analytical specificity for isomers since CBD and CBDA have one more –OH group in their structures than THC and THCA, so their methylated products would differ by a $–CH_2$ group (14 Da). The objectives of this study were to (i) investigate the flash derivatization of cannabinoids in the DART gas stream and optimize reaction conditions; (ii) modify the DART-MS inlet to assist the derivatization reaction and eliminate interference between THC and CBD analysis; (iii) evaluate the analytical performance and apply the method to study cannabis extract.

Materials and methods

Chemicals and materials

LC-MS grade formic acid, water, methanol, acetonitrile, and 0.1 M trimethylphenyl ammonium hydroxide in methanol solution (TMPAH) were purchased from Fisher Scientific, Inc. (Fair Lawn, NJ, United States). Cannabinoid standards, including Δ9-tetrahydrocannabinol (THC, 1 mg/mL), cannabidiol (CBD, 1 mg/mL), cannabidiolic acid (CBDA, 1 mg/mL), Δ9-tetrahydrocannabinolic acid (THCA, 1 mg/mL), Δ9-tetrahydrocannabinol-D_3 (THC-D_3, 100 μg/mL), Δ9-tetrahydrocannabinolic acid A-D_3 (THCA-D_3, 100 μg/mL), and 25 wt% tetramethylammonium hydroxide solution in methanol (TMAH) were purchased from Sigma-Aldrich (St. Louis, MO, United States). Various *Cannabis* hemp varieties, including the Cherry, Cherry Blossom, Eletta, and Carmagnola varieties, were provided by the Tennessee Center for Botanical Medicine Research at Middle Tennessee State University. All the samples were collected in the spring of 2018 and stored in a dedicated laboratory cabinet at room temperature.

Instrumental analysis

Mass spectra were acquired using a DART JumpShot® ion source (Bruker-IonSense, Inc., Saugus, MA, United States) equipped with a Bruker Compact QTOF mass spectrometer (Bruker Daltonics Inc., Billerica, MA, United States) in the positive ion mode with an exit grid voltage of 350 V. Helium was used as ionization gas for all experiments and a gas heater was set to 500 °C. All mass spectra were obtained in an *m/z* range of 50–800. The pre-loaded "QuickStrip" method was used: Heater Wait Time 30 s; Sample Speed 0.5 mm/s; Contact Closure Delay 5 s; Pulse Time 10 s; Standby Temperature 345 °C. A volume of 3 μL sample was spotted on the QuickStrip™ sample card in four replicates on positions 2, 5, 8, and 11, leaving other blank spots on each QuickStrip™ sample card for mass spectrum background subtraction and

examination of possible carry-over. An automated sample introduction apparatus consisting of a Linear Rail Enclosure that holds QuickStrip™ sample cards was used for all the DART-MS analysis. QuickStrip™ Sample Cards were purchased from Bruker Daltonics Inc., Billerica, MA, United States. The Vapur interface, included with the DART system, was used to connect the DART source to the MS instrument. This atmospheric-to-vacuum interface, consisting of a ceramic transfer tube mounted on a custom flange with an auxiliary pump, is essential for reducing the pumping burden on non-JEOL mass spectrometers.[21] However, this interface lacks temperature control. To address this, a customized heating tube was implemented to facilitate the flash derivatization during DART-MS analysis (Fig. 1). A heated copper tube (19 × 16 mm ID) was applied over the ceramic transfer tube for the ion transmission from the DART source to the MS capillary inlet. A high-temperature heating cord (HWC1040, BriskHeat, Columbus, OH, United States) with an SDC temperature controller (max temperature 371 °C, BriskHeat, Columbus, OH, United States) was used to maintain the copper tube at the optimized temperature.

The LC/MS method was adopted from a previous publication in the group.[22] In short, the sample was separated on a Waters Acquity UPLC BEH shield RP18 column (50 × 2.1 mm ID, 1.7 μm, 130 Å) with an UltraLine UHPLC In-Line Filter (RESTEK, Bellefonte, PA, United States) at a flow rate of 0.3 mL/min. The mobile phase consisted of A (0.2 % formic acid in water, *v/v*) and B (100 % acetonitrile). The linear gradient used was 50 % B (*v/v*), hold for 2 min, ramp from 50 to 85 % B at 5 min, hold at 85 % B to 10.5 min, from 85 to 50 % B at 10.6 min, and hold to 12 min. The eluted compounds were detected by the LC-MS instrument consisting of a Thermo LTQ XL mass spectrometer with a Dionex™ UltiMate™ LPG-3400SD Standard Quaternary Pump, a Dionex™ UltiMate™ Standard Well Plate Autosampler, and a Dionex 3000 column chamber (Thermo Scientific, San Jose, CA, United States). The mass spectrometer was operated in positive mode using electrospray ionization (ESI), and the conditions were set as follows: sheath gas at 35 (arbitrary units), auxiliary and sweep gas at 15 (arbitrary units), spray voltage at 4.5 kV, capillary temperature at 500 °C, capillary voltage at 10 V, and tube lens at 100 V. The mass range was from *m/z* 100 to 1000.

Sample preparation

The cannabis plant samples were ground in a stainless steel coffee grinder and then extracted by methanol using the optimized method described by Y-C. Cheng et al.[2] Briefly, dry plant material (50 ± 5 mg) was weighed and mixed with methanol at the ratio of 5 mg plant material per 1 mL methanol using the Eppendorf Research Plus Adjustable Volume Single Channel Pipette (1,000–10,000 μL, Eppendorf North America, Inc., Enfield, CT, United States). The sample was then vortexed for 10 s and allowed to stand for 5 min. An aliquot of 1 mL extract was filtered by a 0.22 μm nylon syringe filter (13 mm, Celltreat Scientific Products, Pepperell, MA, United States). All the plant samples were processed in triplicate.

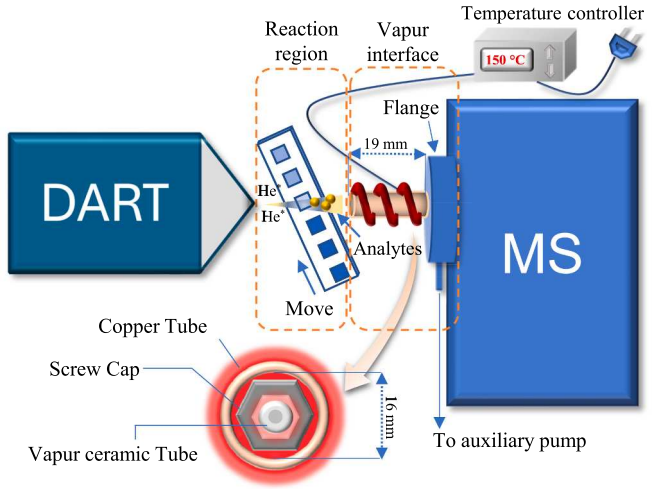

Fig. 1. Schematics of the zone heat-assisted DART-MS with QuickStrip™ module.

For DART-MS analysis, 20 μL plant extract was mixed with 20 μL internal standard solution containing 50 μg/mL THC-D_3 and 3 μg/mL THCA-D_3 in TMPAH methanol solution. For LC/MS analysis, 100 μL plant extract was mixed with 100 μL internal standard solution containing 1 μg/mL THC-D_3 in methanol containing 1 % formic acid, and then 10 μL was injected into LC/MS instrument for analysis.

Derivatization method optimization

Two reagents, including TMAH and TMPAH for in-situ flash derivatization, were selected and investigated in this study due to their reported performance on on-column alkylation of active hydrogens from various functional groups, including hydroxyl groups and carboxylic acids in GC analysis.[23,24] The optimum DART helium gas stream temperatures and reagent/sample ratios were examined. The TMAH and TMPAH solutions were diluted to various concentrations with methanol and mixed with THC and CBD standard solutions (0.1 mg/mL) at a 1:1 ratio. In addition, the derivatization under the zone heat-assisted DART-MS approach was employed and compared with the traditional DART-MS method.

Calibration and method validation for DART-MS analysis

THC, CBD, THCA, and CBDA stock solutions (1 mg/mL) were used to prepare the calibration standard solutions. Concentrations of THC and CBD ranged from 15, 50, 100, 200, and 500 μg/mL, and those of THCA and CBDA were 0.5, 1, 3, 10, and 20 μg/mL. The blank matrix extracts were prepared from the *Cannabis* hemp plant extracts. The methanol extract of an Eletta campana hemp variety was tested by LC-MS and proven to have THC/THCA levels of less than 0.05 μg/mL (or equivalent to 0.001 % by dry weight), so it was used as the blank matrix for THC/THCA analysis. A cherry variety hemp was tested with low CBD and CBDA levels (2.05 % and 1.97 %, respectively), and a second methanol extract from the same plant materials showed undetectable levels of CBD and CBDA (less than 0.01 %). Therefore, the second methanol extract was used as the blank matrix for CBD/CBDA analysis. The accuracy (as relative error, RE) and precision (as relative standard deviation, RSD) of the method were determined by assay of four replicates at three concentrations. The matrix effects were evaluated by spiking the same concentrations of analytes into the blank matrix or methanol and comparing the ion intensity changes. Recoveries of this method have been systematically investigated in the previous report, with recoveries between 80–92 % for THC.[2]

Data processing

The DART-MS data was processed with Otof Control (Bruker Daltonics Inc., Billerica, MA, United States), and LC/MS data was processed with Xcalibur 2.1 (Thermo Scientific, San Jose, CA, United States) and then plotted using Microsoft Office Excel 2021 (Seattle, WA, United States) and MagicPlot 3.0.1 (Magicplot Systems, LLC., Saint Petersburg, Russia).

Results and discussions

Analysis of cannabinoids by flash alkylation and zone heat-assisted DART-MS

THC/CBD and THCA/CBDA are two structural isomer pairs with a formula of $C_{21}H_{30}O_2$ and $C_{22}H_{30}O_4$, respectively. These isomer pairs

exhibit almost identical precursor ions and fragmentation products and are thus challenging to distinguish by mass spectrometry alone.[25] Previous work took advantage of the binding affinity difference between Ag(I) and olefinic groups in THC/CBD to differentiate them based on their product ions in paper spray mass spectrometry.[10] While the unique CBD product ions were observed in this method, the characteristic THC fragment ion (i.e., *m/z* 313) would have interfered with CBD when both THC and CBD were present in the samples. Therefore, the quantitative analysis of the THC/CBD ratio based on their corresponding product ion ratios was achieved with additional mathematical subtraction of contributions from CBD interference. In our study, we propose to utilize the other key difference of the two isomer pairs, the additional –OH group in CBD and CBDA, with an in-situ flash alkylation, making the differentiation and quantitation of individual THC, CBD, THCA, and CBDA possible.

When TMPAH was employed as a reagent for flash derivatization, the mono-O-methylation of the hydroxyl group occurred at the C1 position for THC, while both –OH groups at the1′ and 3′ positions on CBD were methylated, producing derivatives with unique protonated ions in DART-MS spectrum (i.e., *m/z* 329.2475 and 343.2632, respectively, Fig. 2A). Similarly, the derivative products from the THCA and CBDA isomer pairs were also observed to differ in one methyl group. For example, the major ions that appeared in the mass spectrum for THCA derivatives were the protonated ions of the monomethylated THCA with trimethylphenyl ammonium (TMPA) complex with an accurate masses of 508.3422 for $[C_{32}H_{45}NO_4 + H]^+$. As a minor ion corresponding to the methylation of THCA on both hydroxyl and carboxylic groups, *m/z* 387.2530 for $[C_{24}H_{34}O_4 + H]^+$ was also observed. For CBDA, similar derivatives were identified with a 14 Da difference in the mass spectrum from their THCA counterparts corresponding to the $–CH_2$ group (Fig. 2). Since TMPA-THCA/CBDA complexation produced the highest intensity peak in the mass spectrum, *m/z* 508 and 522 were used to monitor and quantify THCA and CBDA in this study. This result suggests that in-situ flash derivatization of THC/CBD and THCA/CBDA pairs occurs in the DART gas stream, which leads to substantial differences in their mass spectra. In addition to the characteristic derivatives, protonated ions for native THC and CBD (i.e., $[C_{21}H_{30}O_2 + H]^+$, *m/z* 315.2308, mass error: −3.2 ppm) were observed, indicating the presence of underivatized THC and CBD. However, for THCA and CBDA, the flash methylation was highly complete, evidenced by the absence of their protonated ions in the mass spectrum.

The QuickStrip (QS) module coupled with DART-MS was initially employed to further study the derivatization reactions for cannabinoids. For each QS sample card, THC, CBD, THCA, and CBDA samples were mixed with derivatization reagents such as TMPAH and then loaded on position numbers 2, 5, 8, and 11 on the QS sample card, leaving the other spots as blanks (Fig. 3). Several issues were identified for the analysis of THC and CBD from this process. First, there was a significant carry-over effect. For example, the ion intensities for derivatives of THC (*m/z* 329) and CBD (*m/z* 343) from the first adjacent QS blank spots (i.e., 3, 6, 9, and 12) were about 15 ± 2 % and 31 ± 7 % of their respective intensities from the sample spots (i.e., 2, 5, 8, and 11), and significant signals (about 20 ± 4 %) were observed even from the second adjacent QS blank spots for CBD samples (Fig. 3C), suggesting more profound carry-over effect for CBD analysis. Second, the ion intensities of underivatized THC and CBD (i.e., *m/z* 315 in Fig. 3A and C) accounted for about 87 % and 71 % of their derivative counterparts and varied significantly among the four replicates with the coefficient of variations (C.V.) of 28 % and 69 % for THC and CBD, respectively. Third, there were *m/z* 329 ions in the mass spectra when analyzing CBD (Fig. 3C), indicating that only one of the two –OH groups on CBD was methylated in this process with a 40 ± 8 % intensity relative to fully methylated CBD (*m/z* 343). The *m/z* 329 ions resulting from the incomplete derivatization of CBD interfere with THC analysis, leading to false positive signals for THC and limiting the method's reliability. Since both CBD and THC naturally occur in cannabis plants, the contribution of *m/z* 329 from CBD can lead to inaccurate measurement and overestimation of THC content, which has a significant impact on legal compliance.

For THCA and CBDA, the in-situ derivatization worked well under this condition. CBDA did not interfere with THCA or vice versa, and they were not decarboxylated into CBD and THC in the DART helium gas stream under 500 °C. This may result from TMPAH stabilization of THCA and CBDA which prevents them from decarboxylation. Therefore, while THCA and CBDA can be quantified using their respective characteristic ions, their presence in the cannabis plant extract would not interfere with the analysis of THC and CBD.

Typically, TMAH and TMPAH require high temperatures (i.e., 400–800 °C) to exceed the energy barrier necessary for online derivatization in pyrolysis GC applications because the derivatization yield is low at lower temperatures.[26] However, the reaction temperature requirement can be lower at the DART source due to the presence of excited state helium to initiate the methylation reaction and ionize the

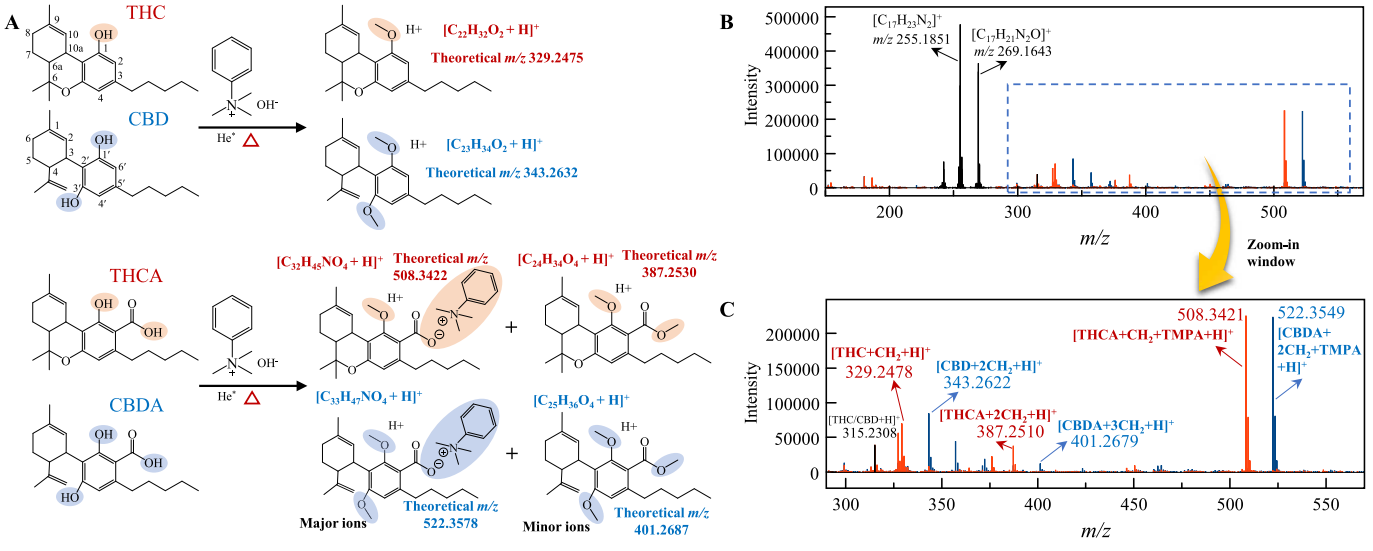

Fig. 2. Flash derivatization reactions for THC, CBD, THCA, and CBDA and the ionized products from the DART ion source (A) and a mass spectrum (B) with a zoom-in window between *m/z* 290 and 570 (C) from a mix standard solution containing 0.15 mg/mL THC and CBD and 0.05 mg/mL THCA and CBDA. Note: ions colored in red correspond to THC/THCA derivatives; ions in blue are for CBD/CBDA derivatives; and ions in black are for TMPAH reagents. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

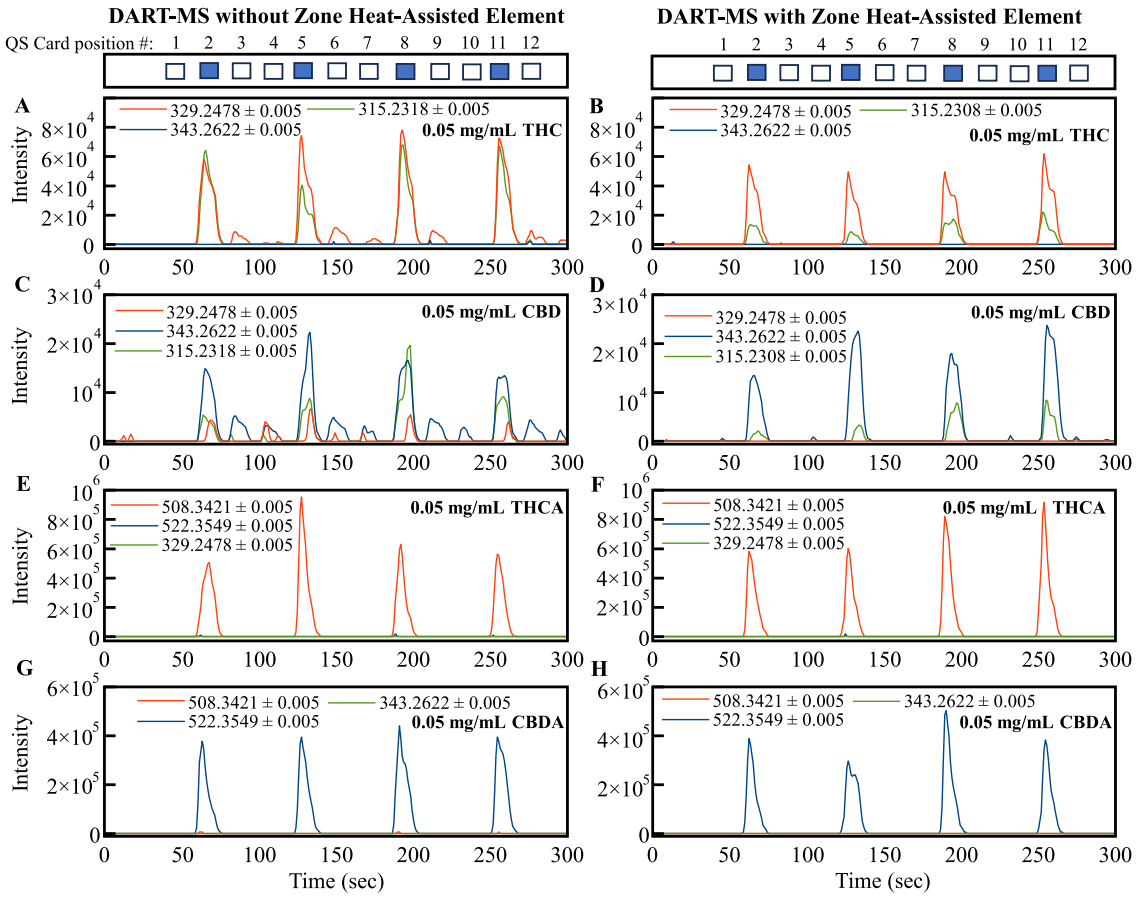

Fig. 3. EIC profiles of characteristic ions (±0.005 Da) for THC, CBD, THCA, and CBDA derivatives at 0.05 mg/mL by QS DART-MS with/without zone heat-assisted device. Note: the DART helium gas temperature was set to 500 °C in all experiments. Blue squares represent QS card positions with spiked samples, while clear squares denote positions for blanks. *m/z* 315.2318: $[THC/CBD + H]^+$; *m/z* 329.2478: $[THC/CBD + CH_2 + H]^+$; *m/z* 343.2622: $[THC/CBD + 2CH_2 + H]^+$; *m/z* 508.3421: $[THCA/CBDA + CH_2 + TMPA + H]^+$; *m/z* 522.3549: $[THCA/CBDA + 2CH_2 + TMPA + H]^+$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

methylation products. Another key difference between GC and DART is that GC can maintain a consistent desired temperature throughout the entire process—from the introduction of the sample and derivatization reagent to the pyrolyzer and through to the GC analysis. In contrast, the excited helium temperature in the DART source drops significantly after exiting the ceramic cap. When the flow of helium gas transports the molecules to the unheated ceramic tube between the DART and MS, the low temperature in the region is unfavorable to the reaction. Both TMAH and TMPAH are less volatile with vapor pressure 1.2×10^{-6} mm Hg[27] and 4.8×10^{-3} mm Hg, respectively, at 25 °C[28]. Thus, they tend to be deposited within the ceramic tube due to the low temperature, which leads to the carryover effect (Fig. 3A and C). To overcome the issues for THC and CBD online derivatization, we proposed modification of the ion transfer tube with a zone heat-assisted device to promote complete methylation. As illustrated in Fig. 1, the heated copper tube was set to 150 °C to reduce the deposition of reactants in the ceramic tube and improve the derivation efficiency in the reaction region of the DART-MS. Other temperatures were attempted, but the current heated zone setup could not maintain a stable temperature higher than 150 °C. With the zone heat-assisted element, the singly methylated CBD (*m/z* 329) was eliminated (Fig. 3D), which is significant to the analysis of samples with both CBD and THC present. Under this condition, *m/z* 343 and *m/z* 329 can be used as the characteristic ions for CBD and THC, respectively, without interference with each other (Fig. 3B and D). The THCA and CBDA derivatives (Fig. 3F and H) remained unaffected as they were under the unheated DART-MS conditions (Fig. 3E and G). As the reaction efficiency improved with the zone heat-assisted element, evidenced by the decreased intensity of *m/z* 315 for the protonated ion of native THC and CBD, more methylated products were expected. However, the ion intensities of the characteristic ions for THC, CBD, THCA, and CBDA did not change significantly compared to their counterparts in the unheated DART-MS condition (Fig. S1). This result could be due to the raised temperature in the DART-MS reaction region increasing the volatility of the derivative products, some of which escaped into the ambient air instead of entering the ceramic tube. Future studies could adjust the diaphragm pump flow rate, which could provide stronger suction of ions into the MS and optimize the distance between the ceramic cap for DART and the entrance of the ceramic tube to reduce the loss of derivative products into the ambient air. It is important to note that the presence of other THC isomers, such as Δ8-THC and cannabichromene (CBC), can interfere with the quantitation of Δ9-THC, as these compounds also contain a single –OH group in their structures, leading to false positive detection. In such cases, confirmation using standard HPLC or GC-based methods would be necessary.

In-situ flash derivatization optimization

Two derivatization reagents, TMAH and TMPAH, were tested in this study as they are both commonly used for inlet derivatization in the GC/MS analysis of organic compounds.[26] The concentrations of derivatizing reagents and the DART gas stream temperature were optimized, primarily based on the THC results, because of their critical role in the cannabis regulatory process. Six concentrations of TMPAH were mixed with 0.05 mg/mL THC and analyzed by DART-MS. As illustrated in

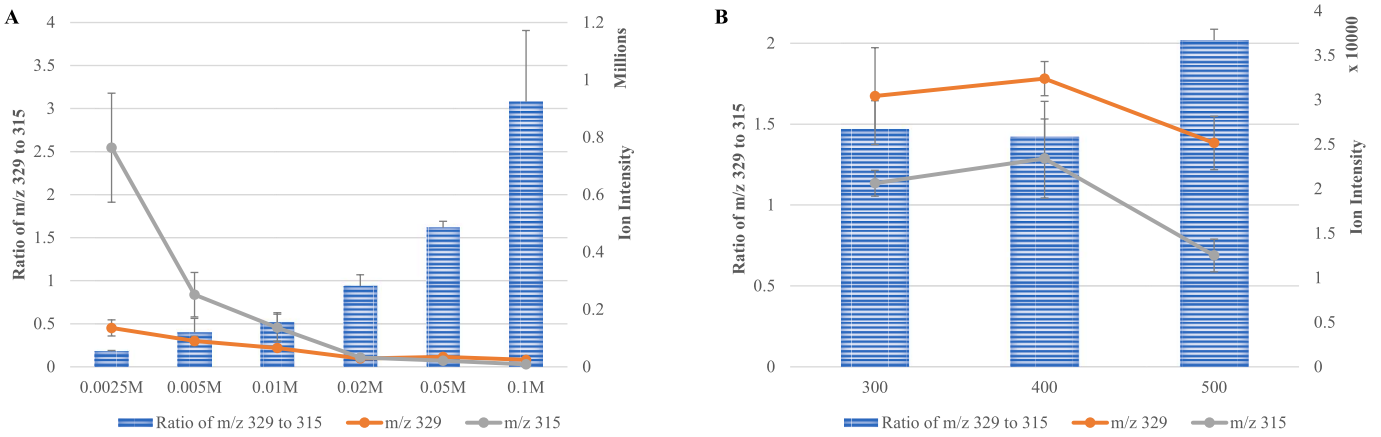

Fig. 4. Comparison of ion intensities of derivatized THC (*m/z* 329), underivatized THC (*m/z* 315), and their ratios (*m/z* 329 to 315) under different conditions: (A) Effect of TMPAH concentrations (DART helium gas temperature: 500 °C). (B) Effect of DART helium gas temperature (TMPAH concentration: 0.1 M). THC concentration was 0.05 mg/mL for all the experiments (n = 3).

Fig. 4A, increasing the TMPAH concentration results in a higher relative ratio of derivatized THC to underivatized THC. However, the *m/z* 315 signal corresponding to underivatized THC remained detectable even at the highest TMPAH concentration tested (0.1 M), indicating that not all THC reacted under this condition. In this reaction, the TMPAH concentration (0.1 M) was significantly higher than the THC concentration (1.6 μM), suggesting that further increasing the TMPAH concentration would not substantially improve THC methylation. The persistent presence of *m/z* 315 may be attributed to some THC molecules being desorbed and ionized by DART before reacting with TMPAH. It is also important to note that the absolute intensity of *m/z* 329, corresponding to the methylated THC derivative, decreases as the TMPAH concentration increases. This reduction can be explained by ionization suppression caused by the presence of excess TMPAH, as DART preferentially ionizes TMPAH. Consequently, the decreased intensity of *m/z* 329 leads to reduced sensitivity in THC quantitation. Additionally, when CBD at 0.05 mg/mL was analyzed with a lower concentration of TMPAH (i.e., 0.0025 M), partial methylation of CBD occurred, resulting in the formation of a product with a relative ion intensity (i.e., *m/z* 329) of 78 % ± 8 % (n = 3) compared to the fully methylated derivative (i.e., *m/z* 343). The presence of *m/z* 329 from partially methylated CBD can interfere with THC analysis, as previously discussed, making complete CBD derivatization essential for accurate quantitation of both CBD and THC when they coexist in a sample. In contrast, analyzing CBD at the same concentration (0.05 mg/mL) with 0.1 M TMPAH resulted in the exclusive detection of *m/z* 343, with no *m/z* 329 observed in the MS spectra. Therefore, under this condition, *m/z* 329 can be reliably used for THC quantification, while *m/z* 343 can be used for CBD quantification, without cross-interference. Although complete methylation of THC was not achieved with 0.1 M TMPAH, both THC and CBD could be analyzed without mutual interference. As a result, 0.1 M TMPAH was selected as the optimal concentration for the in-situ derivatization experiment. For quantitative analysis, the use of an appropriate internal standard effectively compensated for variations caused by the derivatization process, as discussed in the "Method performance and application" section.

Temperature is another crucial factor influencing the derivatization process. Three DART gas temperatures, including 300, 400, and 500 °C, were tested with a mixture containing 0.05 mg/mL THC and 0.1 M TMPAH. As shown in Fig. 4B, higher temperatures enhance the conversion of THC to its derivative product, as indicated by an increased ratio of *m/z* 329 to *m/z* 315 at 500 °C. Although the absolute intensity of *m/z* 329 at 500 °C was 22 % lower than that at 400 °C ($p = 0.046$, n = 3), the 500 °C DART gas temperature was adopted in this experiment because the higher gas temperature reduced the possibility of carryover issues in the QS DART-MS analysis.

TMAH was evaluated as an alternative derivatization reagent for in-situ DART-MS analysis at concentrations ranging from 0.025 % to 0.5 % in methanol. However, the main challenge with the TMAH system was the carryover issue. For example, when a mixture of 0.05 mg/mL THC and 0.25 % TMAH in methanol was analyzed on the first position of the QS sample card at a DART helium gas temperature of 500 °C, the ion intensity of *m/z* 329 (THC derivative) remained elevated in the subsequent three blank QS spots (Fig. S2). This indicated an incomplete reaction during the continuous flow of the ionization gas. As noted, TMAH has a much lower vapor pressure of 1.2×10^{-6} mm Hg compared to TMPAH of 4.8×10^{-3} mm Hg at 25 °C, which necessitates a higher temperature to convert it to the gas phase for the derivatization reaction. However, our DART gas temperature was limited to 500 °C, and the current heated zone setup could not sustain temperatures above 150 °C. Consequently, TMAH was deemed ineffective as a derivatization reagent for this experiment.

Method performance and application

Due to significant variations in ion intensities between different runs or even across various positions on the same QS card during DART-MS analysis, internal standards (IS) were essential for accurate quantification. Isotopic analogs of the analytes with three or more ^{2}H-atoms or ^{13}C-atoms at appropriate positions are the most effective as IS.[29] In this study, THC-D_3 was used as IS for THC and CBD analysis, while THCA-D_3 was used for THCA and CBDA. After TMPAH flash derivatization, the protonated ion of methylated THC-D_3 ($[C_{21}H_{27}D_3O_2 + CH_2 + H]^+$) was detected at *m/z* 332.2670 with a mass error of 2.0 ppm. Similarly, the THCA-D_3 derivative ($[C_{22}H_{27}D_3O_4 + CH_2 + TMPA + H]^+$) was observed at *m/z* 511.3615 with a mass error of 1.0 ppm. Neither ion interferes with the ions of THC, CBD, THCA, or CBDA derivatives in the DART-MS spectrum. The intensities of the analytes and their isotopic counterparts (i.e., THC and THC-D_3, THCA and THCA-D_3) derivative ions are comparable, indicating similar derivatization efficiency and MS sensitivity for both during DART-MS analysis. Calibration curves for THC, CBD, THCA, and CBDA were constructed by plotting the peak area ratios of each analyte to their respective IS against the analyte concentrations in the samples (Fig. S3). The curves demonstrated good linearity ($R^2 \geq 0.9973$) across the full working range: 7.5–250 μg/mL for THC and CBD, and 0.5–20 μg/mL for THCA and CBDA. Accuracy (as relative error, RE) and precision (as relative standard deviation, RSD) were evaluated at low, medium, and high concentration levels, as summarized in Table 1. The accuracy was in the range of −12.6 to 15.9 % and the precisions were ≤ 15 %. The limits of quantification (LOQ) for THC/

Table 1
Accuracy and precision for the analysis of THC, CBD, THCA, and CBDA.

Analytes	Concentration (μg/mL)		Accuracy (RE, %)	Precision (RSD, %)
	Spiked	Mean calculated		
THC	7.5 (LOQ)	7.0	−6.1	14.0
	25	28.9	15.7	7.4
	100	103.0	3.0	2.7
	250	246.0	−1.6	0.7
CBD	7.5 (LOQ)	6.9	−7.9	9.3
	25	26.4	5.5	7.0
	100	115.9	15.9	4.9
	250	263.9	5.6	12.0
THCA	0.5 (LOQ)	0.5	−8.3	2.4
	1.5	1.4	−7.9	2.1
	10	10.0	0.0	0.8
	20	19.9	−0.5	4.8
CBDA	0.5 (LOQ)	0.4	−10.7	8.4
	1.5	1.3	−12.6	3.6
	10	10.5	4.8	7.1
	20	19.7	−1.7	7.0

CBD and THCA/CBDA were determined to be 7.5 μg/mL and 0.5 μg/mL, respectively. The LOQ for THC set at 7.5 μg/mL, corresponds to 0.3 % THC on a dry weight basis using 50 mg of cannabis for extraction. If a lower LOQ is required, several strategies could be implemented, such as increasing the amount of cannabis used in the extraction, concentrating the THC by drying the methanol extract, or applying a larger volume of the extract onto the QS card. To evaluate the matrix effect for THC and THCA quantification, the cannabis matrix was prepared by extracting the cannabis hemp samples and verifying the absence of THC and THCA by the LC/MS method. For CBD and CBDA, the blank matrix was prepared by extracting cannabis hemp samples twice until both compounds were undetectable by LC/MS. The matrix effect was assessed by comparing the intensities of characteristic ions in spiked methanol and spiked matrix extracts. While pure methanol contains no matrix compounds, the cannabis matrix sample does, potentially causing ion suppression or enhancement. The ion intensity ratios for THCA, CBDA, and CBD between samples with and without matrix were 99 ± 6 %, 96 ± 9 %, and 102 ± 4 % respectively, indicating no significant ion suppression or enhancement for these compounds. However, for THC, the intensity ratio was 83 ± 2 %, indicating significant ion suppression from the cannabis matrix. A similar suppression effect was also observed for THC-D_3 (85 ± 1 %), demonstrating that the isotopic internal standard can effectively compensate for the matrix-related variations, which is crucial for accurate THC quantitation. Notably, the blank samples used for the CBD matrix effect were cleaner due to the extensive extraction, and no significant ion suppression or enhancement was observed for THC-D_3 (103 ± 13 %) in these evaluations. It is important to note that the matrix effects can vary across individual cannabis samples. Therefore, the use of IS is essential to minimize the impact of matrix effects in the quantitative analysis of cannabinoids.

Cannabis hemp samples were analyzed using both DART-MS and LC/MS methods, and the concentrations of CBD and CBDA were compared and plotted in Fig. 5. To simulate marijuana samples with THC content ≥ 0.3 %, known amounts of THC and THCA were spiked into the cannabis hemp samples and analyzed using the DART-MS method (Fig. 5C and D). When the concentration exceeded the linear dynamic

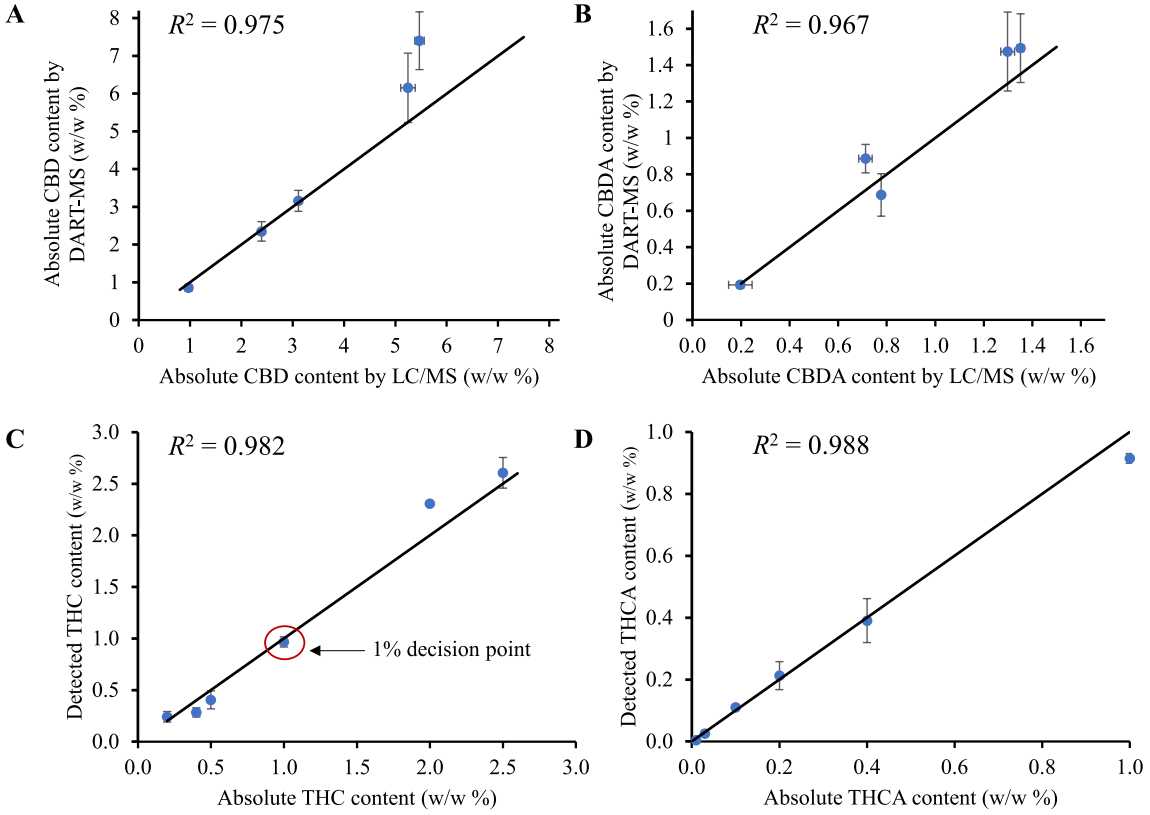

Fig. 5. Absolute CBD and CBDA content detected by DART-MS vs LC/MS methods from cannabis samples (A, B); Absolute THC and THCA content detected by DART-MS from spiked cannabis samples (C, D). The straight lines in the plots represent the y = x function, indicating perfect agreement. Error bars represent standard deviation (n = 4).

range of the calibration curve, the sample extracts were diluted with methanol and then mixed with IS in TMPAH methanol solution for DART-MS analysis. Overall, the quantitative results for the cannabinoids show good agreement with those obtained from the reference LC/MS method or spiked concentrations. At the 1 % decision threshold for THC, the DART-MS method demonstrated accurate predictions, yielding results of 0.97 ± 0.05 % (Fig. 5C), which is significant for forensic applications. Although the LC/MS method exhibits better reproducibility across replicates, as indicated by the smaller horizontal error bars in Fig. 5A and B, DART-MS offers the advantage of high throughput, analyzing extracts in just 10 s. For samples with predicted results near the legal limit, further confirmation using standard HPLC or GC-based methods (*e.g.*, ASTM D8375 or ASTM D8442) is recommended. Implementing an efficient screening method like DART-MS could significantly alleviate the backlog in forensic laboratories.

Conclusion

This study presents a novel approach for the quantitative analysis of cannabinoids using zone heat-assisted DART-MS with in-situ flash derivatization. By employing TMPAH as a derivatization reagent, the method achieves effective differentiation and quantitation of Δ9-THC, CBD, and their acidic precursors THCA and CBDA. The introduction of a heated transfer zone significantly improved the derivatization efficiency and minimized carryover, enhancing the reliability of THC and CBD analysis. The proposed method demonstrated strong agreement with reference LC/MS results, providing accurate and precise quantitation across a range of cannabinoid concentrations. Moreover, the rapid analysis time of approximately 10 s per sample highlights the potential of DART-MS for high-throughput screening, which could alleviate the burden on forensic laboratories facing significant backlogs. However, for samples near the legal decision threshold or those containing high levels of isomeric THC, such as Δ8-THC and CBC, further confirmation using standard HPLC or GC-based methods remains essential. Overall, this study establishes DART-MS with in-situ flash derivatization as a promising tool for fast, reliable cannabinoid analysis, offering both analytical efficiency and forensic applicability.

CRediT authorship contribution statement

Wen Dong: Writing – original draft, Investigation, Formal analysis, Data curation. **Junxiaohan Yuan:** Writing – review & editing, Methodology, Formal analysis. **Xin Yang:** Writing – review & editing, Supervision, Resources. **Ning Zhang:** Writing – review & editing, Resources, Project administration. **Mengliang Zhang:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial 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.2025.100641.

Data availability

Data will be made available on request.

References

[1] M. Durose, A.M. Burch, K. Walsh, E.J.I. Tiry, Publicly funded forensic crime laboratories: Resources and services, 2014, 50(44) (2014) 40.
[2] Y.-C. Cheng, S. Kerrigan, Differentiation of hemp from marijuana using a qualitative decision-point assay, Forensic Chem. 37 (2024) 100541.
[3] ASTM D8442-22, Standard Test Method for Determination of Cannabinoids in Cannabis Raw Materials and Resin Cannabis Products by Gas Chromatography and Flame Ionization Detection, ASTM International, www.astm.org, 2022, p. 7.
[4] ASTM D8375-22, Standard Test Method for Determination of Cannabinoid Concentration in Dried Cannabis and Hemp Raw Materials using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), ASTM International www.astm.org, 2023, p. 17.
[5] A.D. Jacobs, R.R. Steiner, Detection of the Duquenois–Levine chromophore in a marijuana sample, Forensic Sci. Int. 239 (2014) 1–5.
[6] United Nations Office on Drugs, Recommended methods for the identification and analysis of cannabis and cannabis products: manual for use by National Drug Testing Laboratories, United Nations Publications 2009.
[7] N.A. dos Santos, L.M. Souza, E. Domingos, H.S. França, V. Lacerda, A. Beatriz, B. G. Vaz, R.R.T. Rodrigues, V.V. Carvalho, B.B. Merlo, R.M. Kuster, W. Romão, Evaluating the selectivity of colorimetric test (Fast Blue BB salt) for the cannabinoids identification in marijuana street samples by UV–Vis, TLC, ESI(+)FT-ICR MS and ESI(+)MS/MS, Forensic Chem. 1 (2016) 13–21.
[8] H.S. França, A. Acosta, A. Jamal, W. Romao, J. Mulloor, J.R. Almirall, Experimental and ab initio investigation of the products of reaction from Δ9-tetrahydrocannabinol (Δ9-THC) and the fast blue BB spot reagent in presumptive drug tests for cannabinoids, Forensic Chem. 17 (2020) 100212.
[9] N.B. Valdes, R. Gorziza, J.R. Almirall, Quantitative analysis of Δ9-tetrahydrocannabinol (Δ9-THC) in cannabis plants using the Fast Blue BB (FBBB) and 4-aminophenol (4-AP) colorimetric tests, Talanta Open 9 (2024) 100287.
[10] S. Huang, F.W. Claassen, T.A. van Beek, B. Chen, J. Zeng, H. Zuilhof, G.I. J. Salentijn, Rapid distinction and semiquantitative analysis of THC and CBD by silver-impregnated paper spray mass spectrometry, Anal. Chem. 93 (8) (2021) 3794–3802.
[11] A.N. Couch, J.M. Lanza, C.M. Zall, J.T. Davidson, Ag-Ligand Ion Complexation: An Alternative Approach for Hemp and Marijuana Differentiation, 76th AAFS Annual Scientific Conference, The American Academy of Forensic Sciences, Dever, Colorado, 2024.
[12] C. Ieritano, P. Thomas, W.S. Hopkins, Argentination: A silver bullet for cannabinoid separation by differential mobility spectrometry, Anal. Chem. 95 (22) (2023) 8668–8678.
[13] G.P. Jackson, S.A. Mehnert, J.T. Davidson, B.D. Lowe, E.A. Ruiz, J.R. King, Expert algorithm for substance identification using mass spectrometry: statistical foundations in unimolecular reaction rate theory, J. Am. Soc. Mass Spectrom. 34 (7) (2023) 1248–1262.
[14] A.I. Adeoye, J. King, G.P. Jackson, The Application of the Expert Algorithm for Substance Identification (EASI) to Resolve THC and CBD ESI-Mass Spectra, 76th AAFS Annual Scientific Conference, The American Academy of Forensic Sciences, Dever, Colorado, 2024.
[15] B. Fodor, I. Molnár-Perl, The role of derivatization techniques in the analysis of plant cannabinoids by gas chromatography mass spectrometry, TrAC Trends Anal. Chem. 95 (2017) 149–158.
[16] B. Garosi, M.I. Chambers, R.A. Musah, Utilizing derivatizing agents for the differentiation of cannabinoid isomers in complex food, beverage and personal-care product matrices by ambient ionization mass spectrometry, Talanta Open 10 (2024) 100328.
[17] L.K. Ritchie, Y.H. Caplan, J. Park, Δ-9-tetrahydrocannabinol analysis in forensic blood specimens using capillary column gas chromatography with nitrogen sensitive detection, J. Anal. Toxicol. 11 (5) (1987) 205–209.
[18] S. Watts, E.R. de la Rie, Gcms Analysis of triterpenoid resins: in situ derivatization procedures using quaternary ammonium hydroxides, studies in conservation 47(4) (2002) 257-272.
[19] R.B. Cody, J.A. Laramée, H.D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, Anal. Chem. 77 (8) (2005) 2297–2302.
[20] R.S. Borisov, C. Esparza, S.V. Goriainov, V.G. Zaikin, Suitable in-situ derivatization of alcohols by reaction with basic amines in Direct Analysis in Real Time mass spectrometry, Talanta 200 (2019) 31–40.
[21] E.S. Chernetsova, G.E. Morlock, I.A. Revelsky, DART mass spectrometry and its applications in chemical analysis, Russ. Chem. Rev. 80 (3) (2011) 235.
[22] W. Dong, J. Liang, I. Barnett, P.C. Kline, E. Altman, M. Zhang, The classification of Cannabis hemp cultivars by thermal desorption direct analysis in real time mass spectrometry (TD-DART-MS) with chemometrics, Anal. Bioanal. Chem. 411 (30) (2019) 8133–8142.

[23] A. Piccirillo, D. Scalarone, O. Chiantore, Comparison between off-line and on-line derivatisation methods in the characterisation of siccative oils in paint media, J. Anal. Appl. Pyrol. 74 (1) (2005) 33–38.
[24] Y. He, A. Buch, C. Szopa, A.J. Williams, M. Millan, M. Guzman, C. Freissinet, C. Malespin, D.P. Glavin, J.L. Eigenbrode, D. Coscia, S. Teinturier, L. Pin, M. Cabane, P.R. Mahaffy, The search for organic compounds with TMAH thermochemolysis: From Earth analyses to space exploration experiments, TrAC Trends Anal. Chem. 127 (2020) 115896.
[25] N.A. Desrosiers, K.B. Scheidweiler, M.A. Huestis, Quantification of six cannabinoids and metabolites in oral fluid by liquid chromatography-tandem mass spectrometry, Drug Test. Anal. 7 (8) (2015) 684–694.
[26] D. Boulesteix, A. Buch, A.J. Williams, Y. He, C. Freissinet, M.G. Trainer, J.C. Stern, C. Szopa, Comparison of tetramethylammonium hydroxide (TMAH), trimethylsulfonium hydroxide (TMSH), and trimethylphenylammonium hydroxide (TMPAH) thermochemolysis for in situ space analysis of organic molecules in planetary environments, Talanta 257 (2023) 124283.
[27] National Center for Biotechnology Information, PubChem Compound Summary for CID 60966, Tetramethylammonium hydroxide., PubChem.ncbi.nlm.nih.gov, 2024.
[28] National Center for Biotechnology Information. Information, PubChem Compound Summary for CID 15913, Trimethylphenylammonium hydroxide, 2024.
[29] W.T. Chang, D.L. Lin, R.H. Liu, Isotopic analogs as internal standards for quantitative analyses by GC/MS — evaluation of cross-contribution to ions designated for the analyte and the isotopic internal standard, Forensic Sci. Int. 121 (3) (2001) 174–182.