



# Rapid analysis of $\Delta 8$ -tetrahydrocannabinol, $\Delta 9$ -tetrahydrocannabinol, and cannabidiol in $\Delta 8$ -tetrahydrocannabinol edibles by Ag(I) paper spray mass spectrometry after simple extraction

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## ABSTRACT

$\Delta 8$ -Tetrahydrocannabinol (THC) products are increasingly popular despite uncertainties regarding their legality and safety. Based on the hypothesis that unique double-stage tandem mass spectrometry ( $MS^2$ ) and triple-stage tandem mass spectrometry ( $MS^3$ ) fragments would allow unambiguous identification of cannabinoids, a simple and rapid method was developed for the differentiation of  $\Delta 8$ -THC,  $\Delta 9$ -THC, and cannabidiol (CBD) within 1 min. This approach combines methanol extraction and silver(I)-impregnated paper spray mass spectrometry (AgPS-MS) for the analysis of  $\Delta 8$ -THC products. Extraction recovery exceeded 85 % for all three cannabinoids from  $\Delta 8$ -THC edibles, with acceptable matrix effects observed in both brownie and vape oil samples. The  $\Delta 9$ -THC: $\Delta 8$ -THC ratios in acid-treated cannabidiol mixtures were quantified with results comparable to GC-FID method (0 to -11 % deviation). Analysis of commercial  $\Delta 8$ -THC products revealed four contained illegal  $\Delta 9$ -THC levels (> 0.3 %) and all contained various other THC isomers, e.g.,  $\Delta 8$ -iso-THC,  $\Delta(4)8$ -iso-THC, or  $\Delta 3$ -THC. This method offers a reliable solution for  $\Delta 8$ -THC product screening.

## 1. Introduction

$\Delta 8$ -THC products have experienced a rapid and significant rise in popularity in recent years (Peng & Shahidi, 2021). One of the major reasons is that the U.S. Agriculture Improvement Act of 2018 (2018 Farm Bill) defined the legal limit of  $\Delta 9$ -THC as 0.3 % (w/w%) (Congress, 2018), and CBD as legal. Thus, the easy conversion of cheap CBD to  $\Delta 8$ -THC (Huang, van Beek, et al., 2024) created a legal loophole. As a structural isomer of  $\Delta 9$ -THC,  $\Delta 8$ -THC differs only in the position of the olefinic double bond. In terms of psychoactivity,  $\Delta 8$ -THC possesses roughly half of the potency of  $\Delta 9$ -THC (Casajuana Kögel et al., 2018; Hollister & Gillespie, 1973).  $\Delta 8$ -THC, as a constituent of many different types of products, especially edibles, is marketed as a “legal high” (Babalonis et al., 2021; Peng et al., 2021). However, many issues have been identified with  $\Delta 8$ -THC edibles. For example, from a forensic perspective, more than 50 % of tested commercial  $\Delta 8$ -THC products were found to contain illegal levels of  $\Delta 9$ -THC (> 0.3 %) (Council, U. S.

C, 2021; Gleb, 2022). Simultaneously, from a food safety standpoint, cases of intoxication associated with the consumption of  $\Delta 8$ -THC edibles are increasingly being reported. (Simon et al., 2023). These issues can be partly explained by the production process of  $\Delta 8$ -THC, in which inevitably byproducts such as  $\Delta 9$ -THC and other THC isomers are formed, which are difficult to remove before incorporation into food matrixes (Golombek et al., 2020; Peng et al., 2021). Moreover, the lack of regulations, limited information about some of these isomers and wrong/unclear labeling of  $\Delta 8$ -THC and CBD content can easily result in overdose consumption and, thus rising hospitalization (Kaczor et al., 2024). Therefore, it is crucial to know the isomeric cannabinoid compositions of  $\Delta 8$ -THC edibles.

Analysis and distinction of cannabinoid isomers, e.g.,  $\Delta 8$ -THC,  $\Delta 9$ -THC, and CBD, are challenging. Normally, high-resolution chromatographic separation is needed because of the structural similarities (Chan-Hosokawa et al., 2022; Huang, van Beek, et al., 2024; Reber et al., 2022). NMR can be used as an alternative, but compounds with lower

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concentrations are easily overlooked (Draper & McCarney, 2023; Marzullo et al., 2020; Meehan-Atrash & Rahman, 2021). Combining speed and sensitivity, ion mobility spectrometry (IMS) has been employed to resolve isomeric cannabinoids. However, the resolution between  $\Delta$ 8-THC and  $\Delta$ 9-THC is still an issue (Kiselak et al., 2020; Tose et al., 2017), unless silver ions are introduced into the IMS (Ieritano et al., 2023). The use of the unique adduct-formation behavior of cannabinoids with silver ions amplifies structural differences and enhances isomer separation in IMS (Huang, Righetti, et al., 2024; Zietek et al., 2018). While this IMS approach is very powerful, it is also expensive and requires dedicated, state-of-the-art equipment that is not readily available in most labs. In recent work, we demonstrated that Ag(I) enables the distinction of isomeric cannabinoids in both chromatographic and mass spectrometric analyses due to their different affinities for Ag(I) (Huang, van Beek, et al., 2024). The normal-phase/argentation (silica-Ag(I))-high performance liquid chromatography (HPLC)-diode array detector (DAD)/MS provided a detailed and accurate cannabinoid profile for  $\Delta$ 8-THC gummies. However, this method required extensive sample pretreatment and prolonged instrumental analysis, adding significant complexity and time to the process.

Thus, rapid, sensitive, easy, and cheap methods for routine analysis of  $\Delta$ 8-THC products are still needed. Ambient ionization mass spectrometry (AIMS), which is characterized by high speed, acceptable sensitivity, and ease of use (with no or limited sample pretreatment), stands out as an option (Brown et al., 2020). Falconer et al. (Falconer & Morales-Garcia, 2023) applied direct analysis in real-time mass spectrometry (DART-MS) for rapid screening and characterization of approximately 500 vaping liquid samples related to lung injury outbreaks. Within  $\sim$ 1.5 min, multiple analytes, including cannabinoids, could be analyzed qualitatively. However, the distinction of cannabinoid isomers (e.g.,  $\Delta$ 8-THC,  $\Delta$ 9-THC, CBC, and CBD) failed. Similarly, Chambers et al. (Chambers & Musah, 2022) qualitatively analyzed cannabinoid-infused products, personal-care products, and hemp materials in their native forms by DART-high-resolution mass spectrometry (HRMS), yet here again, the ability to distinguish isomers was insufficient. Compared to DART-MS, paper spray mass spectrometry (PS-MS), another widely used AIMS technique, offers enhanced selectivity through paper modification (Liu et al., 2010). This selectivity can be achieved on the paper tip, for instance, by selectively adsorbing or desorbing phospholipids under different pH using multiple-step modified  $\text{Ti}^4$  paper (Luo et al., 2023). Alternatively, selectivity can be achieved during the ionization or MS fragmentation process through the formation and subsequent fragmentation of metal ion adducts (Huang et al., 2021). For example, in our previous work, we successfully demonstrated the first differentiation of  $\Delta$ 9-THC and CBD by fragmenting Ag(I) adducts using Ag(I)-impregnated paper spray mass spectrometry (AgPS-MS). Although the method is characterized by its simplicity, rapid analysis, and low cost, it is primarily designed for screening  $\Delta$ 9-THC:CBD ratios in CBD oils, where interference from multiple isomers and complex matrixes is minimal (Huang et al., 2021). To the best of our knowledge, there have been no reports about the distinction of  $\Delta$ 8-THC and  $\Delta$ 9-THC by AIMS, which is of course essential for the analysis of  $\Delta$ 8-THC products.

In the current study, we therefore investigated the possibility of using the AgPS-MS method as a rapid, easy, and cheap strategy for the distinction between  $\Delta$ 8-THC and  $\Delta$ 9-THC and rapid screening of  $\Delta$ 8-THC products.  $\Delta$ 8-THC and  $\Delta$ 9-THC are structurally more similar, having only different positions of a single olefinic double bond instead of having different numbers of olefinic bonds (which is the case for  $\Delta$ 9-THC and CBD). As a result,  $\Delta$ 8-THC and  $\Delta$ 9-THC are expected to have similar affinities for Ag(I). Therefore, multi-stage fragmentation might be necessary to produce more diagnostic fragments. Moreover, considering the huge variety of  $\Delta$ 8-THC products, a generic, yet simple extraction method that could be used for different types of matrixes would be preferable in combination with rapid instrumental analysis. Lastly, to further reduce the technical expertise required for this analytical

method, 3D-printed accessories to simplify paper spray mass spectrometric operations would enable broader applicability in forensic and food safety screening. Therefore, in this paper, we aimed to develop a simple and robust procedure to analyze  $\Delta$ 8-THC containing samples by the combination of a simple extraction, an easy-to-make 3D-printed paper spray device and AgPS-MS analysis.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Silver nitrate ( $\text{AgNO}_3$ , analytical grade) was obtained from Fisher Scientific (Loughborough, UK). Methanol (MeOH, HPLC-grade) was purchased from VWR Chemicals (Gliwice, Poland). Chloroform ( $\text{CHCl}_3$ ,  $\geq 99\%$ ) was bought from Sigma-Aldrich (St. Louis, MO, USA). Chromatography paper was purchased from Hangzhou Special Paper Co., Ltd. (Hangzhou, China). Crystalline CBD (declared purity 99%) was obtained from CBDolie.nl (Utrecht, the Netherlands).  $\Delta$ 8-THC (purity  $>98\%$ ),  $\Delta$ 9-THC (purity  $>98\%$ ),  $\Delta$ 9-THCV (purity  $>90\%$ ),  $\Delta$ 8-iso-THC (purity  $>98\%$ ), and  $\Delta$ 3-THC (purity  $>90\%$ ) were isolated and identified as in our previous studies, and their purities were determined by NMR and HPLC-UV peak integrations at 215 nm (Huang, Righetti, et al., 2024; Huang, van Beek, et al., 2024).  $\Delta$ (4)-iso-THC was provided by Prof. Danielle Passarella (Dipartimento di Chimica, Università degli Studi di Milano, Milano, Italy).  $\Delta$ 8-THC infused brownies, rice crackers, Cannabis leaves, and vape oils were purchased online (Supporting Information (SI), Table S1). Normal brownies and vape oils without cannabinoids were obtained in local shops (Wageningen, the Netherlands). Different acid-treated CBD mixtures were obtained in our previous study (SI, Table S2) (Huang, van Beek, et al., 2024).

### 2.2. Design and fabrication of 3D-printed paper spray device/3D-printing settings

A 3D-printed paper spray device was designed and produced to stabilize the paper tip, facilitate the sample and solvent application, and position it in front of the MS. All components of the paper spray device (SI, Fig. S1) were designed and assembled using computer-aided design (CAD) software SolidWorks 2021 (Dassault Systèmes SolidWorks Corporation, Waltham, USA), and exported in a 3D manufacturing format (.3MF). The sample well slide was fabricated using a high-resolution stereolithography (SLA) printer (Form 3, FormLabs, Somerville, MA, USA). The print file was generated using Preform version 3.33.0 (FormLabs) at a layer resolution of 100  $\mu\text{m}$  and printed using FormLabs clear resin (V4). Other paper spray accessories, including an arm for anchoring the whole device to the MS, a slide holder, a paper cartridge holder, a slide button for sliding sample wells, and paper cartridge parts, were printed using the Original Prusa i3 MK3S+ (Prusa, Prague, Czech Republic) fused deposition modeling (FDM) printer. These parts were converted into G-Code for 3D printing with PrusaSlicer 2.7.1 (Prusa), using the 0.10 mm "Detail" preset and the following parameters: nozzle diameter 0.4 mm, nozzle temperature 210  $^\circ\text{C}$ , bed temperature 60  $^\circ\text{C}$ , and organic supports on the build plate only. All FDM parts were printed using biobased filament (PolyLite PLA PRO, Polymaker, Utrecht, the Netherlands).

### 2.3. Preparation of paper tips, Ag(I)-impregnated paper tips and Ag(I)-impregnated paper tips for 3D-printed cartridge

Clean paper tips and Ag(I)-impregnated tips were prepared as described in our previous study (Huang et al., 2021). Briefly, isosceles triangle paper tips with a height of 10 mm and a base of 5 mm were obtained by cutting chromatography paper with a homemade paper cutter. The paper tips were thoroughly washed by immersing them in MeOH in a sonication bath for half an hour and dried in a vacuum oven overnight. These paper tips are referred to as *clean*. Ag(I)-impregnated

tips were prepared from *clean* paper tips following the same procedure, except that a  $0.10 \text{ mol} \cdot \text{L}^{-1}$   $\text{AgNO}_3$  MeOH solution instead of MeOH was used for immersing paper tips. Ag(I)-impregnated paper tips for the 3D-printed cartridge were placed in the fixed position on the 3D-printed cartridge, after which the cartridge cover was closed for subsequent attachment to the cartridge holder (SI, Fig. S2).

## 2.4. Linear ion trap mass spectrometer

A Thermo Velos Pro linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was used in positive mode with a spray voltage of 4 kV, S-lens RF level 68.6 %, and capillary temperature of  $250^\circ\text{C}$ , unless specified otherwise. All full-scan measurements were performed with an  $m/z$  100–2000 scan range. Two MS scanning events, including  $\text{MS}^2$  and  $\text{MS}^3$ , were set to get both acquisitions in one single analysis (SI, Table S3). MS transitions of cannabinoids investigated in this study are shown in Table S4 (SI, Table S4). For  $\Delta^9\text{-THC}:\Delta^8\text{-THC}$  ratio measurements, the selected reaction monitoring (SRM) mode was used with the precursor ions  $m/z$   $422 \pm 2$  for  $\text{MS}^2$  fragmentation and  $m/z$   $313 \pm 1$  for  $\text{MS}^3$  fragmentation.

## 2.5. Paper spray setup

### 2.5.1. Conventional paper spray

The standard paper spray experiments were conducted as outlined in our previous study (Huang et al., 2021). Briefly, the paper tip was held by an alligator clip on a modified desorption electrospray ionization (DESI) ion source (Prosolia, Indianapolis, IN, USA) with a rotational and x-y-z positioner. This setup was directly connected to the high voltage (HV) supply of the instrument. The front of the paper tip was carefully positioned 4–6 mm from the MS inlet. A voltage of 4 kV was applied after a  $15 \mu\text{L}$  sample solution was put on the paper tip for spray generation unless otherwise indicated. Each sample was measured five times.

### 2.5.2. 3D-printed cartridge paper spray

A 3D-printed cartridge was used for paper spray experiments. The setup involved pre-assembling a (i) paper spray cartridge holder to a (ii) rotator and (iii) slide holder positioned in front of the MS inlet, which could be done easily by two screws. Samples were deposited in wells of the sample well slide. Then, one-time-use paper spray cartridges could be positioned with the rotator to contact the samples, allowing them to become wetted, after which spray could be generated. Switching between samples was accomplished by sliding the sample well slide and replacing the paper cartridge (SI Video).

## 2.6. GC-FID/MS

GC-FID/MS analysis was achieved using the method developed in our previous work (Huang, Righetti, et al., 2024; Huang, van Beek, et al., 2024). Briefly, a DB-5MS UI capillary column (Agilent J&W GC column, Agilent, Santa Clara, CA, USA) was connected to an Agilent 5975C VL MSD GC-FID/MS system (Agilent, Amstelveen, the Netherlands). The initial column temperature was set to  $200^\circ\text{C}$  and increased by  $1^\circ\text{C} \cdot \text{min}^{-1}$  to  $222.5^\circ\text{C}$ . Then, the temperature was raised at  $5^\circ\text{C} \cdot \text{min}^{-1}$  to  $250^\circ\text{C}$  and held for 5 min, totaling 33 min of analysis. A  $1 \mu\text{L}$  sample was injected with a 1:10 split ratio at an injection temperature of  $200^\circ\text{C}$ . Each sample was injected once. Helium was the carrier gas at a constant flow with a linear velocity of  $26 \text{ cm} \cdot \text{s}^{-1}$ . The mass spectrometer was operated in 70 eV electron ionization (EI) mode, scanning from  $m/z$  35 to 500 at  $4 \text{ spectra} \cdot \text{s}^{-1}$ . Measurements were delayed by 3 min after injection to protect the mass spectrometer filament.

## 2.7. Sample extraction and extraction efficiency

### 2.7.1. Sample extraction

$10.0 \pm 1.0 \text{ mg}$  ( $n = 3$  per sample) of  $\Delta^8\text{-THC}$  infused brownies or rice

crackers were accurately weighed (M5, Mettler Instrumente AG, Zurich, Switzerland) in 1.5 mL Eppendorf safe-lock tubes (Eppendorf Nederland B.V., Nijmegen, the Netherlands) followed by adding 1.00 mL of MeOH to each tube with a micropipette ( $100\text{--}1000 \mu\text{L}$ , Eppendorf research plus, Nijmegen, the Netherlands). For  $\Delta^8\text{-THC}$  infused Cannabis leaves,  $10.0 \pm 1.0 \text{ mg}$  ( $n = 3$  per sample) was weighed in 15 mL polypropylene screw cap centrifuge tubes (Sarstedt, Nümbrecht, Germany), and 10.0 mL of MeOH was added with a pipette ( $1\text{--}10 \text{ mL}$ , Eppendorf research plus, Nijmegen, the Netherlands). These tubes were put into a tube box (or tube rack for 15 mL tubes) and manually shaken for 10 min to extract cannabinoids unless otherwise specified. Obtained solutions were filtered over  $0.2 \mu\text{m}$  PTFE membrane syringe filters ( $\varnothing$  13 mm, Pall Corporation, Port Washington, NY, USA) and then used for subsequent analysis. Three consecutive MeOH extractions were performed by removing the supernatant after 10-min manual shaking and adding 1.00 mL of fresh MeOH. For tubes containing Cannabis leaves, 10.0 mL instead of 1.00 mL of fresh MeOH was added. The fourth extraction followed a standard procedure described in literature (Di Marco Pisciotto et al., 2018; Di Marco Pisciotto et al., 2021). Specifically, 1.00 mL (or 10.0 mL for Cannabis leaves) of MeOH: $\text{CHCl}_3$  ( $v:v = 9:1$ ) was added to the sample, followed by vigorous vortexing (Fisherbrand, WhirliMixer, Loughborough, UK) for 1 min. After that, the mixture was centrifuged (Eppendorf 5424R centrifuge, Eppendorf AG, Hamburg, Germany) at 14000 relative centrifugal force (rcf) for 15 min, and the supernatant was used for subsequent analysis. For  $\Delta^8\text{-THC}$  infused vape oils, an easy dilution-and-analysis strategy was used. Namely,  $10.0 \pm 1.0 \text{ mg}$  ( $n = 3$  per sample) of vape oils was weighed and diluted by MeOH to  $1.00 \text{ mg} \cdot \text{mL}^{-1}$  and then subjected to analysis.

### 2.7.2. Extraction efficiency

The extraction efficiency of each extraction was evaluated by the GC-FID method and expressed as: extraction efficiency (%) =  $A_{\text{single}}/A_{\text{total}} \times 100$ , where  $A_{\text{single}}$  means the characteristic GC-FID peak area of the single extraction, and  $A_{\text{total}}$  means the summed-up peak areas from all four extractions, assuming 100 % extraction recovery.

## 2.8. Matrix effects

$100.0 \pm 0.3 \text{ mg}$  of a normal brownie was extracted by adding 10.0 mL of MeOH and hand-shaking for 10 min and filtered as described above, resulting in the “normal brownie extract”.  $100.0 \pm 0.3 \text{ mg}$  of a normal vape oil was diluted by 10.0 mL of MeOH, resulting in the “normal vape extract.” These extracts were spiked with cannabinoids and referred to as sample type II. The MeOH standard solution containing corresponding concentrations of cannabinoids was referred to as sample type I. Sample types I and II were prepared ( $n = 3$ ) containing 0.1 %, 0.3 %, and 1 % of  $\Delta^8\text{-THC}$ ,  $\Delta^9\text{-THC}$ , and CBD, as well as 0.5 % of  $\Delta^9\text{-THCV}$  (IS) ( $w/w\%$ ) (SI, Table S5). These samples were analyzed by the described AgPS-MS and GC-FID methods.

### 2.8.1. Matrix effect for each cannabinoid with the AgPS- $\text{MS}^2$ and AgPS- $\text{MS}^3$ methods

The matrix effect for each cannabinoid with both the AgPS- $\text{MS}^2$  and AgPS- $\text{MS}^3$  methods was calculated as: matrix effect (%) =  $((A_{\text{II}}/A_{\text{I}}) - 1) \times 100$ . First, the extracted ion chromatogram (EIC) of each cannabinoid was normalized to the total ion chromatogram (TIC) (EIC/TIC) to correct for spray instability.  $A_{\text{II}}$  and  $A_{\text{I}}$  were the normalized characteristic EIC areas of each cannabinoid ( $m/z$  245 for  $\Delta^8\text{-THC}$  in the  $\text{MS}^2$  spectrum,  $m/z$  313 for  $\Delta^9\text{-THC}$  in the  $\text{MS}^2$  spectrum, and  $m/z$  353 for CBD in the  $\text{MS}^2$  spectrum;  $m/z$  217 for  $\Delta^9\text{-THC}$  in the  $\text{MS}^3$  spectrum,  $m/z$  245 for  $\Delta^8\text{-THC}$  in the  $\text{MS}^3$  spectrum) in sample type II and sample type I, respectively.

### 2.8.2. Matrix effect for the $\Delta^9\text{-THC}:\Delta^8\text{-THC}$ ratio with the AgPS- $\text{MS}^2$ and AgPS- $\text{MS}^3$ methods

The matrix effect for the  $\Delta^9\text{-THC}:\Delta^8\text{-THC}$  ratio with the AgPS- $\text{MS}^2$

and AgPS-MS<sup>3</sup> methods were expressed as:  $(R_{\text{matrix}} - R_{\text{solvent}})/R_{\text{solvent}} \times 100$ , where  $R_{\text{matrix}}$  represents the characteristic peak area ratio of  $\Delta 9$ -THC: $\Delta 8$ -THC ( $m/z$  313: $m/z$  245 in MS<sup>2</sup> spectrum and  $m/z$  217: $m/z$  245 in MS<sup>3</sup> spectrum) in the matrix, and  $R_{\text{solvent}}$  represents the characteristic peak area ratio of  $\Delta 9$ -THC: $\Delta 8$ -THC ( $m/z$  313: $m/z$  245 in MS<sup>2</sup> spectrum and  $m/z$  217: $m/z$  245 in MS<sup>3</sup> spectrum) in the solvent.

### 2.8.3. Matrix effect for each cannabinoid with the GC-FID method

The matrix effect for each cannabinoid with the GC-FID method was calculated as: matrix effect (%) =  $((A_{\text{II}}/A_{\text{I}}) - 1) \times 100$ .  $A_{\text{II}}$  and  $A_{\text{I}}$  were the characteristic GC-FID areas of each cannabinoid.

## 2.9. Calibration curve construction and evaluation of LOD

Stock solutions of  $\Delta 8$ -THC at 1.00 mg·mL<sup>-1</sup>,  $\Delta 9$ -THC at 1.00 mg·mL<sup>-1</sup>, CBD at 1.00 mg·mL<sup>-1</sup>, and internal standard (IS)  $\Delta 9$ -THCV at 5.00 mg·mL<sup>-1</sup> were prepared in MeOH.

### 2.9.1. $\Delta 9$ -THC: $\Delta 8$ -THC ratio calibration curve

$\Delta 8$ -THC and  $\Delta 9$ -THC stock solutions were used to prepare calibration solutions with various  $\Delta 9$ -THC: $\Delta 8$ -THC ratios (0.00100, 0.00200, 0.00500, 0.0100, 0.0200, 0.0500, 0.100, 0.200, 0.500, and 1.00;  $n = 3$  per ratio), maintaining the  $\Delta 8$ -THC concentration at a constant 50.0  $\mu\text{g}\cdot\text{mL}^{-1}$ . To achieve this, specific volumes of methanolic stock solutions were mixed and diluted with MeOH to a final volume of 2.00 mL. These samples were then analyzed with the AgPS-MS method in selected reaction monitoring (SRM) mode with precursor ions at  $m/z$  422  $\pm$  2 for MS<sup>2</sup> fragmentation and MS<sup>2</sup> fragments at  $m/z$  313  $\pm$  2 for MS<sup>3</sup> fragmentation. The characteristic MS<sup>2</sup> and MS<sup>3</sup> extracted ion chromatogram (EIC) area ratios of  $\Delta 9$ -THC: $\Delta 8$ -THC were plotted against the concentration ratios of  $\Delta 9$ -THC: $\Delta 8$ -THC to construct calibration curves for AgPS-MS<sup>2</sup> and AgPS-MS<sup>3</sup>. A correction was made by subtracting the signal of  $m/z$  313 from  $\Delta 8$ -THC in MS<sup>2</sup> spectra to obtain a net MS<sup>2</sup> signal intensity of  $\Delta 9$ -THC. Similarly, the signal of  $m/z$  245 from  $\Delta 9$ -THC in MS<sup>3</sup> spectra was subtracted to obtain a net MS<sup>3</sup> signal intensity of  $\Delta 8$ -THC. The characteristic MS<sup>2</sup> EIC area ratios of  $\Delta 9$ -THC: $\Delta 8$ -THC were obtained after subtracting the MS<sup>2</sup> background value of  $0.53 \times \text{EIC}(m/z$  245) from  $\Delta 8$ -THC and expressed as  $(A_{\text{EIC}(m/z$  313) - (0.53  $\times$   $A_{\text{EIC}(m/z$  245)))/ $A_{\text{EIC}(m/z$  245). Similarly, the characteristic MS<sup>3</sup> EIC area ratios of  $\Delta 9$ -THC: $\Delta 8$ -THC were obtained after subtracting the MS<sup>3</sup> background value of  $0.074 \times \text{EIC}(m/z$  217) for  $\Delta 8$ -THC and expressed as  $A_{\text{EIC}(m/z$  217)/( $A_{\text{EIC}(m/z$  245) - (0.074  $\times$   $A_{\text{EIC}(m/z$  217)).

### 2.9.2. $\Delta 8$ -THC, $\Delta 9$ -THC, and CBD concentration calibration curve

Three-standard solutions of 200, 100, 50.0, 10.0, 5.00, and 1.00  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $n = 3$  per concentration), each containing 50.0  $\mu\text{g}\cdot\text{mL}^{-1}$  of the internal standard, were prepared by mixing stock solutions of  $\Delta 8$ -THC,  $\Delta 9$ -THC, and CBD and serially diluting them with MeOH. These solutions were analyzed using the AgPS-MS and GC-FID methods. Characteristic peak areas were normalized by the area of the internal standard and the obtained ratios were plotted against the concentrations to construct calibration curves for quantification. The limit of detection (LOD) of  $\Delta 8$ -THC,  $\Delta 9$ -THC, and CBD by the AgPS-MS method was estimated by:  $\text{LOD} = 3 \times \text{SD}$  of the lowest concentration of the calibration curve/slope of the calibration curve (Little, 2015).

## 2.10. Sample analysis

### 2.10.1. $\Delta 9$ -THC: $\Delta 8$ -THC ratio analysis of acid-treated CBD mixtures

Acid-treated CBD mixtures were prepared in MeOH at 100  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $n = 3$  per sample) and analyzed by the AgPS-MS method and the GC-FID method

### 2.10.2. Screening analysis of $\Delta 8$ -THC products

$\Delta 8$ -THC products were extracted or diluted as described above. The internal standard was added to the sample extracts prior to AgPS-MS

analysis to achieve the same final internal standard concentration as used in the calibration curves. Concentrations of  $\Delta 8$ -THC,  $\Delta 9$ -THC, and CBD were calculated with the constructed calibration curves, and their absolute percentages were expressed as below, considering any dilution factors applied:

$$\text{Weight percentage (\%)} = \frac{C \times V}{W} \times 100,$$

with: C = calculated concentration in extract ( $\mu\text{g}\cdot\text{mL}^{-1}$ ); V = volume of extract (mL); W = total sample weight ( $\mu\text{g}$ ).

### 2.10.3. Standard addition of $\Delta 8$ -THC products

A 1.00 mg·mL<sup>-1</sup>  $\Delta 8$ -THC vape oil extract was diluted with MeOH to 0.250 mg·mL<sup>-1</sup>. A 10.0 mg·mL<sup>-1</sup>  $\Delta 8$ -THC brownie extract was blow-dried and reconstituted in MeOH to 20.0 mg·mL<sup>-1</sup>. The resulting “ $\Delta 8$ -THC edible extracts” were spiked with various volumes of  $\Delta 8$ -THC and  $\Delta 9$ -THC stock solutions, along with a fixed volume of  $\Delta 9$ -THCV (IS) stock solution to make solutions containing 200, 150, 100, 50.0, and 0  $\mu\text{g}\cdot\text{mL}^{-1}$  of  $\Delta 8$ -THC and 40.0, 30.0, 20.0, 10.0, and 0  $\mu\text{g}\cdot\text{mL}^{-1}$  of  $\Delta 9$ -THC, in addition to what was already present in the samples ( $n = 3$  per concentration). Each solution also contained 50.0  $\mu\text{g}\cdot\text{mL}^{-1}$  of the internal standard. Ratios of the characteristic EIC of  $\Delta 8$ -THC ( $m/z$  245 in the MS<sup>3</sup> spectrum) and  $\Delta 9$ -THC ( $m/z$  217 in the MS<sup>3</sup> spectrum) to the internal standard ( $m/z$  189 in the MS<sup>3</sup> spectrum) were calculated and plotted against the added concentrations of  $\Delta 8$ -THC and  $\Delta 9$ -THC to construct calibration curves. The X-intercept of each calibration curve was determined and used to identify the concentration of  $\Delta 8$ -THC and  $\Delta 9$ -THC in the non-spiked  $\Delta 8$ -THC edible extracts. The absolute percentages of  $\Delta 8$ -THC and  $\Delta 9$ -THC were calculated as previously described, accounting for any dilution factors applied.

## 3. Results and discussion

### 3.1. Ag(I)-impregnated paper spray mass spectrometric distinction of $\Delta 8$ -THC and $\Delta 9$ -THC

Distinguishing isomeric cannabinoids using AIMS is challenging. In 2021, we demonstrated for the first time that by using a AgPS-MS<sup>2</sup> method, cannabinoid isomers ( $\Delta 9$ -THC and CBD) with identical mass spectra and MS/MS product ion spectra of protonated precursor ions (Wishart et al., 2024) show completely different product ion spectra when selecting silver adducts as precursor ions (Huang et al., 2021). Similarly, using this AgPS-MS<sup>2</sup> method,  $\Delta 8$ -THC and  $\Delta 9$ -THC produce distinct MS<sup>2</sup> spectra with a base peak at  $m/z$  313 for  $\Delta 9$ -THC and  $m/z$  245 for  $\Delta 8$ -THC (Fig. 1A and B) (Huang, Righetti, et al., 2024; Huang, van Beek, et al., 2024). However, in the MS<sup>2</sup> spectrum of  $\Delta 8$ -THC (Fig. 1A), there is also a significant (~50 %) peak at  $m/z$  313. This equals the mass of the characteristic MS<sup>2</sup> fragment of  $\Delta 9$ -THC. While this is not an issue in combination with a chromatographic separation (Huang, van Beek, et al., 2024) or IMS separation (Huang, Righetti, et al., 2024), it presents a challenge in paper spray analysis, where there is no physical separation of molecules or ions prior to the MS analysis.

To rule out the possibility of conversion from  $\Delta 8$ -THC to  $\Delta 9$ -THC and thus leading to the observed signal of  $m/z$  313 in the MS<sup>2</sup> spectrum of  $\Delta 8$ -THC, the peak area ratio of  $m/z$  313 to  $m/z$  245 was measured for pure  $\Delta 8$ -THC under various conditions, including different CID energies, capillary temperatures, and spray voltages. This is based on the assumption that if any degradation were to occur, it would likely happen during the spray process, influenced by one or more of the factors mentioned above. The ratio remained constant within the range of 0.46–0.56 regardless of the parameter changes. When MS parameters are constant, the ratio of  $m/z$  313: $m/z$  245 is  $0.53 \pm 0.01$  across a wide concentration range of 0.5–1000  $\mu\text{g}\cdot\text{mL}^{-1}$  (SI, Fig. S3). This suggests that the fragment at  $m/z$  313 in the MS<sup>2</sup> spectrum of  $\Delta 8$ -THC is a genuine fragment of  $\Delta 8$ -THC and not due to *in situ* conversion to  $\Delta 9$ -

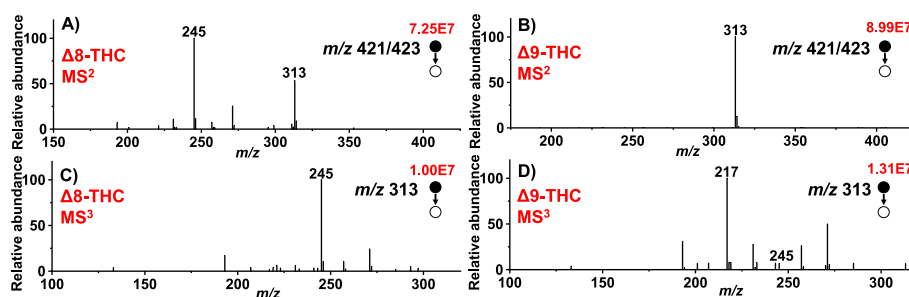


Fig. 1. AgPS-MS<sup>2</sup> spectra of A) Δ8-THC and B) Δ9-THC ( $m/z$  422  $\pm$  2 as precursor ions), as well as AgPS-MS<sup>3</sup> spectra of C) Δ8-THC and D) Δ9-THC (product ions from the MS<sup>2</sup> fragment at  $m/z$  313  $\pm$  2).

THC, as such conversion would likely cause a change in the ratio of  $m/z$  313: $m/z$  245 with parameter adjustments.

Considering the above, the  $m/z$  313 signal in the Δ8-THC spectrum can be subtracted as the background value in the MS data from samples. Quantitative analysis of the Δ9-THC to Δ8-THC ratio in samples can be achieved by first subtracting the background value of  $0.53 \times \text{EIC}$  ( $m/z$  245) in pure Δ8-THC and then determining the EIC area ratio of  $m/z$  313: $m/z$  245 in the MS<sup>2</sup> spectra. A similar strategy was shown to be effective in analyzing Δ9-THC:CBD ratios in commercial CBD oils in our previous study (Huang et al., 2021).

For Δ8-THC products, it is important to obtain content information for both Δ8-THC and Δ9-THC as both possess psychoactive properties. When Δ8-THC and Δ9-THC coexist,  $m/z$  245 in the MS<sup>2</sup> spectrum can be used to quantify Δ8-THC. To quantify Δ9-THC, the  $m/z$  313 signal first requires subtracting a high background value ( $0.53 \times \text{EIC}$  ( $m/z$  245)) produced by Δ8-THC during the MS<sup>2</sup> stage. This high background value potentially hinders direct and sensitive Δ9-THC analysis. To further improve the selectivity, MS<sup>3</sup> fragmentation was performed by fragmenting the  $m/z$  313 fragments from the MS<sup>2</sup> stage. The MS<sup>3</sup> spectrum of Δ8-THC is dominated by a fragment at  $m/z$  245 (Figs. 1C), and the MS<sup>3</sup> spectrum of Δ9-THC is dominated by a fragment at  $m/z$  217 (Fig. 1D). A mechanism for the MS<sup>3</sup> fragmentation of Δ8-THC and Δ9-THC was proposed (SI, Fig. S4). For Δ8-THC, our previous study demonstrated that the fragment at  $m/z$  245 could be generated via a *retro*-Diels–Alder rearrangement (Huang, van Beek, et al., 2024). Previously, we proposed that the fragmentation of Δ9-THC involves the loss of C<sub>6</sub>H<sub>12</sub> through partial cleavage of the benzene ring and alkyl side chain (Huang et al., 2021). However, the MS fragmentation of Δ9-THCV observed in this study negates the possibility of alkyl side-chain loss (SI, Fig. S15). Therefore, we proposed an alternative pathway for the formation of the fragment at  $m/z$  217, involving the neutral losses of methylcyclohexadiene and H<sub>2</sub>. Nonetheless, further research is required to validate this mechanism.

Even though there is a minor peak at  $m/z$  245 in the MS<sup>3</sup> spectrum of Δ9-THC, the signal intensity is only around 7 % of the base peak ( $m/z$  217). The EIC peak area ratio of  $m/z$  245 to  $m/z$  217 in the MS<sup>3</sup> spectra of pure Δ9-THC was consistently  $0.074 \pm 0.0038$ , regardless of different CID energies and isolation windows (SI, Table S6 and Fig. S5). When using the AgPS-MS<sup>3</sup> method, the MS<sup>3</sup> fragment at  $m/z$  217 can be used to characterize Δ9-THC, and the MS<sup>3</sup> fragment at  $m/z$  245 can be used to characterize Δ8-THC after subtracting the background value of  $0.074 \times \text{EIC}$  ( $m/z$  217).

### 3.2. Extraction efficiency

The broad scope of Δ8-THC products poses great challenges for sample extraction and pretreatment due to the diversity of the matrixes and their physicochemical properties (Chambers et al., 2022). Generally, extraction procedures are needed according to the different forms of products, e.g. solid-phase extraction, ultrasound-assisted extraction, and Soxhlet extraction (Christodoulou et al., 2023; Di Marco Pisciotano

et al., 2018; Di Marco Pisciotano et al., 2021). These sample pretreatment operations require expertise, resources, and time, which limits large scale screening of different forms of Δ8-THC products. Since the objective of the current work is to develop an easy and rapid method for screening of different types of Δ8-THC products, a simple and straightforward sample extraction step, which is suitable for both solid and liquid products, is deemed preferable prior to the rapid analysis by AgPS-MS. In our previous work, extraction by MeOH vortexing was demonstrated as an efficient strategy for extracting Δ9-THC and CBD from CBD oils in a short time, with minimal matrix effects (Huang et al., 2021). Moreover, MeOH has been demonstrated to be one of the most effective solvents for extracting cannabinoids from Cannabis plant materials and food matrixes (Brighenti et al., 2017; Escrivá et al., 2017). Other solvents, such as ethanol (EtOH) and acetonitrile (ACN), have also been employed for cannabinoid extraction (Vella Szijj, 2024). However, in this study, the extraction solvent also serves as the spray solvent for subsequent AgPS-MS analysis. ACN has a tendency to form very strong complexes with Ag(I), which competed with cannabinoids during the argmentation process (SI, Fig. S6). Furthermore, MeOH was shown to produce stronger cannabinoid signal intensities compared to ACN and EtOH (SI, Fig. S6). This is likely due to its high dielectric constant and low boiling point (Mukta et al., 2024; Zhang et al., 2012), which enhance ionization efficiency and make it widely applicable in PS-MS analysis of cannabinoids (Borges et al., 2019; Espy et al., 2014; Ma et al., 2015). Therefore, MeOH was selected as the extraction solvent to extract Δ8-THC, Δ9-THC, and CBD from Δ8-THC brownies, rice crackers, Cannabis leaves, and vape oils; handshaking instead of vortexing was used for reducing dependency on instruments.

To evaluate the recovery of a single extraction by such a simple method, firstly, four repeated extractions of the solid samples were conducted, aiming to achieve exhaustive extraction of Δ8-THC, Δ9-THC, and CBD. Three consecutive extractions in MeOH were performed, followed by a fourth extraction using a commonly applied extraction method involving vortexing and centrifuging that was assumed would recover the remaining cannabinoids almost completely (recoveries of 80 %–114 % have been reported) (Di Marco Pisciotano et al., 2018; Di Marco Pisciotano et al., 2021). Here, this fourth extraction yielded almost no additional Δ8-THC, Δ9-THC, and CBD, supporting that assumption. Secondly, the combined recoveries from the four extractions were considered quantitative and the recoveries of the individual extraction steps were normalized against this total recovery (100 %). As a result (Fig. 2), a single extraction by handshaking already resulted in recoveries of  $94.4 \pm 2.9$  % for Δ8-THC and  $97.7 \pm 2.1$  % for Δ9-THC in Δ8-THC brownies;  $87.9 \pm 16$  % for Δ8-THC and  $85.7 \pm 17$  % for Δ9-THC in Δ8-THC rice crackers;  $96.7 \pm 0.6$  % for Δ8-THC,  $95.8 \pm 1.3$  % for Δ9-THC and  $96.0 \pm 0.2$  % for CBD in Δ8-THC-coated Cannabis leaves. The higher RSD values observed for the rice crackers were likely attributable to the inhomogeneity of the sample, as evidenced by the appearance of differently colored particles within the sample (SI, Fig. S7). Increasing the sample mass tenfold, from 10 mg to 100 mg (separate experiment, with 3 methanol extractions), significantly

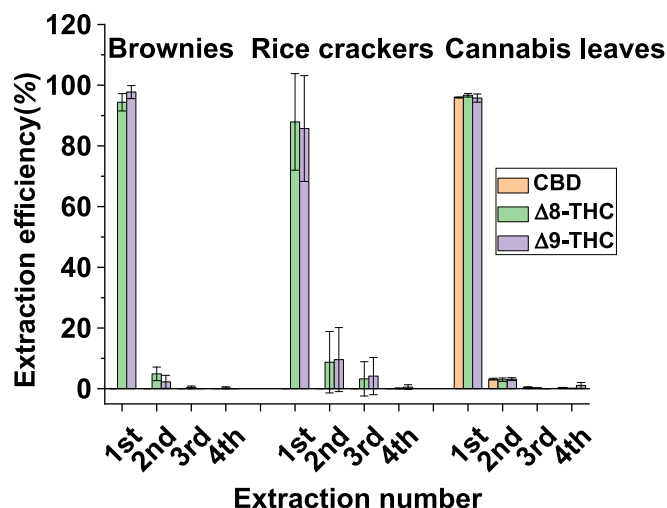


Fig. 2. Extraction efficiency for Δ8-THC, Δ9-THC, and CBD from solid Δ8-THC products as a function of the number of extractions. Error bars represent the standard deviation of three independently prepared sample replicates, with each sample measured once ( $n = 3$ ).

improved the RSD values, with Δ8-THC decreasing from 12.6 % to 5.4 % and Δ9-THC decreasing from 13.1 % to 6.3 % (SI, Table S7). The inhomogeneity of this product raises concerns about dose consistency for consumers, which warrants further attention (Blake & Nahtigal, 2019). For liquid products, namely vape oils in this study, MeOH was used for dilution, and the resulting clear mixtures obtained were directly used for subsequent analysis. Therefore, a recovery of 100 % was assumed for these liquid products.

### 3.3. Matrix effects

To evaluate the matrix effects in the detection of Δ8-THC, Δ9-THC, and CBD in combination with this extraction method, normal brownies and vape oils without cannabinoids were used as representative matrices for solid and liquid products, respectively (Table 1, SI, Table S8). Three different spiking weight percentages, 0.1 %, 0.3 %, and 1 % (low, medium, and high) for Δ8-THC, Δ9-THC, and CBD were assessed. Using the AgPS-MS<sup>2</sup> method, matrix effects were generally more pronounced, ranging from  $-40 \pm 1.1$  % to  $-19 \pm 2.0$  % for Δ8-THC,  $-35 \pm 0.7$  % to  $-4.5 \pm 0.5$  % for Δ9-THC, and  $7.3 \pm 1.7$  % to  $17 \pm 2.4$  % for Δ9-THC:Δ8-THC ratio. In contrast, the AgPS-MS<sup>3</sup> method showed significantly reduced matrix effects for Δ8-THC, with acceptable values ranging from  $-7.1 \pm 1.4$  % to  $17 \pm 2.9$  % across two different matrices

at three concentration levels (SWGTOX, 2013). Similarly, matrix effects for Δ9-THC (signal enhancement from  $2.2 \pm 0.3$  % to  $17 \pm 1.8$  %) were acceptable except in the vape oil matrix at a spiking percentage of 0.1 %, where a matrix effect of  $34 \pm 2.2$  % was observed. For the ratio of Δ9-THC:Δ8-THC, satisfactory matrix effects ( $-25$  %– $25$  % (SWGTOX, 2013)) were observed in all these situations ( $7.4 \pm 1.0$  % to  $15 \pm 1.5$  %). The overall better performance observed with the AgPS-MS<sup>3</sup> method indicates improved selectivity for Δ8-THC and Δ9-THC. This enhancement is attributed to the additional fragmentation step, which increases the signal-to-noise ratio. Moreover, since Δ8-THC and Δ9-THC are structural isomers, differing only in the position of the C=C bond, they were similarly affected by the matrices and thus the ratio Δ9-THC:Δ8-THC was less affected (all within  $\pm 25$  %) (SWGTOX, 2013) by matrix effects in both the AgPS-MS<sup>2</sup> ( $7.3 \pm 1.7$  % to  $17 \pm 2.4$  %) and AgPS-MS<sup>3</sup> ( $7.4 \pm 1.0$  % to  $15 \pm 1.5$  %) methods compared with the individual compounds. Besides, the matrix effects for Δ9-THCV ( $-60 \pm 1.2$  % to  $-33 \pm 0.6$  % in the AgPS-MS<sup>2</sup> and  $-39 \pm 2.1$  % to  $-12 \pm 1.0$  % in the AgPS-MS<sup>3</sup>) were quite different (more suppressed) from Δ8-THC and Δ9-THC, indicating that more suitable internal standards e.g., deuterated internal standards are desired to improve the analysis performance (Stokvis et al., 2005). For CBD, which was only identified by the AgPS-MS<sup>2</sup> method, there were pronounced signal enhancements of over 25 % (SWGTOX, 2013) with only one exception of the brownie matrix at a spiking percentage of 1 % (matrix effect of  $16 \pm 3.6$  %). On the other hand, due to the chromatographic separation procedure (33 min), a GC-FID based method developed previously (Huang, van Beek, et al., 2024) showed minor matrix effects ( $-1.6 \pm 0.0$  % to  $7.2 \pm 0.1$  %) for all the three cannabinoids in the two matrices at three spiking weight percentages (SWGTOX, 2013). Therefore, such a simple sample pretreatment method, if combined with the GC-FID method, would have very limited matrix effects on the analysis of the three isomeric cannabinoids. If combined with the much faster AgPS-MS method (tens of seconds vs. 33 min), matrix effects for Δ8-THC and Δ9-THC were generally acceptable ( $-25$  %– $25$  %) (SWGTOX, 2013). However, the matrix effects were more pronounced and significant for CBD, exceeding the tolerant limit (SWGTOX, 2013). Since CBD is legal and non-psychoactive, its measurement in this study is primarily to determine whether it is present in high abundance and thus could interfere with the Δ8-THC and Δ9-THC determinations. Therefore, minimizing the matrix effect for CBD is not as crucial as it is for Δ8-THC and Δ9-THC in Δ8-THC samples (as opposed to CBD-based products, such as CBD oils). In short, such a simple extraction could result in satisfactory recoveries ( $85.7 \pm 17$  % to  $97.7 \pm 2.1$  %) of Δ8-THC, Δ9-THC, and CBD (Christodoulou et al., 2023; Di Marco Pisciotano et al., 2018; Di Marco Pisciotano et al., 2021), and acceptable matrix effects ( $7.4 \pm 1.0$  % to  $15 \pm 1.5$  %) for Δ9-THC:Δ8-THC ratios when combined with rapid and direct paper spray analysis (SWGTOX, 2013).

Table 1

Matrix effect of AgPS-MS<sup>3</sup> and GC-FID methods.

| Spiking matrix and concentration (w/w%) | AgPS-MS          |                 |                |                 | GC-FID             |                    |                   |
|-----------------------------------------|------------------|-----------------|----------------|-----------------|--------------------|--------------------|-------------------|
|                                         | Δ8-THC           | Δ9-THC          | CBD*           | Δ9-THC:Δ8-THC   | Δ8-THC             | Δ9-THC             | CBD               |
| Blank vape (0.1 %)                      | $17 \pm 2.9$ %   | $34 \pm 2.2$ %  | $49 \pm 2.6$ % | $14 \pm 3.4$ %  | $-1.6 \pm 0.02$ %  | $-0.9 \pm 0.02$ %  | $0.85 \pm 0.01$ % |
| Blank vape (0.3 %)                      | $1.9 \pm 0.1$ %  | $17 \pm 1.8$ %  | $36 \pm 1.7$ % | $15 \pm 1.5$ %  | $2.2 \pm 0.02$ %   | $0.8 \pm 0.005$ %  | $3.3 \pm 0.03$ %  |
| Blank vape (1 %)                        | $-7.0 \pm 0.9$ % | $3.8 \pm 0.5$ % | $24 \pm 0.2$ % | $12 \pm 0.5$ %  | $0.8 \pm 0.002$ %  | $0.1 \pm 0.0005$ % | $2.1 \pm 0.007$ % |
| Blank brownie (0.1 %)                   | $1.5 \pm 0.1$ %  | $12 \pm 1.9$ %  | $49 \pm 1.2$ % | $10 \pm 1.8$ %  | $-1.6 \pm 0.02$ %  | $0.06 \pm 0.002$ % | $4.2 \pm 0.05$ %  |
| Blank brownie (0.3 %)                   | $1.3 \pm 0.1$ %  | $8.9 \pm 0.7$ % | $30 \pm 1.4$ % | $7.4 \pm 1.0$ % | $3.2 \pm 0.02$ %   | $7.2 \pm 0.06$ %   | $5.9 \pm 0.03$ %  |
| Blank brownie (1 %)                     | $-7.1 \pm 1.4$ % | $2.2 \pm 0.3$ % | $16 \pm 3.6$ % | $9.9 \pm 1.9$ % | $-1.4 \pm 0.003$ % | $-0.8 \pm 0.003$ % | $0.7 \pm 0.004$ % |

\* CBD was measured by the AgPS-MS<sup>2</sup> method;  $\pm$ SD (for AgPS-MS, SD represents the variability across three independently prepared sample replicates, with each sample measured five times using AgPS-MS ( $n = 15$ ); for GC-FID, the SD represents the variability across three independently prepared sample replicates, with each sample measured once using GC-FID ( $n = 3$ )).

### 3.4. Calibration curve and LOD

$\Delta 8$ -THC is normally produced from CBD accompanied by side products (such as  $\Delta 9$ -THC) as well as residual CBD, and all these compounds can end up in commercial  $\Delta 8$ -THC products (Huang, van Beek, et al., 2024). Based on our previous work, the AgPS-MS<sup>2</sup> method can be used to resolve  $\Delta 9$ -THC and CBD, with characteristic fragments at  $m/z$  313 for  $\Delta 9$ -THC and  $m/z$  353/355 for CBD (Huang et al., 2021). Here, the quantification performance of  $\Delta 8$ -THC,  $\Delta 9$ -THC, and CBD by the AgPS-MS<sup>2</sup> and AgPS-MS<sup>3</sup> methods was evaluated. It is worth mentioning that with the MS settings of two scan events, both MS<sup>2</sup> and MS<sup>3</sup> acquisitions can be obtained in one spray experiment, and thus, the quantification of  $\Delta 8$ -THC,  $\Delta 9$ -THC, and CBD can be achieved in a single analysis.

### 3.5. Calibration curve of $\Delta 9$ -THC: $\Delta 8$ -THC ratio

To investigate the relative quantification potential of the developed method, calibration curves for the  $\Delta 9$ -THC: $\Delta 8$ -THC ratio with the AgPS-MS<sup>2</sup> method and the AgPS-MS<sup>3</sup> method were established in MeOH (SI, Fig. S8). Concentration dependency was found for both the AgPS-MS<sup>2</sup> method ( $R^2 = 0.9854$ ) and the AgPS-MS<sup>3</sup> method ( $R^2 = 0.9764$ ) within the  $\Delta 9$ -THC: $\Delta 8$ -THC ratio range of  $1.00 \times 10^{-3}$  to 1.00. This demonstrates that the developed method can be effectively used to determine the ratio of these two isomers in the absence of other cannabinoids, and is suitable for relatively simple samples.

### 3.6. Calibration curve of $\Delta 8$ -THC, $\Delta 9$ -THC, and CBD and their LODs

To explore the potential for absolute quantification,  $\Delta 9$ -THCV was used as an internal standard (IS) and added to samples just prior to the analysis.  $\Delta 9$ -THCV, as a phytocannabinoid, is structurally similar to  $\Delta 8$ -THC and  $\Delta 9$ -THC but not likely to exist in synthetic  $\Delta 8$ -THC products (Walsh et al., 2021). The linear range for  $\Delta 8$ -THC,  $\Delta 9$ -THC, and CBD is  $1.00$ – $200 \mu\text{g}\cdot\text{mL}^{-1}$  ( $R^2 = 0.9973$  for  $\Delta 8$ -THC,  $R^2 = 0.9994$  for  $\Delta 9$ -THC, and  $R^2 = 0.9852$  for CBD) with an LOD of  $0.1 \mu\text{g}\cdot\text{mL}^{-1}$  for  $\Delta 8$ -THC and  $\Delta 9$ -THC and  $0.2 \mu\text{g}\cdot\text{mL}^{-1}$  for CBD when using the AgPS-MS<sup>2</sup> method. The AgPS-MS<sup>3</sup> method had the same linear range of  $1.00$ – $200 \mu\text{g}\cdot\text{mL}^{-1}$  for  $\Delta 8$ -THC and  $\Delta 9$ -THC ( $R^2 = 0.9977$  for  $\Delta 8$ -THC and  $R^2 = 0.9959$  for  $\Delta 9$ -THC) but gave a slightly higher LOD of  $0.3 \mu\text{g}\cdot\text{mL}^{-1}$  for  $\Delta 8$ -THC and  $\Delta 9$ -THC (SI, Fig. S9, S10). Despite the slight compromise in sensitivity from MS<sup>2</sup> to MS<sup>3</sup>, the enhanced selectivity through two rounds of selecting the targeted signal and fragmenting is expected to decrease interference as discussed above. Moreover, in the MS<sup>3</sup> spectrum,  $m/z$  217 only exists in the spectrum of  $\Delta 9$ -THC, while  $m/z$  245 is mainly originating from  $\Delta 8$ -THC with a minor contribution from  $\Delta 9$ -THC. This makes the analysis and identification of  $\Delta 8$ -THC and  $\Delta 9$ -THC more straightforward – again, in the absence of other isomers that might produce the same fragments. For CBD, the characteristic (most intense) fragment occurs at  $m/z$  353/355 in the MS<sup>2</sup> spectrum, with a minor fragment at  $m/z$  313 (around 2.8 % of the base peak) (Huang et al., 2021). Further fragmenting the  $m/z$  313 signal of CBD results in the MS<sup>3</sup> spectrum with  $m/z$  217 as the base peak accompanied by a minor peak at  $m/z$  245 (around 5 % of the base peak), which equals the mass of the characteristic fragments of  $\Delta 9$ -THC and  $\Delta 8$ -THC, respectively. Therefore, the contribution of  $m/z$  217 and  $m/z$  245 in the MS<sup>3</sup> stage of CBD would interfere with the quantification of  $\Delta 8$ -THC and  $\Delta 9$ -THC, especially when large amounts of CBD exist. Moreover, since CBD (containing two C=C bonds) has stronger affinities for Ag(I) compared with  $\Delta 8$ -THC and  $\Delta 9$ -THC (containing only one C=C bond), a substantial CBD content would likely affect quantitative performance via argenta-tion competition. Consequently, CBD levels should be monitored as well.

### 3.7. Analysis of acid-treated CBD mixtures

As mentioned, producing  $\Delta 8$ -THC from CBD regularly results in  $\Delta 9$ -

THC and other THC isomers (Golombek et al., 2020). In our previous study (Huang, van Beek, et al., 2024), we applied different conversion methods, including methods that could be used in a home kitchen, to produce  $\Delta 8$ -THC from CBD, aiming to understand the possible cannabinoid profile in  $\Delta 8$ -THC infused edibles. Chromatographic methods (with run times of 30–40 min) were needed to distinguish and quantify isomeric cannabinoids in different acid-treated CBD mixtures. Such acid-treated CBD protocols are commonly used to produce  $\Delta 8$ -THC for infusion into food matrixes. Rapid and easy methods for screening for  $\Delta 8$ -THC and  $\Delta 9$ -THC content would allow for faster optimization of the production process and thereby ensuring that  $\Delta 9$ -THC does not end up in  $\Delta 8$ -THC products. Therefore, the ratios of  $\Delta 9$ -THC: $\Delta 8$ -THC in seven acid-treated CBD mixtures were measured by the AgPS-MS method and results were compared with those obtained by the GC-FID method in our previous study (SI, Fig. S11, S12, Table S9) (Huang, van Beek, et al., 2024). For samples #1 and 2, there were minor deviations (0 to –11 %) between the results obtained by the two methods, probably because  $\Delta 8$ -THC and  $\Delta 9$ -THC are much more abundant than other THC isomers. For sample #7, no peak at  $m/z$  245 and dominant signals at  $m/z$  353/355 were observed in the MS<sup>2</sup> spectrum, indicating no  $\Delta 8$ -THC and abundant CBD, which matches the result obtained by GC-FID. For samples #3, 4, 5, and 6, in which  $\Delta 8$ -iso-THC and  $\Delta(4)$ -iso-THC were present in higher amounts than either  $\Delta 8$ -THC (sample #6) or  $\Delta 9$ -THC (samples #3, #4 and #5), varying deviations were observed for the AgPS-MS<sup>2</sup> (–36 % to 200 %) and AgPS-MS<sup>3</sup> methods (–36 % to 69 %). An overall better result was achieved by the AgPS-MS<sup>3</sup> method than the AgPS-MS<sup>2</sup> method, indicating that an additional fragmentation step effectively reduces interference from other THC isomers. Besides, the results show that the AgPS-MS<sup>2</sup> and AgPS-MS<sup>3</sup> methods are suitable to determine the ratios of  $\Delta 9$ -THC: $\Delta 8$ -THC in samples containing minor other THC isomers.

It is clear that samples that contain high amounts of other isomers are problematic. First, it complicates the accurate analysis of  $\Delta 8$ -THC and  $\Delta 9$ -THC content. Second, from a consumer perspective, these isomers are concerning because they are not well-studied and may have negative health effects. In order to provide a strategy to flag these samples for further analysis, purified cannabinoid isomers with MW = 314 from our previous work (Huang, Righetti, et al., 2024) were investigated by the AgPS-MS method and the spectra were compared with  $\Delta 8$ -THC and  $\Delta 9$ -THC (SI, Fig. S13, S14).  $\Delta 8$ -iso-THC produced MS<sup>2</sup> fragments at  $m/z$  313 (20 % of the base peak) and MS<sup>3</sup> fragments at  $m/z$  217 and  $m/z$  245 (both 40 % of the base peak) that would certainly interfere with the  $\Delta 8$ -THC and  $\Delta 9$ -THC quantification. However, the pronounced MS<sup>2</sup> fragment at  $m/z$  299 and MS<sup>3</sup> fragment at  $m/z$  243 of  $\Delta 8$ -iso-THC, which are barely visible in  $\Delta 8$ -THC and  $\Delta 9$ -THC can be used to flag its presence. Similarly,  $\Delta(4)$ -iso-THC produced MS<sup>2</sup> fragments at  $m/z$  313 (35 % of the base peak) and MS<sup>3</sup> fragments at  $m/z$  217 and  $m/z$  245 (both 25 % of the base peak), interfering with the analysis of  $\Delta 8$ -THC and  $\Delta 9$ -THC. At the same time, a strong MS<sup>2</sup> signal at  $m/z$  299 and an MS<sup>3</sup> signal at  $m/z$  223, which were very minor or absent in  $\Delta 8$ -THC and  $\Delta 9$ -THC allows discrimination of  $\Delta(4)$ -iso-THC from both  $\Delta 8$ -THC and  $\Delta 9$ -THC.  $\Delta 3$ -THC produced MS<sup>2</sup> fragments at  $m/z$  313 (55 % of the base peak) and MS<sup>3</sup> fragments at  $m/z$  217 (55 % of the base peak) and  $m/z$  245 (20 % of the base peak) — as well as a specific and discriminatory MS<sup>2</sup> fragment at  $m/z$  299 and MS<sup>3</sup> fragment at  $m/z$  243, compared to  $\Delta 8$ -THC and  $\Delta 9$ -THC. In summary, the existence of such isomeric cannabinoids would contribute to different extents in overestimating  $\Delta 8$ -THC and  $\Delta 9$ -THC and thus result in the deviation of  $\Delta 9$ -THC: $\Delta 8$ -THC ratios from those measured by the GC-FID method. At the same time, the presence of diagnostic fragments ( $m/z$  299 in MS<sup>2</sup> spectra and  $m/z$  223 or  $m/z$  243 in MS<sup>3</sup> spectra) for their presence can be used to observe whether this potential problem really occurs, and – only in that case – flag samples for more detailed follow-up analysis in the lab.

### 3.8. Analysis of commercial $\Delta$ 8-THC products

#### 3.8.1. Rapid screening analysis

To broaden the application of the developed method for analyzing commercial  $\Delta$ 8-THC products, it would be advantageous if it could rapidly determine the following: i) whether the detected  $\Delta$ 8-THC content matches the declared  $\Delta$ 8-THC content on the label; ii) whether the products contain other THC/CBD isomers and iii) whether the products contain a high content of  $\Delta$ 9-THC. Therefore, separate quantification of  $\Delta$ 8-THC and  $\Delta$ 9-THC was performed by using an internal standard. Additionally, CBD was quantified to prevent excessive CBD in the products from causing false indications of  $\Delta$ 8-THC and  $\Delta$ 9-THC levels. Finally, samples were monitored for the presence of  $MS^2$  signals at  $m/z$  299 and  $MS^3$  signals at  $m/z$  223 to flag samples suspected of containing substantial amounts of, and thus interference from, other cannabinoid isomers. Since the used internal standard  $\Delta$ 9-THCV produced  $MS^3$  signals at  $m/z$  243 (SI, Fig. S15),  $m/z$  243 cannot be used for flagging in this situation. A more appropriate internal standard, such as heavy-isotope labeled standard, would solve this issue. Moreover, it would suffer less from (differential) matrix effects, as indicated above.

From their  $MS^2$  spectra (SI, Fig. S16), it is quite obvious that  $\Delta$ 8-THC rice crackers contained much more  $\Delta$ 9-THC than  $\Delta$ 8-THC. The dominant signal at  $m/z$  313 and the almost invisible signal at  $m/z$  245 highlight this difference, a quite disturbing result in itself. When focusing on the analytical chemistry again, this discrepancy suggests that the AgPS- $MS^2$  method was expected to give a more accurate quantification of  $\Delta$ 8-THC. Alternatively, the signal at  $m/z$  245 produced by  $\Delta$ 9-THC in the  $MS^3$  stage could be subtracted (namely a background value of  $0.074 \times EIC(m/z\ 217)$ ) when using the AgPS- $MS^3$  method, as discussed in the analysis of acid-treated CBD mixtures. Signals at  $m/z$  353/355 indicated the presence of CBD in  $\Delta$ 8-THC Cannabis leaves and  $\Delta$ 10-THC vape oil, and thus overestimation of  $\Delta$ 8-THC and  $\Delta$ 9-THC. The quantification results were compared with those obtained by GC-FID and summarized in Table 2 and Table S10 (SI, Table S10). For  $\Delta$ 8-THC, there was a good correspondence between the results obtained by the AgPS- $MS^3$  method and the GC-FID method with minor deviations of 0–12 % in four out of the five  $\Delta$ 8-THC products. In the rice crackers, indeed the AgPS- $MS^2$  method gave a more accurate quantification with a much smaller deviation (409 % by AgPS- $MS^3$  and – 14 % by AgPS- $MS^2$ ) from the results of GC-FID. When the subtraction strategy was applied to the AgPS- $MS^3$  method, the deviation could be reduced from 409 % to – 45 % ( $0.56 \pm 0.08$  % vs.  $0.061 \pm 0.01$  % of  $\Delta$ 8-THC before and after subtraction). When comparing with the claimed contents, except for two  $\Delta$ 8-THC products without  $\Delta$ 8-THC information, the other three  $\Delta$ 8-THC products overclaimed their  $\Delta$ 8-THC content, as reported before (Gleb, 2022). For  $\Delta$ 9-THC, an overall overestimation (deviation from 65 % to

427 %) compared to the results obtained by GC-FID was encountered for the AgPS-MS method with the exception of  $\Delta$ 8-THC rice crackers (acceptable deviation of 13 %). The overestimations can be partly explained by the abundant presence of CBD, as detected in  $\Delta$ 8-THC Cannabis leaves and  $\Delta$ 10-THC vape oil. Monitoring relevant markers, specifically  $MS^2$  signals at  $m/z$  299 and  $MS^3$  signals at  $m/z$  223, indicated that, in addition to CBD, these products likely contained other isomers, such as  $\Delta$ 8-iso-THC,  $\Delta$ (4)8-iso-THC,  $\Delta$ 3-THC, or a combination of these (SI, Fig. S16, S17). The isomeric cannabinoid compositions were then analyzed by the GC-FID method (SI, Table S11, Fig. S18, S19). Except for the rice crackers, the total amounts of CBD,  $\Delta$ 8-iso-THC,  $\Delta$ (4)8-iso-THC, and  $\Delta$ 3-THC in other products were much higher than those of  $\Delta$ 9-THC, leading to the overestimation of  $\Delta$ 9-THC. These THC isomers, without having more knowledge about pharmacological effects (Michael Geci, 2023), should be given attention. However, methodologically, resolving these isomeric cannabinoids remains challenging even with time-consuming chromatographic methods (Chan-Hosokawa et al., 2022; Huang, van Beek, et al., 2024; Reber et al., 2022). It is noteworthy that in the  $\Delta$ 10-THC vape oil, which claimed to contain 70–75 %  $\Delta$ 8-THC and 15–20 %  $\Delta$ 10-THC (SI, Table S1), no  $\Delta$ 10-THC was detected by NMR. Instead, the main cannabinoids identified by NMR after isolation by preparative HPLC-MS were  $\Delta$ 8-THC and  $\Delta$ 3-THC (Huang, Righetti, et al., 2024). GC-FID/MS analysis revealed major peaks corresponding to  $\Delta$ 8-THC and  $\Delta$ 3-THC, along with minor peaks identified as  $\Delta$ 8-iso-THC,  $\Delta$ (4)8-iso-THC,  $\Delta$ 9-THC, and CBD, with no significant signal for  $\Delta$ 10-THC (SI, Fig. S19). In any case, this highlights the need for analytical methods capable of detecting and distinguishing cannabinoid isomers with minor structural differences, such as C=C bond position. Due to the unavailability of a  $\Delta$ 10-THC standard, no AgPS-MS spectra could be acquired for  $\Delta$ 10-THC. However, it is expected that its  $MS^2$  and  $MS^3$  spectra will allow for a differentiation, as  $\Delta$ 10-THC differs in the position of the C=C bond compared to other THC isomers investigated in this study, all of which exhibit different  $MS^2$  and  $MS^3$  spectra (SI, Figs. S12 and S13).

The developed AgPS-MS method enables rapid distinction of isomeric cannabinoids and is suitable for estimating  $\Delta$ 8-THC with acceptable accuracy (deviations of 0–13 %). Moreover, the developed method is able to flag problematic products containing either too much  $\Delta$ 9-THC (e.g., > 0.3 %), or other THC/CBD isomers or both. The existence of other isomeric cannabinoids could be flagged by characteristic  $MS^2$  and  $MS^3$  signals, which means that these samples should be further analyzed using confirmatory methods. If such isomers are absent but  $\Delta$ 9-THC is present,  $\Delta$ 9-THC can be quantified with acceptable accuracy, as demonstrated in the  $\Delta$ 8-THC rice crackers sample. However, in most of these products, both issues – presence of  $\Delta$ 9-THC and other THC/CBD isomers – occur simultaneously, leading to an overestimation of  $\Delta$ 9-

**Table 2**

Comparison of the absolute percentages of  $\Delta$ 8-THC,  $\Delta$ 9-THC, and CBD in  $\Delta$ 8-THC infused products measured by the AgPS-MS method, GC-FID method, and claimed content.

| Sample                         | $\Delta$ 8-THC (w/w%) |                  |                 | $\Delta$ 9-THC (w/w%) |                   |                 | CBD (w/w%)           |                 |                 |
|--------------------------------|-----------------------|------------------|-----------------|-----------------------|-------------------|-----------------|----------------------|-----------------|-----------------|
|                                | Ag-PSMS <sup>3</sup>  | GC-FID           | Claimed content | Ag-PSMS <sup>3</sup>  | GC-FID            | Claimed content | Ag-PSMS <sup>2</sup> | GC-FID          | Claimed content |
| $\Delta$ 8-THC brownies        | $0.80 \pm 0.04$       | $0.73 \pm 0.01$  | 1.4             | $0.070 \pm 0.01$      | $0.025 \pm 0.002$ | NA              | $0.01 \pm 0.001$     | ND              | NA              |
| $\Delta$ 8-THC rice crackers   | $0.095 \pm 0.01^*$    | $0.11 \pm 0.002$ | 1.4             | $1.7 \pm 0.09$        | $1.5 \pm 0.02$    | NA              | $0.02 \pm 0.004$     | ND              | NA              |
| $\Delta$ 8-THC Cannabis leaves | $10 \pm 1.1$          | $9.9 \pm 0.1$    | NA              | $1.5 \pm 0.1$         | $0.91 \pm 0.01$   | < 0.3           | $3.4 \pm 0.3$        | $4.4 \pm 0.03$  | NA              |
| $\Delta$ 8-THC vape oil        | $62 \pm 5.4$          | $62 \pm 1.2$     | NA              | $7.7 \pm 0.6$         | $3.7 \pm 0.07$    | < 0.3           | $0.62 \pm 0.13$      | ND              | NA              |
| $\Delta$ 10-THC vape oil       | $38 \pm 4.3$          | $34 \pm 0.3$     | 70–75           | $7.9 \pm 0.6$         | $1.5 \pm 0.07$    | < 0.3           | $5.1 \pm 0.5$        | $0.69 \pm 0.01$ | NA              |

NA, not available; ND, non-detectable, i.e., <LOD; \* measured by the AgPS- $MS^2$  method;  $\pm$ SD (for AgPS- $MS^2$  and AgPS- $MS^3$ , SD represents the variability across three independently prepared sample replicates, with each sample measured five times using AgPS- $MS^2$  and AgPS- $MS^3$  ( $n = 15$ ); for GC-FID, the SD represents the variability across three independently prepared sample replicates, with each sample measured once using GC-FID ( $n = 3$ )).

THC. Despite this overestimation, all four products that contained illegal levels of  $\Delta^9$ -THC ( $> 0.3\%$ ) were correctly identified as such. While it is true that when the  $\Delta^9$ -THC content is close to the  $0.3\%$  legal limit, such overestimation might result in a false positive result, this would show up in the confirmatory analysis. This is then needed in any case due to the detection of other THC/CBD isomers, that would have been flagged by characteristic  $MS^2$  and  $MS^3$  signals.

### 3.8.2. Standard addition for quantification

To explore if better accuracy could be obtained by correcting for matrix effects, the standard addition of  $\Delta^8$ -THC and  $\Delta^9$ -THC into  $\Delta^8$ -THC brownie and  $\Delta^8$ -THC vape oil extracts was performed (SI, Fig. S20). The results are summarized in Table 3. Similar to rapid screening results, quantification of the dominant cannabinoid  $\Delta^8$ -THC by the AgPS-MS method exhibited acceptable ( $\pm 25\%$ ) deviation from that obtained by the GC-FID method ( $-16\%$  for  $\Delta^8$ -THC brownies and  $-6.1\%$  for  $\Delta^8$ -THC vape oil), showing minor interferences from matrixes and other isomers. For  $\Delta^9$ -THC, higher accuracy was achieved compared to the rapid screening method. However, significant interferences from abundant isomeric cannabinoids resulted in deviations of  $76\%$  for  $\Delta^8$ -THC brownies and  $56\%$  for  $\Delta^8$ -THC vape oil. Additionally, this method is much more labor-intensive. While standard addition allows for better accuracy, interference from other isomers still causes substantial deviations, making the effort unjustified. Therefore, we did not apply this method to all samples.

Based on the performance comparison between AgPS-MS and GC-FID, as well as previously reported methods for the analysis of isomeric cannabinoids discussed in the Introduction, Table S12 (SI, Table S12) summarizes their performance in terms of sample type, pretreatment requirements, analysis time, solvent consumption, LOD, and analyzed cannabinoids. Unlike methods such as DART-MS (Chambers et al., 2022; Falconer et al., 2023), which are limited to qualitative analysis and unable to differentiate isomers such as  $\Delta^8$ -THC and  $\Delta^9$ -THC, the AgPS-MS method presented in this work can distinguish up to six cannabinoid isomers:  $\Delta^8$ -THC,  $\Delta^9$ -THC,  $\Delta^8$ -iso-THC,  $\Delta^4$ -iso-THC,  $\Delta^3$ -THC, and CBD. Other methods, such as HPLC-UV combined with offline NMR (Marzullo et al., 2020) or NMR coupled with GC-MS (Meehan-Atrash et al., 2021), can differentiate some isomers but require significantly longer analysis times, larger sample sizes, or higher solvent consumption. In contrast, the AgPS-MS method offers substantial advantages in both speed and efficiency. The instrumental analysis time of AgPS-MS is in seconds, which is far superior to chromatography-based methods such as 2D-HPLC-MS-MS (10 min) (Chan-Hosokawa et al., 2022) and UHPLC-TWIM-MS (14 min) (Tose et al., 2017). Moreover, it requires only  $15\text{--}20\ \mu\text{L}$  of solvent, a fraction of the volume consumed by other methods, such as silica-Ag(I)-HPLC-DAD/MS (Huang, van Beek, et al., 2024), which utilizes tens of milliliters. In terms of sensitivity, the LOD of AgPS-MS ( $0.1\text{--}0.3\ \mu\text{g}\cdot\text{mL}^{-1}$ ) is highly competitive, especially considering its exceptional isomer differentiation capability. Additionally, this method requires minimal sample preparation, involving only dilution and shaking, thereby eliminating the need for labor-intensive steps and complex instrumentation often associated with other techniques. In summary, the AgPS-MS method proposed in this work combines rapid analysis, low solvent consumption, and strong isomer differentiation capabilities (up to six isomers). These attributes make it a highly efficient, cost-effective solution for cannabinoid analysis,

particularly in complex matrixes such as acid-treated CBD mixtures and  $\Delta^8$ -THC edibles.

### 3.9. Prototype 3D-printed device for AgPS-MS

In conventional PS-MS, a paper tip is positioned with its tip directed towards the MS orifice, with a metal clip; then  $15\text{--}20\ \mu\text{L}$  of sample/spray solution is dropped on the paper tip, followed by application of a high voltage to commence spray. Despite PS-MS being easy and rapid to use, manually positioning the paper tip in front of the MS inlet somewhat limits its user-friendliness and throughput (Brown et al., 2020). Moreover, conventional paper spray is characterized by quite an unstable spray and no separation, due to, amongst other factors, the single deposition of solvent for spray generation. In previous research (Salentijn et al., 2014), 3D printing has been demonstrated to allow the design and production of paper spray devices to improve such aspects. Therefore, a 3D-printed device, consisting of a one-time-use paper cartridge, sample well slide, cartridge holder, and slide holder (Fig. 3A) was designed. The slide holder and paper cartridge holder were first fixed in front of the MS inlet, so that the cartridge holder only needs to be turned vertically when loading paper cartridges or loading samples (Fig. 3B). Subsequently, the sample well slide with multiple sample wells was put in the slide holder, and the slide button (Fig. 3A) was used to switch between samples, by moving the slide. Spray was produced when the paper tip was in contact with samples and wetted the paper. A video of the operation of the device can be found in the SI. By using this device, (i) there was no need for manual positioning of the paper tip, which enhances operational simplicity; (ii) cross-contamination introduced by the metal clip was avoided; (iii) preliminary, low-resolution paper-chromatographic separation of  $\Delta^9$ -THC and CBD was achieved (Fig. 3C and D), as the sample with all compounds is introduced in a repeatable manner at the back of the paper tip. Since CBD has more hydroxyl groups that can interact with the very polar paper, maximum intensity was observed later than for the THC molecules. Despite the separation being low resolution, it might be further optimized, e.g., based on silver-affinity as demonstrated on TLC plates (Huang et al., 2022), or a continuous solvent supply design (Salentijn et al., 2014). Differences in elution profiles can be leveraged to improve the distinction between isomers, in combination with their  $MS^2/MS^3$  profiles. The total cost of the 3D-printed device was only 1.28 euros with 0.09 euros as the costs for each one-time-use cartridge, which is overall thus quite cost-effective (SI, Table S13).

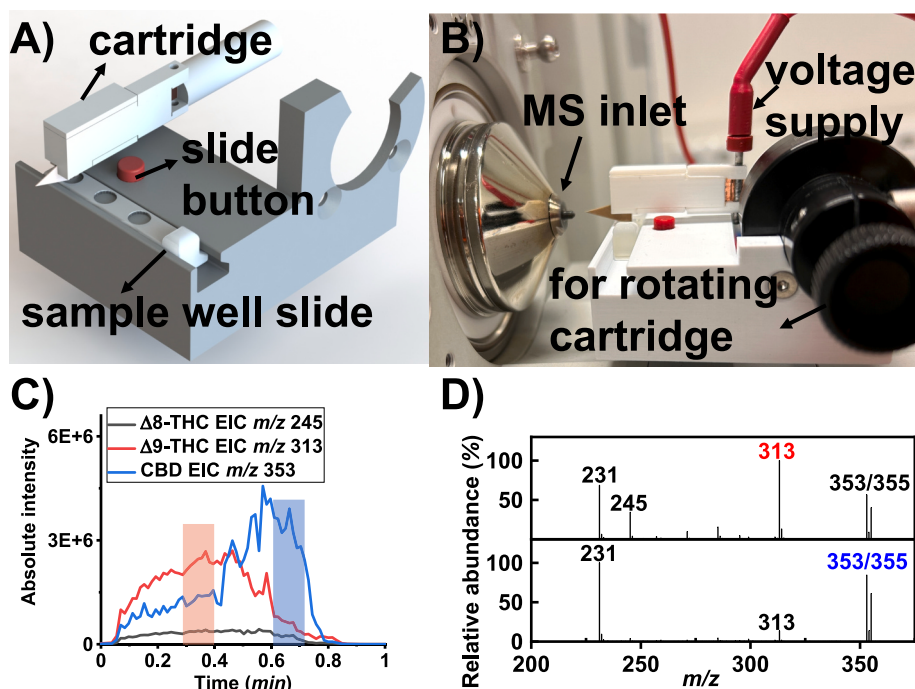
## 4. Conclusions

In conclusion, a rapid and easy method to distinguish between  $\Delta^8$ -THC and  $\Delta^9$ -THC for screening of  $\Delta^8$ -THC products was developed. This method combines a simple MeOH extraction and a rapid AgPS-MS analysis. It enabled the semiquantification of  $\Delta^9$ -THC: $\Delta^8$ -THC ratios and the individual content of  $\Delta^8$ -THC and  $\Delta^9$ -THC in various matrixes. Different acid-treated CBD mixtures and commercial  $\Delta^8$ -THC products were analyzed and benchmarked against the GC-FID method that serves as the current benchmark. For samples mainly containing  $\Delta^8$ -THC,  $\Delta^9$ -THC, and CBD, the quantification of  $\Delta^9$ -THC: $\Delta^8$ -THC was comparable to that of GC-FID. The analysis of commercial  $\Delta^8$ -THC products showed that the developed AgPS-MS method enabled reliable quantification of

**Table 3**  
Absolute percentages of  $\Delta^8$ -THC,  $\Delta^9$ -THC, and CBD in  $\Delta^8$ -THC infused products measured by the standard addition method.

| Sample                   | $\Delta^8$ -THC (w/w%) |                 |           | – | $\Delta^9$ -THC (w/w%) |                   |           |
|--------------------------|------------------------|-----------------|-----------|---|------------------------|-------------------|-----------|
|                          | Ag-PSMS <sup>3</sup>   | GC-FID          | Deviation |   | Ag-PSMS <sup>3</sup>   | GC-FID            | Deviation |
| $\Delta^8$ -THC brownies | $0.61 \pm 0.014$       | $0.73 \pm 0.07$ | $-16\%$   |   | $0.051 \pm 0.006$      | $0.029 \pm 0.004$ | $76\%$    |
| $\Delta^8$ -THC vape oil | $62 \pm 3.8$           | $66 \pm 3.4$    | $-6.1\%$  |   | $6.4 \pm 0.46$         | $4.1 \pm 0.26$    | $56\%$    |

$\pm$ SD (for AgPS-MS<sup>3</sup>, SD represents the variability across three independently prepared sample replicates, with each sample measured five times using AgPS-MS<sup>3</sup> ( $n = 15$ ); for GC-FID, the SD represents the variability across three independently prepared sample replicates, with each sample measured once using GC-FID ( $n = 3$ )).



**Fig. 3.** A) 3D-printed device; B) 3D-printed device mounted to MS; C) MS<sup>2</sup> EIC chromatogram of Δ8-THC ( $m/z$  245), Δ9-THC ( $m/z$  313), and CBD ( $m/z$  353); D) averaged MS<sup>2</sup> spectra of measurements over 0.1 min (red shade area and blue shade area) for the mixture of Δ8-THC, Δ9-THC, and CBD. Note: A more pronounced characteristic signal of Δ9-THC at  $m/z$  313 was found at an earlier elution time (0.3 min), and a more pronounced characteristic signal of CBD at  $m/z$  353/355 was found after a longer elution time (0.6 min). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Δ8-THC, and in fact revealed that four out of five commercial products contained illegal levels of Δ9-THC (> 0.3 %), which was confirmed by GC-FID. Besides, the presence of other isomers like Δ8-iso-THC, Δ(4)8-iso-THC, and Δ3-THC could also be identified. The implementation of a 3D-printed device for the paper spray improved operational simplicity and provides the potential for high-throughput analysis. Further optimization of paper modification and 3D-printed paper cartridges could improve the paper-chromatographic separation and thus decrease the interferences from co-eluting isomers. The developed method is highly promising for user-friendly and routine screening analysis of Δ8-THC products for badly needed forensic and food regulation purposes.

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#### CRediT authorship contribution statement

**Si Huang:** Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Teris A. van Beek:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Erik Beij:** Methodology. **Ming Ma:** Writing – review & editing, Methodology, Funding acquisition. **Bo Chen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Han Zuilhof:** Writing – review & editing, Supervision, Conceptualization. **Gert IJ. Salentijn:** Writing – review & editing, Validation, Supervision, Methodology, 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

Product information, images, and isomeric cannabinoid composition (analyzed by the GC-FID method in this study) of commercial Δ8-THC products; 3D-printed device design, material, printing time, and cost; AgPS-MS settings and MS transitions; preparation of acid-treated CBD mixtures and their isomeric cannabinoid compositions; preparation of sample sets I and II; EIC area ratio of  $m/z$  313:  $m/z$  245 in Δ8-THC AgPS-MS<sup>2</sup> spectrum under different conditions; AgPS-MS mass spectra of CBD and Δ9-THC in ACN; EIC at  $m/z$  421 for CBD and Δ9-THC in various solvents; comparison of extraction efficiency for different sample sizes of Δ8-THC rice crackers; calibration curves of Δ8-THC, Δ9-THC, CBD, and Δ9-THC:Δ8-THC as well as standard addition calibration curves; matrix effects on cannabinoids investigated in this study; comparison of Δ8-THC, Δ9-THC, and CBD percentages, and Δ9-THC:Δ8-THC ratios in acid-treated CBD mixtures and commercial Δ8-THC products measured by the AgPS-MS method and GC-FID method; GC-FID chromatograms of commercial Δ8-THC products; AgPS-MS<sup>2</sup> spectrum and AgPS-MS<sup>3</sup> spectra of Δ8-THC, Δ9-THC, CBD, Δ8-iso-THC, Δ(4)8-iso-THC, Δ3-THC, Δ9-THCV, acid-treated CBD reaction mixtures, and commercial Δ8-THC products; performance comparison of various methods for isomeric cannabinoid analysis; video of the operation on 3D-printed devices. Supplementary data to this article can be found online at [<https://doi.org/10.1016/j.foodchem.2025.143829>].

## Data availability

Data will be made available on request.

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