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RESEARCH ARTICLE

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Cannabis and its cannabinoids analysis by gas chromatography–mass spectrometry with Cold EI

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Abstract

Cannabis extracts and products were analyzed by gas chromatography–mass spectrometry (GC–MS) with Cold EI for their full content including terpenes, sesquiterpenes, sesquiterpinols, fatty acids, delta 9-tetrahydrocannabinol (THC), cannabidiol (CBD), other cannabinoids, hydrocarbons, sterols, diglycerides, triglycerides, and impurities. GC–MS with Cold EI is based on interfacing GC and MS with supersonic molecular beams (SMB) along with electron ionization of vibrationally cold sample compounds in the SMB in a fly-through ion source (hence the name Cold EI). GC–MS with Cold EI improves all the performance aspects of GC–MS, enables the analysis of Cannabinoids with OH groups without derivatization, while providing enhanced molecular ions for improved identification, and enables internal quantitation without calibration. We found over 50 cannabinoid compounds including a new one with a Cold EI mass spectrum very similar to delta 9-THC as well as relatively large cannabinoids with molecular weight above m/z = 400. Because the analysis was universal in full scan and not targeted, we found impurities such as bromo CBD and fluticasone propionate and could monitor the formation of oxidized CBD during decarboxylation. In addition, GC–MS with Cold EI enabled nontargeted full analysis of terpenes, sesquiterpenes, and sesquiterpinols in cannabis extracts with good internal quantitation. GC–MS with Cold EI further served with very good sensitivity for the concentration determination of delta 9-THC in CBD-related products. Finally, cannabis drugs such as EP-1 used in Israel for treatment of epilepsy and for children with autism spectrum disorder (ASD) were analyzed for their full cannabinoids content for learning on the entourage effect and for drug activity optimization.

KEYWORDS
cannabinoids, cannabis, Cold EI, GC–MS, GC–MS with Cold EI, supersonic molecular beams

1 | INTRODUCTION AND BACKGROUND

Cannabis-based drugs and products are becoming more popular for treatment of a variety of diseases and disorders. One of the most promising fields is moderating phenomena such as repetitive behavior, echolalia, and self-stimulatory behavior among children with autism spectrum disorder (ASD). Most of the cannabis products are plant extracts in oil, and thus, their analysis is an area of fast-growing popularity.

Gas chromatography–mass spectrometry (GC–MS) with standard EI serves mostly for terpenes analysis as well as for pesticides analysis, whereas LC–UV and/or LC–MS serve for target cannabinoids

analysis.[1–5] However, universal untargeted cannabinoids analysis is not performed due to unavailability of appropriate tools for such analysis. As known, compounds with OH groups tend to tail and decompose on the metallic surface of standard EI ion sources, exhibit nonlinear response with poor limits of detection, and thus require tedious derivatization prior to their GC–MS analysis.[6,7] In addition, derivatized compounds rarely exhibit molecular ions in standard EI, and thus, derivatization impedes on new compounds identification. In addition, full cannabis extract content is not explored in part because compounds such as diglycerides and triglycerides do not elute from the GC column in standard GC–MS analysis.

The most widely known and potent compounds in cannabis are delta 9-tetrahydrocannabinol (THC) and cannabidiol (CBD) that were initially explored, and their benefits were promoted by Mechoulam.[8] Cannabis-related compounds are described by Brenneisen,[9] which lists and describes 58 cannabinoids, whereas Atakan[10] mentions 60 cannabinoids and over 400 compounds in cannabis. However, there are 256 naturally occurring and genetically engineered cannabis strains in Israel[11] and over 2650 brands in the United States,[12] and it is claimed that each could have different medical effects due to the synergy between THC, CBD, and other (over 100) cannabinoid compounds as found in their different potency as antitumor agents.[13]

The composition of the plant extract is highly dependent on the cannabis brand, growth conditions, and the extraction process. As a result, cannabis products exhibit poor reproducibility compared with drugs manufactured by the pharmaceutical industry. Parents of children with ASD are used to suspect and replace batches of the same product when their child's behavior deteriorates. Therefore, there is a growing need for a reliable and comprehensible analytical method for quality assurance (QA). Accordingly, an important yet unmet challenge is to analyze untargeted cannabinoid compounds in various brands of cannabis and correlate the cannabis brands' medical effectiveness with their cannabinoid compounds content (entourage effect[14,15]).

GC–MS with Cold EI is based on interfacing the GC and MS with supersonic molecular beams (SMB) along with electron ionization of vibrationally cold sample compounds in the SMB in a fly-through ion source (hence the name Cold EI).[16–20] GC–MS with Cold EI improves all the central performance aspects of GC–MS and uniquely provides enhanced molecular ions, improved sample identification, significantly extended range of compounds amenable for analysis, uniform response to all analytes (internal quantitation), faster analysis, greater selectivity, and lower limits of detection.[19,20] The response uniformity is a very important feature of Cold EI as it enables internal quantitation without the addition of a calibration analysis. In Cold EI, the sample compounds fly through the ion source without any contact with its walls, and thus, the original approximately uniform electron ionization yield is retained unlike in standard EI.

Thus, in this paper, we describe the application of GC–MS with Cold EI for the untargeted full range of all compounds in cannabis extracts analysis from the volatile terpenes all the way to triglycerides and include quantitative analysis of the full range of cannabinoids.

2 | EXPERIMENTAL

We used the 5977-SMB GC–MS with Cold EI system that is based on the combination of an Agilent 7890A GC + 5977 MSD (Agilent Technologies, Santa Clara, CA, USA) with the Aviv Analytical SMB interface and its dual-cage fly-through ion source (Aviv Analytical LTD, Hod Hasharon, Israel). The technology of GC–MS with Cold EI is reviewed in literature.[19,20]

In these systems, the GC column output is mixed with helium make-up gas (~60 ml/min typical total column and make-up flow rate combined), in front of a supersonic nozzle located at the end of a heated and temperature controlled transfer line. The helium make-up gas flow can be mixed (via the opening of one valve) with perfluorotributylamine (PFTBA) for periodic system tuning and mass calibration. The sample compounds seeded in the helium gas expand from a 100-μm-diameter supersonic nozzle into an SMB nozzle vacuum chamber that is differentially pumped by a Varian Navigator 301 turbo molecular pump (Varian Inc., Torino, Italy) with 250 L/s pumping speed. The helium pressure at this vacuum chamber is about 6×10^{-3} mbar. The supersonic expansion vibrationally cools the sample compounds, and the expanded supersonic free jet is skimmed by a 0.8-mm skimmer and collimated in a second differentially pumped vacuum chamber, where an SMB is formed. The second vacuum chamber is pumped by the Agilent 5977 system "Performance" turbo molecular pump that pumps the dual-cage fly-through ion source and MS (Pfeiffer 250 L/s pump). The SMB seeded with vibrationally cold sample compounds pass a fly-through dual-cage EI ion source[21] where these beam species are ionized by 70-eV electrons with 6-mA emission current. It is worthwhile to note that the Cold EI ion source was used for over 5 years while maintaining almost the same performance without service. The ions are focused by an ion lens system, deflected 90° by an ion mirror, and enter the Agilent 5977 MS for their mass analysis. The 90° ion mirror is separately heated and serves to keep the mass analyzer clean from possible sample-induced contaminations. The ions that exit the Agilent MS are detected by the Agilent triple axis ion detector, and the data are processed by the Agilent Chemstation software.

GC separation was performed with a 15-m column with 0.32 mm I.D., 0.1 μ DB1HT film, and typically 2 ml/min column flow rate with flow program from 2 to 30 ml/min after 11 min. The GC oven was ramped from 50°C to 330°C at 20°C/min and maintained at 330°C for 3 min. Several cannabis flowers, cannabis extract samples, and cannabis-based drugs were given to us by several people from different sources. The cannabis extracts were typically a viscous liquid that was dissolved at about 0.5–1 mg in 1-ml hexane and injected with split 10 so that the most abundant compound (typically CBD) will be at ~50-ng on-column amount and thus will not saturate or only slightly saturate the column and exhibit tolerable peak fronting. We found that dichloromethane should be avoided as a solvent for cannabis extracts as it induces oxidation, and thus, our solvent of choice was hexane. In our comparison experiments with standard EI, we used an Agilent 7890 GC + 5977 MSD with its Inert ion source (Agilent, Santa Clara, CA, USA). We used 30 m, 0.25 mm I.D. column with 0.25 μ DB-5MS Ultra Inert film, 1.2 ml/min He column flow rate, and GC oven temperature program rate of 10°C/min from 50°C to 320°C.

3 | RESULTS AND DISCUSSION

In Figure 1, we show that GC–MS with Cold EI can uniquely serve for the analysis of cannabis extracts with its full range of all volatile and semivolatile classes of compounds as indicated, including terpenes, sesquiterpenes, fatty acids, cannabinoids, sterols, diglycerides, and triglycerides. Hydrocarbons around the size n-$C_{29}H_{60}$ were also found eluting prior to and near the sterols. Furthermore, Cold EI provides uniform compound-independent response, and thus, the abundance of each compound can be estimated without any additional calibration curve analysis, including for all the cannabinoids.

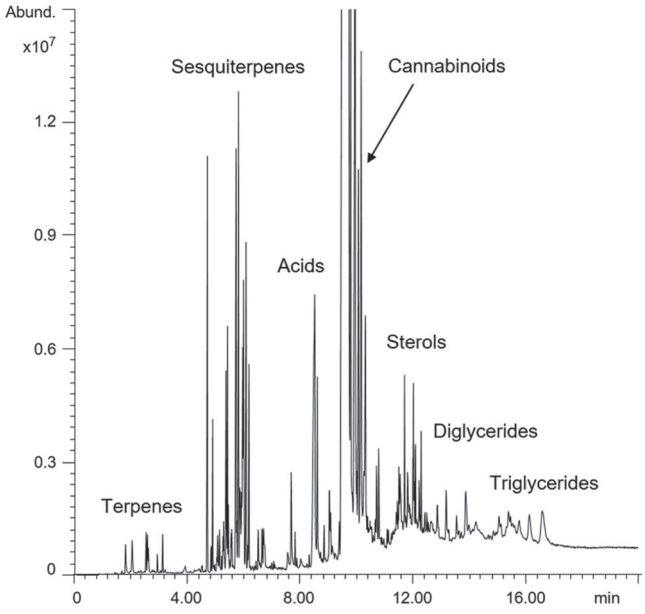

FIGURE 1 Gas chromatography–mass spectrometry with Cold EI analysis of a cannabis extract with its full range of all classes of compounds as indicated

GC–MS with standard EI typically serves for the analysis of terpenes in cannabis extracts[1–5] and for pesticides analysis, but it is avoided in the analysis of cannabinoids as such analyses require derivatization[6,7] (which is tedious and not always practical) because without derivatization it is known that compounds with free one or two groups of OH tend to react with the standard EI metallic ion source surface and decompose plus they exhibit compound-dependent nonlinear response.[22] To demonstrate the magnitude of standard EI problems with underivatized cannabis analysis, we show in Figure 2 a comparison of cannabis extract analysis by GC–MS with standard EI (upper) and Cold EI (bottom) (both are zoomed ×10).

Note the much greater number of cannabinoids observed with Cold EI. In addition, all the cannabinoid compounds' relative abundances to CBD are greater in Cold EI than in standard EI because whereas Cold EI provides uniform response, standard EI provides nonuniform nonlinear response as cannabinoids with free OH decompose on the metallic ion source surface and the degree of such degradation depends on the compound and sample amount. As an example, the THC/CBD ratio is 6 times greater in Cold EI. On the other hand, the peak just after CBD in Cold EI, which is of cannabichromene (CBC), is practically missing in standard EI, whereas the early peak at 6.93 min in Cold EI, which is of cannabidivarin (CBDV), is only twice lower in standard EI. Accordingly and as we know, standard EI should not be used in the analysis of cannabinoids without

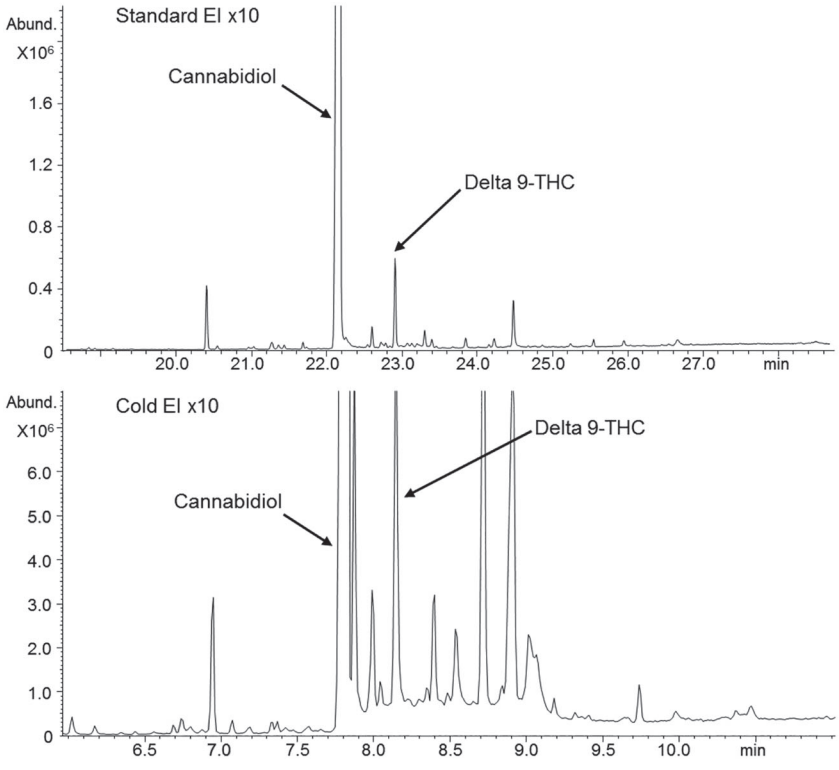

FIGURE 2 A comparison of cannabis extract analysis by gas chromatography–mass spectrometry with standard EI (upper mass chromatogram) and Cold EI (bottom mass chromatogram). Both mass chromatograms are magnified 10 times. The peaks of cannabidiol and delta 9-tetrahydrocannabinol (THC) are indicated by the arrows

derivatization, and thus, LC–MS serves for cannabinoids analysis. However, because Electrospray or APCI ion sources of LC–MS are also characterized by nonuniform response, LC–MS served mostly for target cannabinoids analysis with response calibration with a test mixture, and there are only a few (about 20) cannabinoids available as standards. Moreover, the synergetic effect of the cannabinoids requires quantitative analysis, but with API source, labeled internal standard is needed for each analyte. In addition, cannabinoids derivatization is not only time consuming and not always practical in the viscous cannabis drugs, but the identification of derivatized cannabinoids is also difficult because fewer of them are included in the NIST library and they typically do not exhibit molecular ions. Thus, and as will be shown, Cold EI with its contact-free fly-through ion source is the ideal ion source for cannabinoids analysis as well as for the full content of cannabis extracts and drug analysis.

GC–MS with standard EI serves in the analysis of the volatile terpene and sesquiterpene compounds that affect the taste and smell of cannabis products. However, while commonly being ignored, these groups of terpene and sesquiterpene include compounds with OH group that tend to decompose at the standard EI ion source, and thus, their analysis is problematic without derivatization even with compound-specific calibration curves.

In Figure 3, we compare the analyses of sesquiterpenes in cannabis cancer drug by GC–MS with standard EI (upper traces) and Cold EI (bottom traces). As shown, standard EI exhibits significantly lower relative abundance of sesquiterpinols (sesquiterpene alcohols) (MW = 222, with added H_2O) versus Cold EI plus complete absence of 1-hexadecanol because these compounds decompose on the standard EI metallic ion source surface. In Figure 3, we also show the mass spectra of guaiol (first to elute sesquiterpinol) obtained by standard EI (upper right) and Cold EI (bottom right). As shown, in standard EI, the mass spectrum of guaiol is without any molecular ion and dominated by background interference, and thus, it failed to be identified by the NIST library. In contrast, the Cold EI mass spectrum of guaiol exhibited about 25% relative abundance of its molecular ion at m/z = 222, clean mass spectrum, and 59% NIST library identification probability, which is high for a sesquiterpinol compound in view of the many such isomer compounds in the library. NIST library identification at the isomer level in a group of many isomers is a challenge, and the NIST 2017 library has 575 terpenoids (161 terpenes + 265

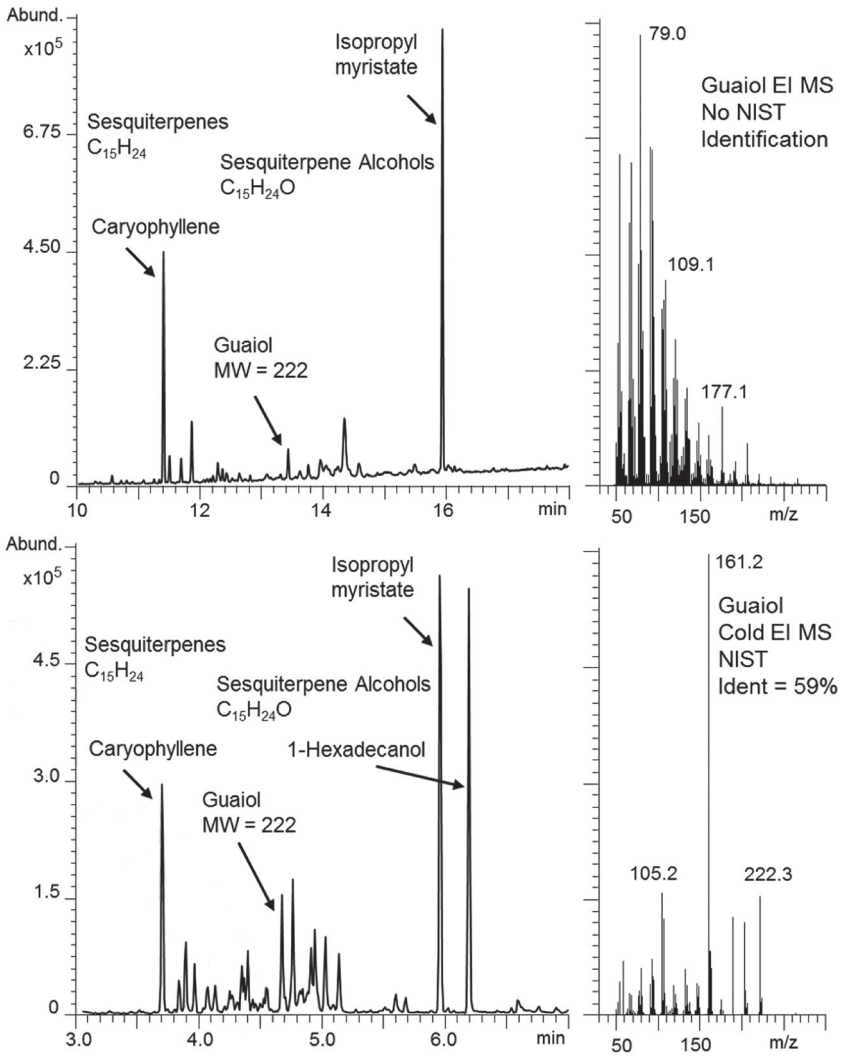

FIGURE 3 A comparison of cannabis cancer drug analysis by gas chromatography–mass spectrometry with standard EI (upper left) and Cold EI (bottom left) zoomed around the elution time of sesquiterpenes plus mass spectra of guaiol obtained by standard EI (upper right) and Cold EI (bottom right)

sesquiterpenes + 149 sesquiterpinols). Thus, from Figure 3, we assert that Cold EI is much better suited for the general untargeted analysis of terpenes and sesquiterpenes compounds in cannabis extracts, cannabis drugs, and other essential oils.

In Figure 4, we show medical cannabis extract analysis by GC–MS with Cold EI zoomed around the elution times of cannabinoid compounds (upper trace) plus zoomed 10 times on the intensity scale and the Cold EI mass spectrum of CBD (bottom trace). The relative abundance of the molecular ion of CBD is enhanced about 10 times in Cold EI compared with standard EI. CBD was identified by the NIST library with 65% identification probability, which was better than in standard EI that resulted in its identification probability with only 50%. Cold EI typically provides higher NIST library identification probabilities due to its enhanced molecular ion as explained in details in previous studies.[23,24] As immediately observed, very large number of cannabinoid compounds are exhibited in Figure 4. We counted more than 50 cannabinoids in this complex Cannabis mixture, and each provided a distinct and rich Cold EI mass spectrum with abundant molecular ions, and several of these compounds could be identified by the NIST library at the isomer level. Another important aspect that

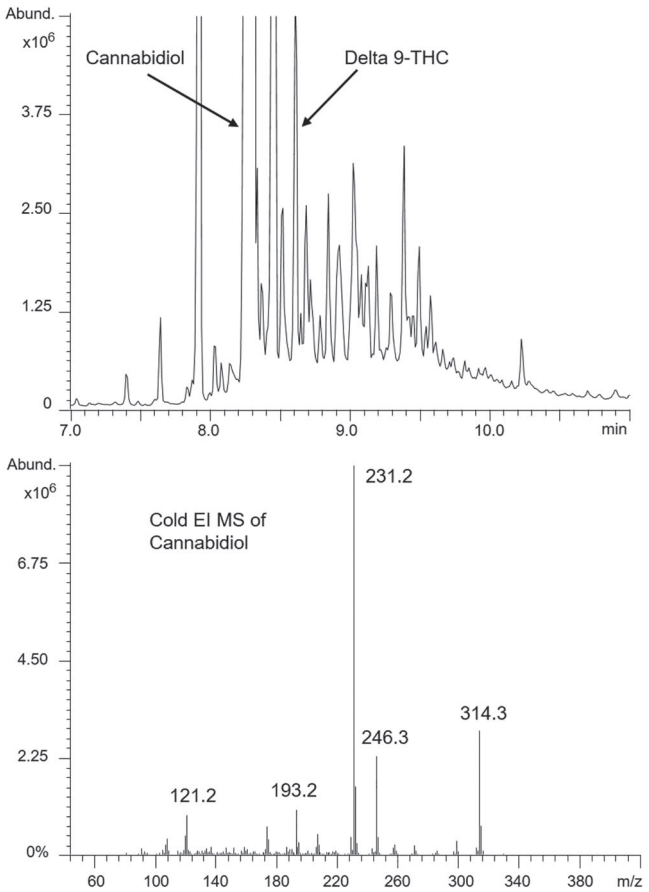

FIGURE 4 Medical cannabis extract analysis by gas chromatography–mass spectrometry (MS) with Cold EI, zoomed around the elution times of cannabinoid compounds (upper trace) plus zoomed 10 times on the intensity scale and the Cold EI mass spectrum of cannabidiol (bottom trace)

can be concluded from Figure 4 is that cannabis should better be analyzed by a universal analysis method to monitor and observe the full picture of its content and not by a targeted method such as LC–MS.

In Figure 5, we provide an unexpected datum that further demonstrates why cannabis should better be analyzed by a universal analysis method. In Figure 5, we show the finding of bromo CBD in the medical cannabis extract mixture. As shown, the Cold EI mass spectrum provides two molecular ions at m/z = 392 and m/z = 394 that correspond to the molecular weight of CBD of 314 plus the atomic weight of bromine (79 and 81) minus one hydrogen atom that it replaces. Naturally, we are unable to confirm the base cannabinoid isomer and assume that it is CBD because it is by far the most abundant compound in this mixture. We do not know the origin of this compound, and it is unlikely natural because bromine compounds are quite rare in natural products, and thus, we assume that it originated from the treatment of the cannabis extract in some solvent with bromine atom or via contact with a plastic with a brominated flame retardant that partially reacted with it.

Another reason why cannabis should be analyzed by a universal analysis method is the potential finding of hazardous and/or illegal compounds. In Figure 6, we demonstrate the finding of fluticasone propionate, which is a manufactured steroid used to treat nasal symptoms in a cannabis flower. The upper mass chromatogram in Figure 6 shows the analysis of a portion of a cannabis flower for its content, and we used the ChromatoProbe sample introduction device[25] (Agilent name "Thermal Separation Probe") for the thermal extraction of a small portion of the cannabis flower via its direct insertion into the heated GC injector. The bottom trace is the Cold EI MS of the arrow indicated peak of fluticasone propionate as confirmed by the NIST library with 98.5% identification probability. Although we do not know why and how fluticasone propionate was added into the cannabis flower, its identification is unambiguous. Possibly it was added to the cannabis flower to increase access of the THC to the blood when inhaled or to ease asthma symptoms. Note the availability of a molecular ion for this compound in Cold EI unlike in the NIST library in which it is absent. GC–MS–MS and LC–MS–MS are target compound-based instruments that serve to detect pesticides below 10 ng/g pesticide levels. However, there are over 1600 pesticides and many more hazardous compounds. Thus, they miss even 10 μg/g unlisted pesticides and/or other hazardous compounds such as fluticasone propionate that was detected at 7200 μg/g (0.72%) from the vaporized matter in the cannabis flower (1.05% from the delta 9-THC). Accordingly, universal untargeted GC–MS with Cold EI analysis as demonstrated in Figure 6 is highly valuable for cannabis products' consumption safety. Figure 6 further shows the dominant delta 9-THC peak, which saturated the column and was zoomed ×10 and a multitude of sesquiterpenes and sesquiterpinols at the early elution time of the mass chromatogram (up to 6 min).

Cold EI also uniquely enables the finding of new cannabinoids, including with molecular weight well above 314 of THC and CBD. These cannabinoids can be of medical value and/or serve as precursors of valuable cannabinoids. In Figure 7, we show such finding of large cannabinoids in a cannabis extract. The upper Cold EI mass

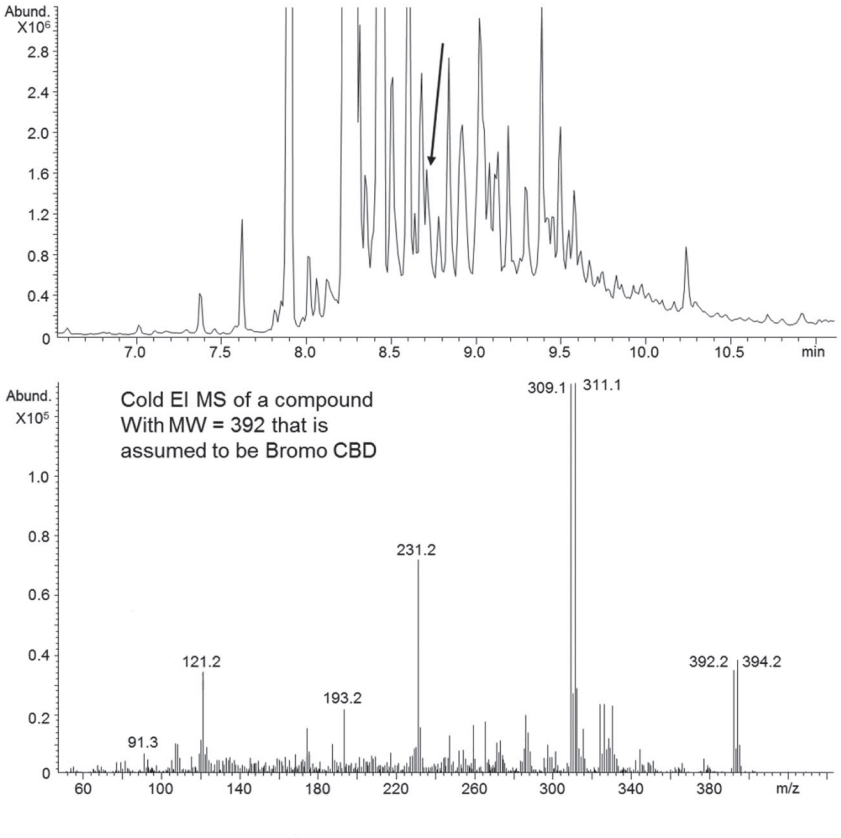

FIGURE 5 Medical cannabis extract analysis by gas chromatography–mass spectrometry (MS) with Cold EI zoomed around the elution times of cannabinoid compounds (upper mass chromatogram) plus zoomed 10 times on the intensity scale. The Cold EI mass spectrum of a compound with molecular weight m/z = 392 and m/z = 394 is also shown (bottom mass spectrum), which is tentatively identified as bromo cannabidiol (CBD). It was obtained at the arrow indicated elution time in the mass chromatogram

chromatogram shows a segment of the mass chromatogram at an elution time window after that of the major cannabinoids. The Cold EI mass spectra are of the two peaks A and B that are indicated by the arrows and each with a distinct molecular ion and high mass fragment ions that include the m/z = 314 and m/z = 231 characteristic fragment ions, and thus, we safely assume that these compounds are cannabinoids. In fact, in view of the feature of enhanced molecular ion of Cold EI, we can characterize several other interesting unknown compounds in the mass chromatogram of Figure 7.

Cold EI can also uniquely provide quantitative assessment of the efficiency of cannabis processing, including of THC acid (THCA) and CBD acid (CBDA) decarboxylation and conversion into THC and CBD. This process may require relatively high temperature and at the presence of air can induce undesirable CBD oxidation. In Figure 8, we show the monitoring of cannabis decarboxylation and its resulting partial conversion of CBDA into oxidized CBD (MW = 328). Cold EI mass chromatogram of a cannabis extract (upper trace) is shown after its high temperature decarboxylation and the Cold EI mass spectrum of a new peak that was induced by this decarboxylation process is shown at the bottom mass spectrum, obtained at the arrow indicated peak. This new peak was identified by the NIST library, and thus, we provide its structure with three oxygen atoms. Clearly monitoring this peak with the goal of its elimination is important for the optimization of the yield of the decarboxylation process. We note that THCA and CBDA mostly decompose at the GC injector and thus cannot be properly analyzed without decarboxylation even by GC–MS with Cold EI.

In several cases, there is a need to measure the relative concentration of delta 9-THC in comparison with CBD. One main reason for

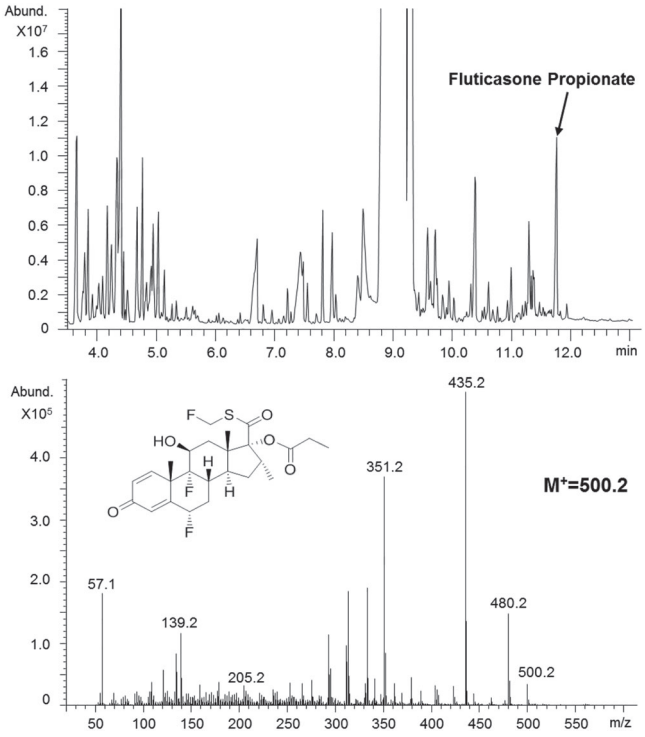

FIGURE 6 The finding of fluticasone propionate nasal spray drug in a cannabis flower. The upper mass chromatogram shows the analysis of a portion of a cannabis flower for its content, whereas the bottom trace is the Cold EI MS of the arrow indicated peak of fluticasone propionate as confirmed by the NIST library with 98.5% identification probability. The structure of fluticasone propionate is given in the bottom insert

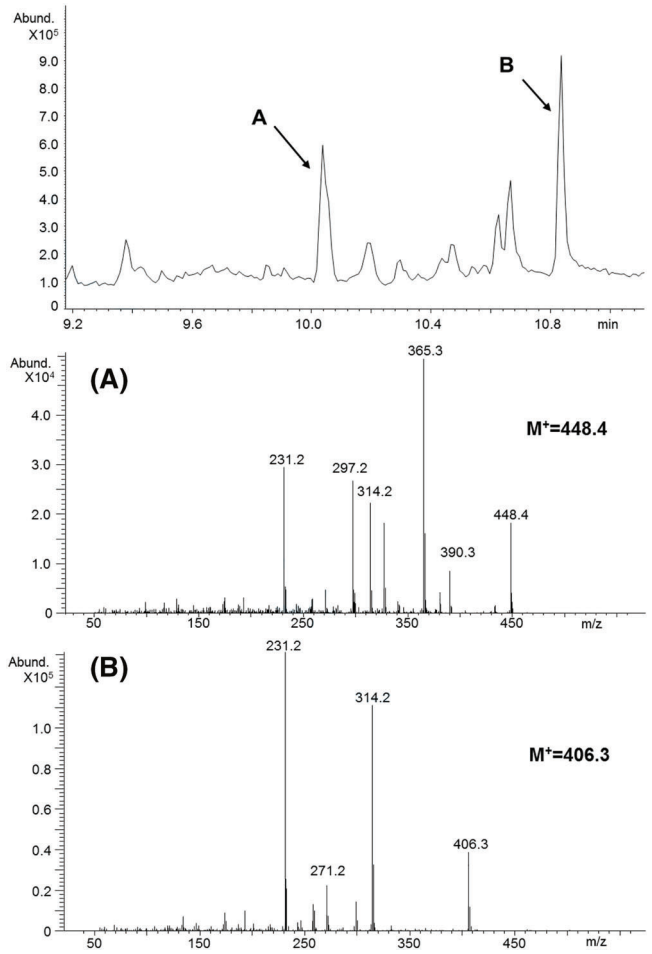

FIGURE 7 The finding of large cannabinoids in a cannabis extract. The upper Cold EI mass chromatogram shows a segment of the mass chromatogram at an elution time window after that of the major cannabinoids. The bottom traces A and B show mass spectra of the two arrows indicated peaks, and each with a distinct molecular ion and the marked high mass fragment ions

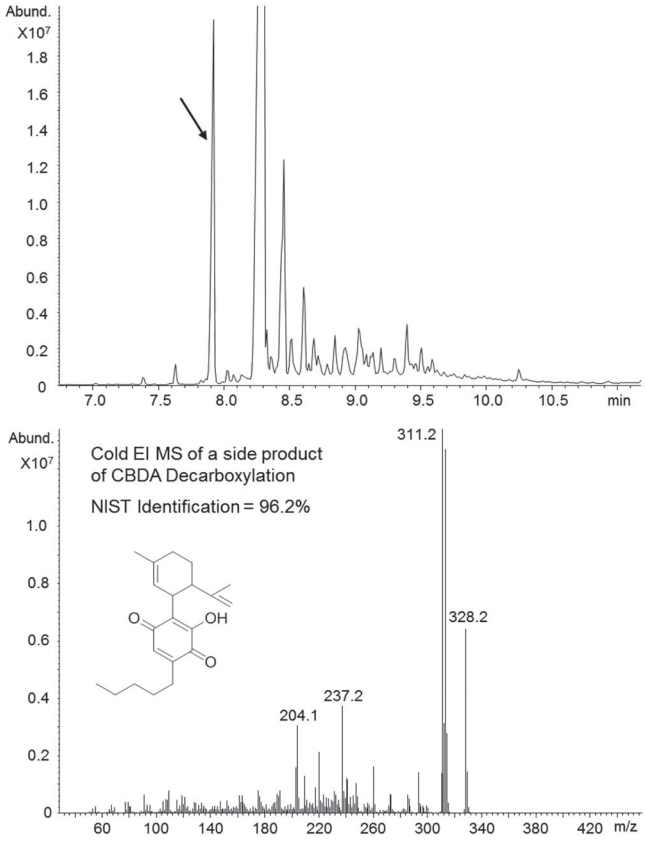

FIGURE 8 Monitoring the partial conversion of cannabidiol acid into oxidized cannabidiol (MW = 328) upon decarboxylation. Cold EI mass chromatogram of a cannabis extract (upper trace) after its high temperature decarboxylation and the Cold EI mass spectrum of a new peak that was induced by this decarboxylation process (bottom mass spectrum obtained at the arrow indicated peak)

this need is that according to local laws and certain regulations, the delta 9-THC concentration should be lower than 0.3% of that of the CBD. In Figure 8, we show the Cold EI single ion monitoring mass chromatogram at m/z = 314.2 of a cannabis extract with 0.66% delta 9-THC compared with CBD. The value of 0.66% was obtained by LC with UV detector, and we found a similar value with our GC–MS with Cold EI. As shown in Figure 9, the signal-to-noise ratio at the delta 9-THC peak is 81,000 (in peak to peak), and thus, the instrumental sensitivity is about 0.1 μg/g. The real limit of detection is about 0.01% (100 μg/g) due to the ever present multitude of cannabinoids and their isomers at that concentration level. Thus, we view GC–MS with Cold EI as the most sensitive system for the determination of the THC/CBD ratio with practical LOD of 0.01%.

An important cannabis-related application is to analyze a cannabis cancer drug for its content, including the relative concentration ratio of CBD/THC, the magnitude and identity of other cannabinoids, and the amount of added oil. This specific cannabis drug serves to help cancer patients sleep and ease their cancer-related pain. In Figure 10,

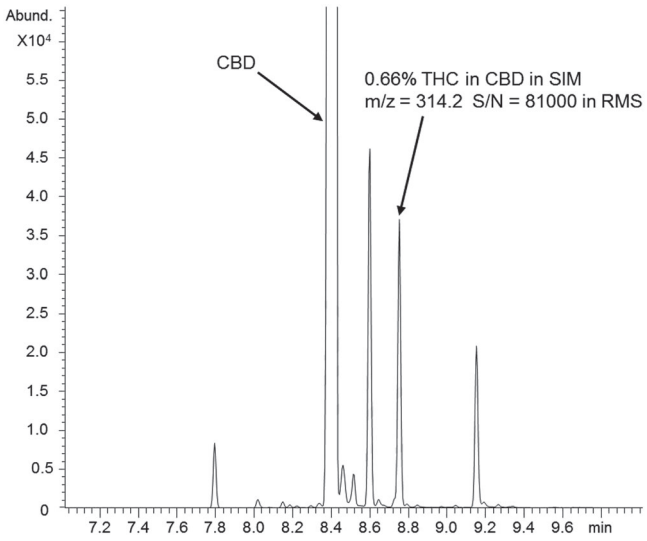

FIGURE 9 Cold EI single ion monitoring mass chromatogram at m/z = 314.2 of a cannabis extract with 0.66% delta 9-tetrahydrocannabinol (THC) compared with cannabidiol (CBD)

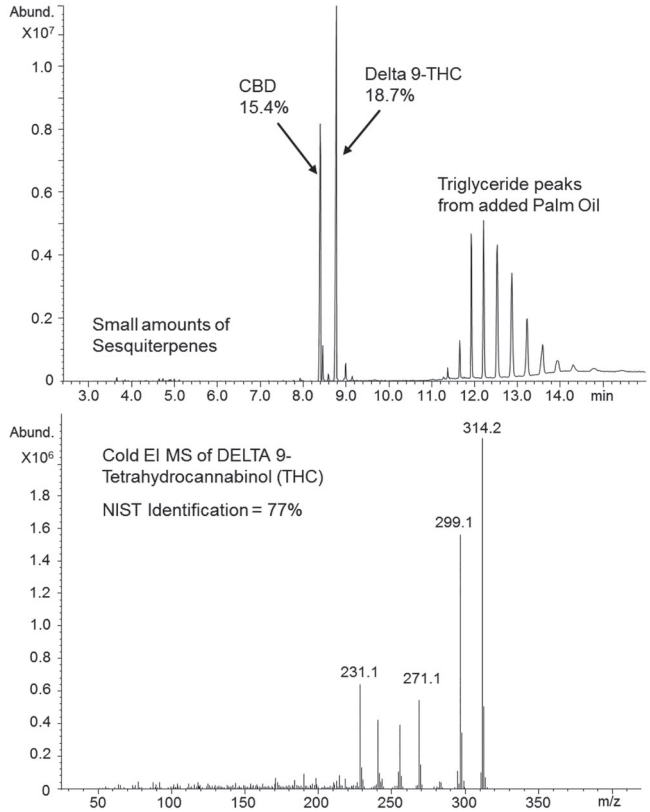

FIGURE 10 Gas chromatography–mass spectrometry (MS) with Cold EI analysis of a cannabis-based cancer drug. The mass chromatogram is shown at the upper trace, whereas the Cold EI mass spectrum of delta 9-THC is shown at the bottom

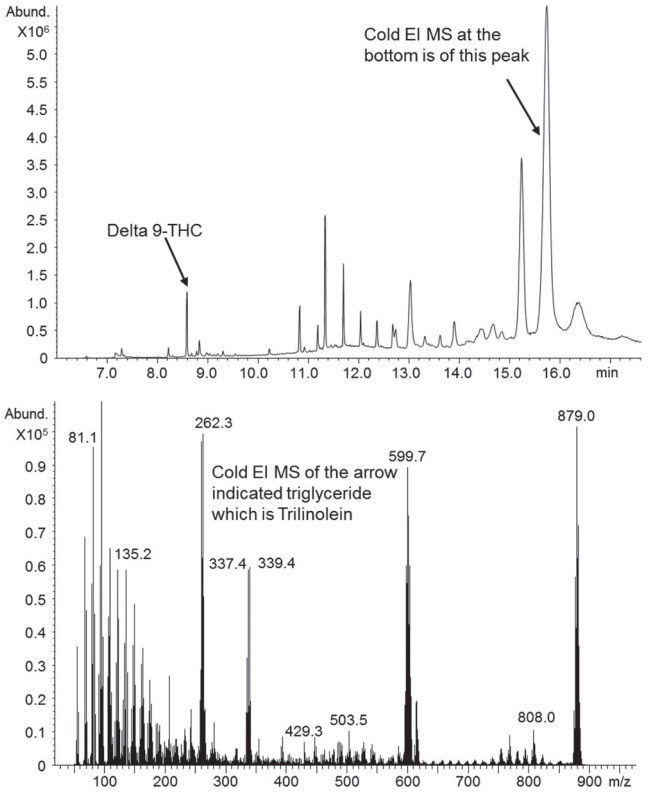

FIGURE 11 Gas chromatography–mass spectrometry (MS) with Cold EI analysis of a Rick Simpson cannabis extract. The mass chromatogram is shown at the upper trace, whereas the Cold EI mass spectrum of the most abundant triglyceride is shown at the bottom trace. THC, tetrahydrocannabinol

we show such a GC–MS with Cold EI analysis of a cannabis-based cancer drug. The mass chromatogram (upper trace) shows both CBD and delta 9-THC plus a few other cannabinoids plus a multitude of triglyceride peaks due to the addition of palm oil. The ability to elute the palm oil components is important in order to allow us to measure without any compound-specific calibration of the actual concentration of both CBD and delta 9-THC as indicated in Figure 10. We also note that the mass chromatogram shows the cleanliness of the drug that includes delta 9-THC, CBD, a few other cannabinoids, and palm oil only. The Cold EI mass spectrum of delta 9-THC is shown at the bottom with a dominant molecular ion yet with 77% NIST library identification probability. The NIST library includes 49 compounds with the elemental formula $C_{21}H_{30}O_2$ of delta 9-THC; thus, 77% identification probability is high. The Agilent Chemstation integration results are that delta 9-THC was found at 20.2%, CBD was found at 16.7%, and about 60% of the drug was its added palm oil.

Rick Simpson cannabis oil is a well-known unrefined, potent cannabis oil, extracted using ethanol and named after the man who created it and first benefited from it.[26] Canadian Rick Simpson claimed that he cured his own skin cancer with a custom blend of cannabis oil, which has come to be known as Rick Simpson Oil (RSO). We used his extraction method on our own cannabis sample and analyzed the RSO, and in Figure 11, the GC–MS with Cold EI analysis of a Rick Simpson cannabis extract is shown. The mass chromatogram is demonstrated at the upper trace, whereas the Cold EI mass spectrum of the most abundant triglyceride is shown at the bottom trace. Note the complexity of the Cold EI mass chromatogram that can be measured only with Cold EI, where molecular ions information is provided for all compounds. The delta 9-THC peak (indicated by the left arrow) was measured at 1.3% of the total ion count mass chromatogram, and a multitude of triglycerides dominated the RSO content.

In Figure 12, we show cannabis EP-1 children with ASD drug analysis. GC–MS with Cold EI mass chromatogram zoomed around the elution time of cannabinoids is shown in the upper trace, whereas the Cold EI mass spectrum of cannabidivarol (also named as CBDV) is shown at the bottom trace. As shown, Cold EI analyzes the full cannabinoids content plus can provide indications to what is unique in it that makes it an effective drug for children with ASD. For example, a related another local drug for children with ASD is named Avidekel, and we found that the main difference between the EP-1 and Avidekel is that the EP-1 has much greater relative amount of CBDV such as 3.9% CBDV/CBD whereas this ratio is only 0.48% in the Avidekel drug.

We measured that the THC/CBD ratio is 5.0% in agreement with LC with diode array and calibration measurement (although achieved without any calibration). In addition, we also found vitamin E in the

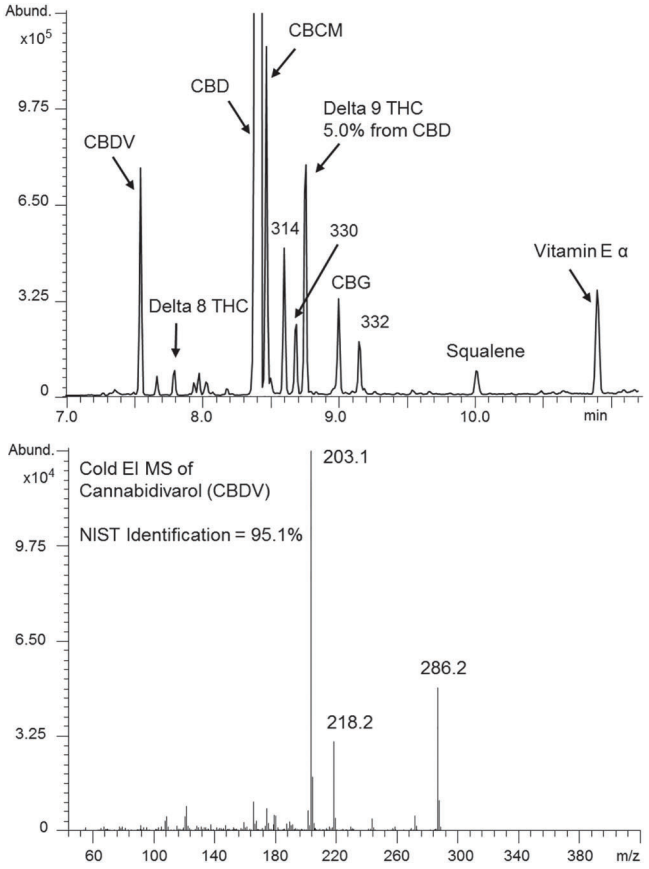

FIGURE 12 Cannabis EP-1 children with autism spectrum disorder drug analysis. Gas chromatography–mass spectrometry with Cold EI mass chromatogram zoomed around the elution time of cannabinoids (upper trace) and the Cold EI mass spectrum of cannabidivarin (bottom). Names and molecular weights of selected cannabinoids and other detected compounds are indicated. CBD, cannabidiol; THC, tetrahydrocannabinol

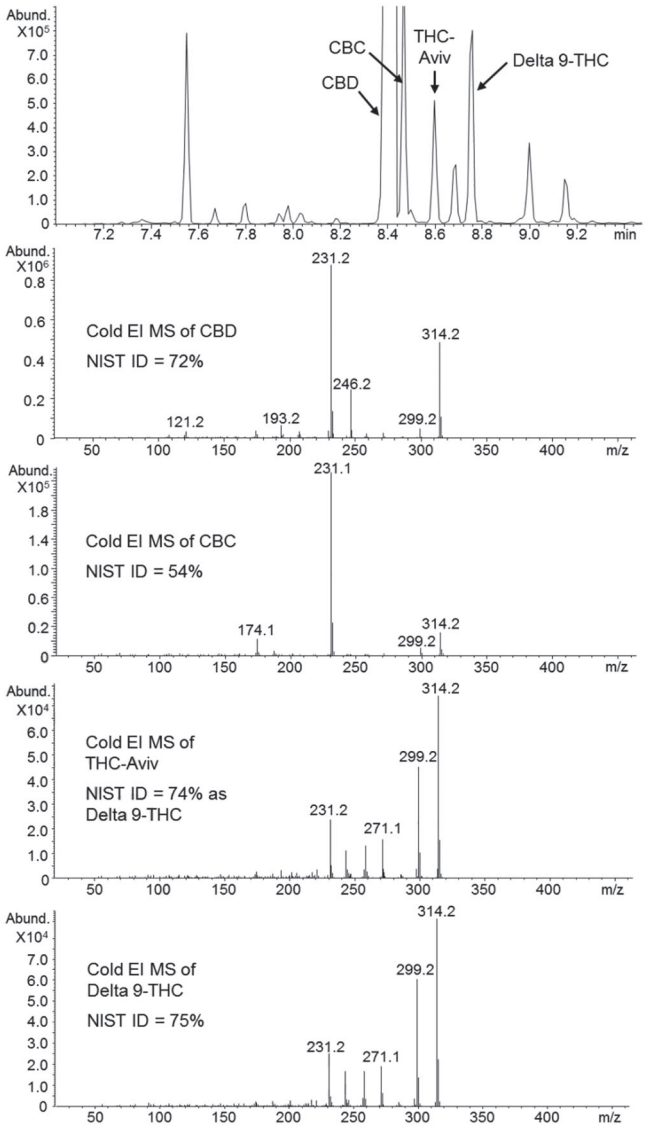

FIGURE 13 The discovery of a new cannabinoid compound named THC-Aviv in cannabis EP-1 drug for children with autism spectrum disorder. The upper trace is the zoomed mass chromatogram of the cannabis EP-I children with autism spectrum disorder drug, and below it, we show the Cold EI mass spectra of the indicated four compounds with the elemental formula $C_{21}H_{30}O_2$ (MW = 314.22). (cannabidiol [CBD], cannabichromene [CBC], THC-Aviv, and delta 9-tetrahydrocannabinol [THC])

form of alpha tocopherol as indicated in Figure 12. Accordingly, the road is now open to explore the entourage effect for the optimization of cannabis-based drugs efficiencies, as it seems that CBDV also helps the therapeutic value of this cannabis-based drug. It is important to note that the entourage effect is debatable and has not gained general acknowledgment by the scientific community yet. However, unlike standard drugs that are mostly based on one active pharmaceutical ingredient, cannabis-based drugs usually include two or more cannabinoids, and thus, the entourage effect is a possibility. As an important example, most cannabis-based drugs include both CBD and delta 9-THC at various proportions, and thus, it seems that likely this combination has some synergy. One of the goals of this paper is to demonstrate that GC–MS with Cold EI can serve as an effective tool for the exploration of the entourage effect.

New tools often bring new findings, and we found a new and unreported compound in cannabis extracts that is potentially of importance, and we named it as THC-Variant or THC-Aviv. In Figure 13, we show the zoomed mass chromatogram of the cannabis EP-I children with ASD drug (upper mass chromatogram) and the Cold EI mass spectra of four compounds with the elemental formula $C_{21}H_{30}O_2$ (MW = 314.22). These isomer compounds from left (early elution time) to right (longer elution time) are CBD, CBC, THC-Aviv, and delta 9-THC. As shown, we found a strange cannabinoid compound with Cold EI mass spectrum that is practically identical to that of delta 9-THC, and thus, we named it as THC-Aviv. We note that this compound elutes between CBD and delta 9-THC, and it is identified by the NIST library as delta 9-THC with about the same identification probability (74% vs. 75% for THC). In some cannabis extracts, we found that this THC-Aviv compound coelutes with a cannabinoid with molecular weight of 330.2 (potentially CBD oxide), but in this EP-1

drug, it eluted clean. We did not find any report about a cannabinoid compound with a mass spectrum that is identical to that of delta 9-THC and thus believe that it is a new and unreported compound. We do not know about the relative potency of this THC-Aviv cannabinoid but that it can be high. However, it could be important for the entourage effect, and it demonstrates the value and significance of Cold EI and universal untargeted analysis.

4 | CONCLUSIONS

In this paper, the analysis of cannabis and cannabinoid compounds by GC–MS with Cold EI was described. GC–MS with Cold EI is based on interfacing the GC and MS with SMB along with electron ionization of vibrationally cold sample compounds in the SMB in a fly-through ion source (hence the name Cold EI). GC–MS with Cold EI improves all the performance aspects of GC–MS including the ability to analyze cannabinoids compounds that contain one, two, or more OH groups without any derivatization and without any ion source-related peak tailing and/or degradation. GC–MS with Cold EI provides uniform compound-independent response that enables quantitation without compound-specific calibration and provides enhanced molecular ions, yet with effective NIST library-based sample identification.[23,24] Accordingly, the main attribute of GC–MS with Cold EI for cannabis analysis is its ability to perform universal untargeted analysis of all the vaporizable compounds and obtain their concentrations this way.

Furthermore, we have described and demonstrated the following:

a. The analysis of cannabis extracts with their full range of all volatile and semivolatile groups of compounds including terpenes, sesquiterpenes, sesquiterpinols, fatty acids, cannabinoids, hydrocarbons, sterols, diglycerides, and triglycerides.
b. The nonuniform response of GC–MS with standard EI for cannabinoids and sesquiterpinols was shown and compared with Cold EI that exhibited uniform response.
c. We analyzed complex cannabis extracts in which we counted over 50 cannabinoid compounds, and each provided a distinct and rich Cold EI mass spectrum with abundant molecular ions. We also found in these mixtures compounds such as bromo CBD that were probably produced during the cannabis extract processing.
d. Because we analyzed cannabis extracts in full scan mode for its universal analysis, we unexpectedly found fluticasone propionate (nasal spray drug) in a cannabis flower. Such a compound cannot be found by standard GC–MS or LC–MS analyses because they serve only for targeted compound analysis.
e. We found relatively large cannabinoids in cannabis extracts with molecular weight above 400 μ with abundant molecular ions and distinct high mass fragment ions.
f. The use of universal untargeted GC–MS with Cold EI analysis enabled the monitoring of partial conversion of CBDA into oxidized CBD (MW = 328) upon decarboxylation of the cannabis extract via its heating.
g. We demonstrated the sensitive monitoring of low levels of delta 9-THC ratio to CBD. We demonstrated having S/N 81000 for 0.66% delta 9-THC compared with CBD, which is far more sensitive than the needed limit of 0.3% (local Israeli regulation).
h. We demonstrated the GC–MS with Cold EI analysis of a cannabis cancer drug, including its added palm oil with its triglycerides. Similarly, we showed the analysis of a complex Rick Simpson cannabis extract (oil) including its heavy triglycerides.
i. Cannabis EP-1 drug for children with ASD was analyzed. The GC–MS with Cold EI mass chromatogram showed the full range of its included cannabinoids and provided their concentrations. Accordingly, GC–MS with Cold EI can serve for learning about the entourage effect for the optimization of cannabis-based drugs efficiency.
j. We presented the discovery of a new cannabinoid compound named THC-Aviv in cannabis EP-1 drug for children with ASD. Whereas the Cold EI mass spectra of a few cannabinoid isomers with the elemental formula $C_{21}H_{30}O_2$ such as CBD, CBC, and delta 9-THC were very different, the THC-Aviv has a very similar Cold EI mass spectrum to that of delta 9-THC. To our knowledge, this is a new and unreported cannabinoid compound that could be of potential medical importance.

Thus, we conclude that GC–MS with Cold EI can serve for the characterization and optimization of cannabis extracts, products, and drugs and provide information on the full content of the cannabis sample compounds and their concentrations including terpenes, sesquiterpenes, sesquiterpinols, fatty acids, delta 9-THC, CBD, other cannabinoids, hydrocarbons, sterols, diglycerides, triglycerides, and impurities.

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