Application Note

Food Testing and Agriculture

Agilent

Trusted Answers

Accurate Mass Library for Natural Products Based on Compounds Identified in Hemp Oil Using High-Resolution GC/Q-TOF

Authors

Sofia Nieto and
Matthew Curtis
Agilent Technologies, Inc.

Abstract

This application note describes the creation of an accurate mass Natural Products library for GC/Q-TOF MS based on hemp CBD oil comprehensive GC × GC data. A comprehensive GC × GC approach was used to achieve better chromatographic separation of a complex matrix than one-dimensional (1D) GC separation. The GC × GC approach was necessary to correctly identify the compounds and extract clean spectra since the library was built directly from the hemp CBD oil samples. This study also provides examples of the target and nontarget screening workflows using the GC/Q-TOF electron ionization (EI) Natural Products spectral library.

Introduction

The use of hemp and CBD oils has become increasingly popular in many parts of the world either as a direct use product or incorporating it into another product. The hemp plant (*Cannabis sativa*) is an extremely rich natural resource used as textile fibers and building materials, among other applications.[1,2] Groups of potentially bioactive chemicals produced in hemp include cannabinoids, terpenes, and flavonoids.[3] One of the important directions of the chemical analysis of hemp and hemp products includes an exploration of the chemical composition of different hemp strains to identify compounds with specific chemical properties.

Concentrated CBD oils derived from hemp are complex samples and typically produce between 350 to 560 chromatographic peaks under a simple 1D configuration. Therefore, a comprehensive GC × GC approach may be beneficial to ensure the chromatographic separation of individual components, while high-resolution accurate mass GC/MS helps to reduce the ambiguity in compound identification. This application note describes the development of a retention index (RI)-based EI accurate mass library for these types of samples using the Agilent 7250 GC/Q-TOF, with the idea that it would reduce the overall data analysis time and allow a focus on the unique components of this type of sample.

Experimental

Samples and extractions

The samples used in this study included five different varieties of hemp CBD oil used in dog treats.

Data acquisition and data processing

CBD oil samples and cannabis extracts were analyzed using high-resolution 7250 GC/Q-TOF MS. The samples were separated on both 1D GC as well as comprehensive GC × GC configuration using the Zoex ZX2 thermal modulator (Figure 1) and Agilent 7890B GC. An Agilent J&W DB-5ms Ultra Inert 30 m column was used to acquire 1D GC data. The GC × GC configuration had an Agilent DB-5ms Ultra Inert 30 m column as the primary column, coupled to a 2.8 m Agilent J&W DB-HeavyWAX as the secondary column. Data were acquired in EI mode at 70 eV. Data acquisition parameters are described in detail in Table 1. The RIs were calculated based on the alkane ladder to assist compound identification and library curation.

The GC/Q-TOF data were processed using the Agilent Unknowns Analysis software tool of Agilent MassHunter Quantitative Analysis software 10.2, as well as Agilent MassHunter Qualitative Analysis software 10 and GC Image GC × GC software 2.9. NIST 17 and NIST 20 libraries were used for initial compound identification.

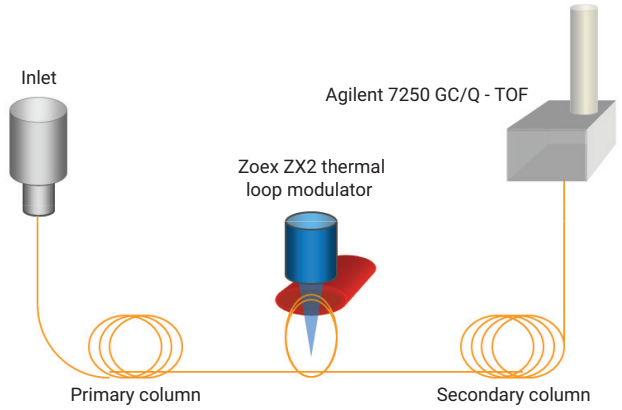


Figure 1. Schematic representation of the Zoex ZX2 thermal modulator.

Table 1. Data acquisition parameters.

GC and MS Conditions	2D	1D
MS	Agilent 7250 GC/Q-TOF	
GC	Agilent 7890B GC	
Inlet	Multimode inlet, 4 mm Agilent Ultra Inert inlet liner, single taper with wool	
Inlet Temperature	280 °C	
Injection Volume	1 µL	
Columns	Primary: Agilent J&W DB-5ms Ultra Inert, 30 m × 0.25 mm, 0.25 µm	Agilent J&W DB-5ms Ultra Inert, 30 m × 0.25 mm, 0.25 µm
	Secondary: Agilent J&W DB-HeavyWAX, 2.8 m × 100 µm, 0.1 µm	–
Oven Temperature Program	60 °C for 5 min; 3 °C/min to 290 °C, 25 min hold	60 °C for 5 min; 4 °C/min to 300 °C, 7 min hold
Carrier Gas	Helium	
Column Flow	1 mL/min constant flow	
Modulation Period	6 s	–
Cold Jet Flow	13 L/min	–
Hot Jet Temperature	300 °C	–
Hot Jet Duration	320 ms	–
Transfer Line Temperature	280 °C	
Quadrupole Temperature	150 °C	
Source Temperature	200 °C	
Electron Energy	70 eV	
Emission Current	5 µA	
Spectral Acquisition Rate	50 Hz	5 Hz
Mass Range	*m/z* 40 to 650	

Results and discussion

Creating the accurate mass library of natural products

The objective of this study was to create a comprehensive accurate mass personal compound database and library (PCDL) based on hemp CBD oil samples for fast screening with high confidence in a standard 1D GC configuration. To achieve adequate chromatographic separation of these complex samples, the data were collected using comprehensive GC × GC configuration. The same set of samples was analyzed using a 1D configuration to validate the accurate mass library as well as screening approach.

The GC × GC data were visualized using the GC Image GC × GC software and compounds were tentatively identified using NIST 17 and NIST 20 libraries. The first-dimension Kovats RIs were calculated using an alkane ladder from C_8 to C_{30} and used for additional compound identification. On a 2D plot, one can clearly see the separation of the different compound classes (Figure 2).

The 2D retention time (RT) significantly helped to increase confidence in compound identification by confirming the chemical class of the compound.

Furthermore, accurate mass of fragment ions and accurate isotope ratios were also used to increase confidence in identifying the components of a CBD oil sample, by reducing the possible elemental compositions. The fragment formula annotation of the compound spectra was performed using MassHunter Qualitative Analysis software (Figure 3A). The annotated spectra were exported to the PCDL following automatic conversion of the measured *m/z* to the theoretical values based on the elemental compositions of the individual ions (Figure 3B). When an unambiguous identification of an isomer was not possible, a compound would be assigned an indexed molecular formula instead of a name. The current PCDL contains approximately 350 compound spectra, of which over 260 entries are assigned a name and a structure.

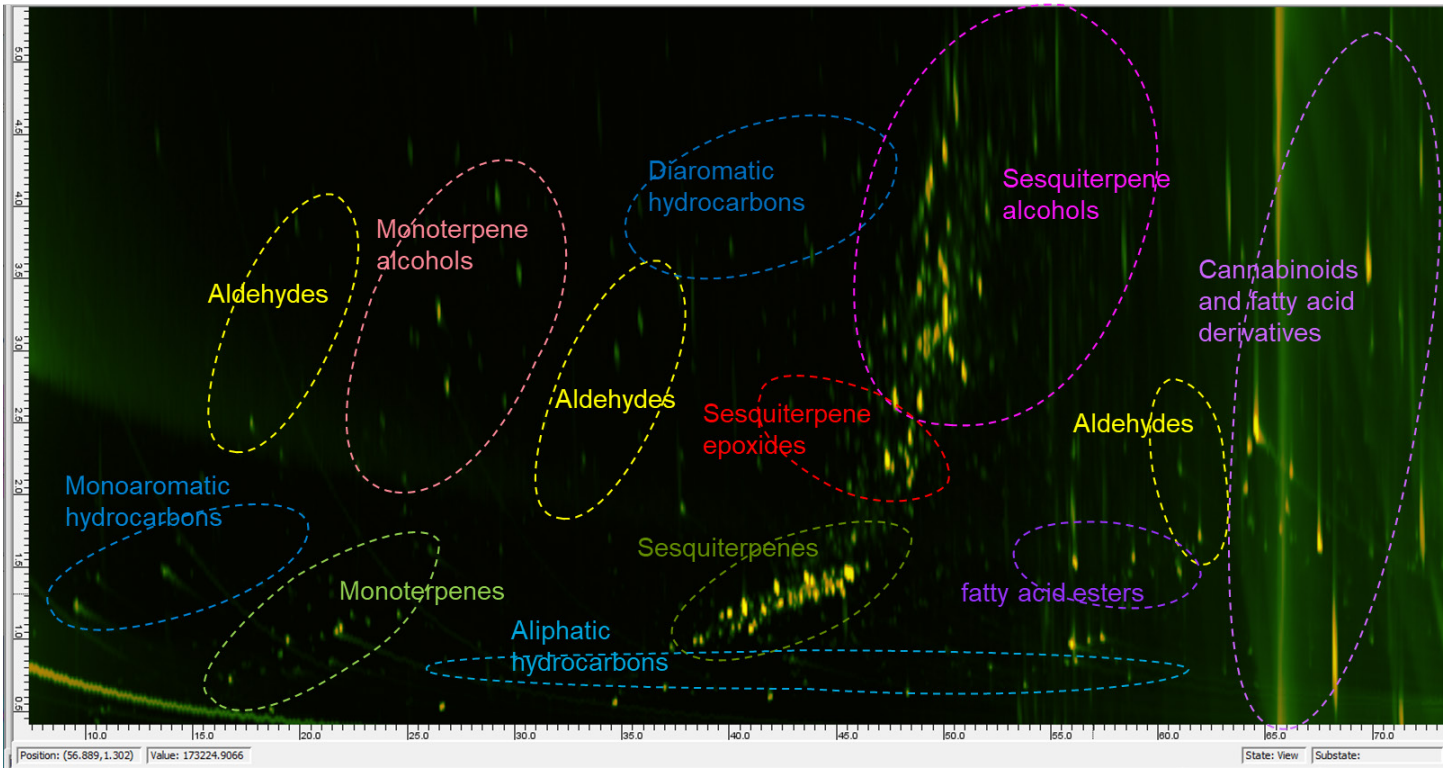
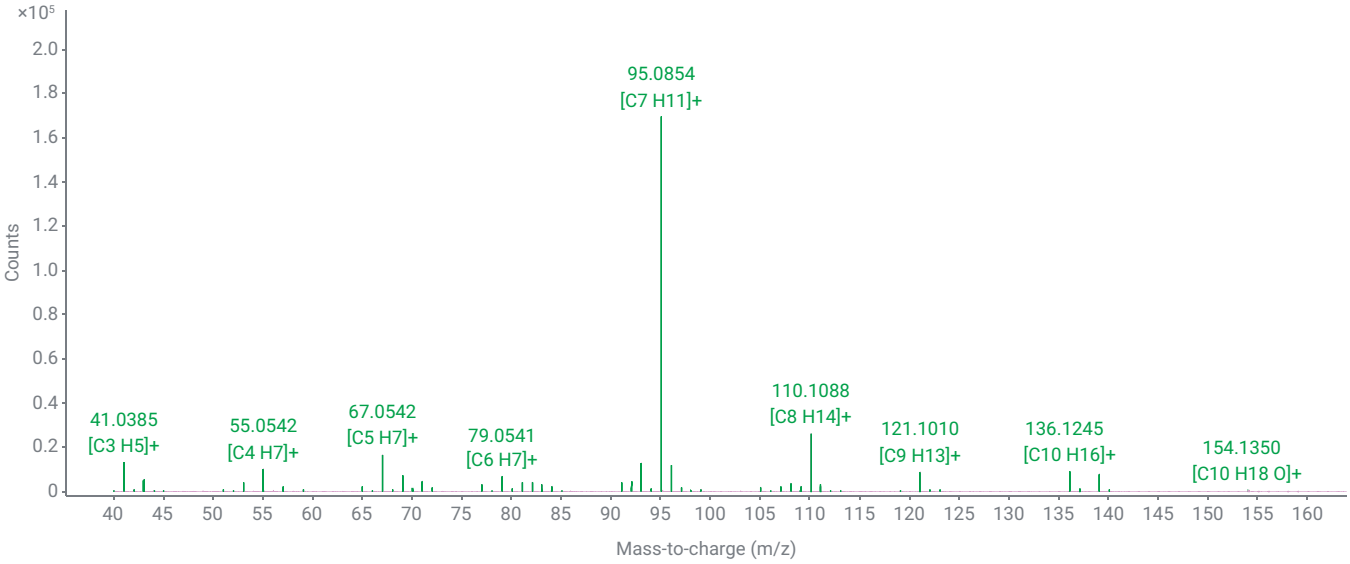


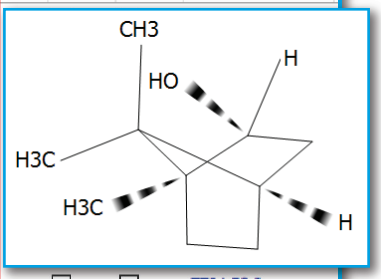
Figure 2. Compound classes mapped on GC × GC/Q-TOF chromatogram of a CBD oil sample.

A



B

Name	Formula	Mass	Retention Time	Retention Index	Cation	Anion	CAS
C11H18-1	C11H18	150.14085	15.03	1134			
C10H16O-2	C10H16O	152.12012	15.13	1137			
cis-2-Nonenal	C9H16O	140.12012	15.508	1147			
Acetic acid, 2-propylpentyl ester	C10H20O2	172.14633	15.535	1148			
C11H18-2	C11H18	150.14085	15.7	1152			
C10H14-4	C10H14	134.10955	15.735	1153			
5-Methylundecane	C12H26	170.20345	15.85	1156			
n-Pentyl benzene	C11H16	148.1252	15.9	1157			
Dihydrocarvone	C10H16O	152.12012	15.9	1157	☐	☐	7764-50-3
trans-2-Nonenal	C9H16O	140.12012	15.99	1160	☐	☐	18829-56-6
endo-Borneol	C10H18O	154.13577	16.45	1171	☐	☐	507-70-0
C10H16O-4	C10H16O	152.12012	16.58	1175	☐	☐	00-00-0



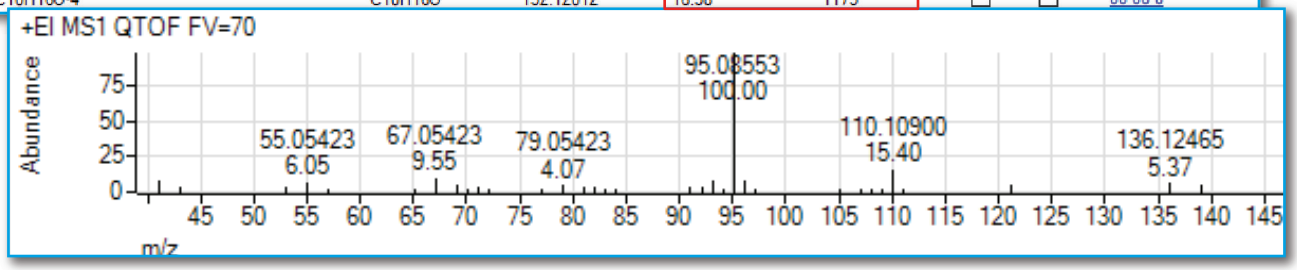


Figure 3. (A) Fragment formula annotation of spectrum is an important step in creating a high-quality accurate mass library. (B) The PCDL of hemp and natural products includes both retention times and retention indices. All the spectra have theoretical *m/z* of the fragment ions.

The distribution of the different compound classes in the PCDL, including, whenever possible, those identified down to the formula, is shown in Figure 4. Monoterpenoids, sesquiterpenoids, and various alcohols represented almost a half of the total number of spectra included in the PCDL.

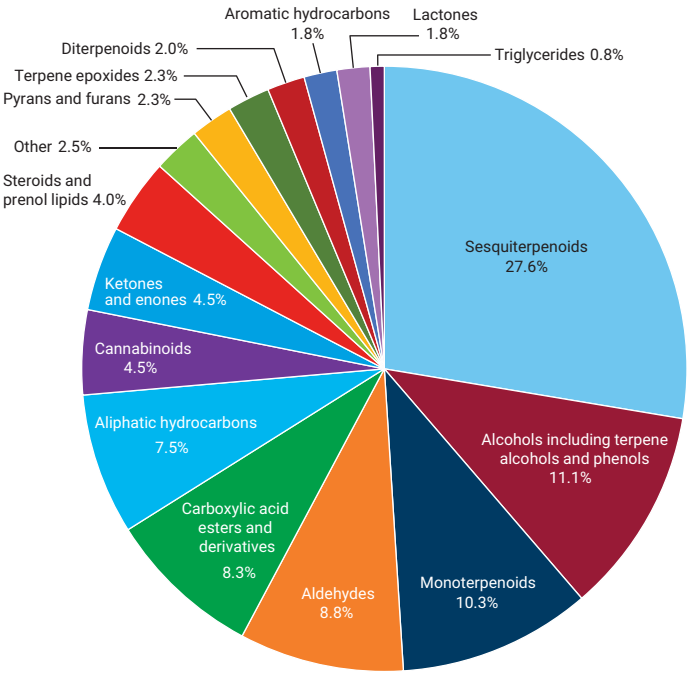


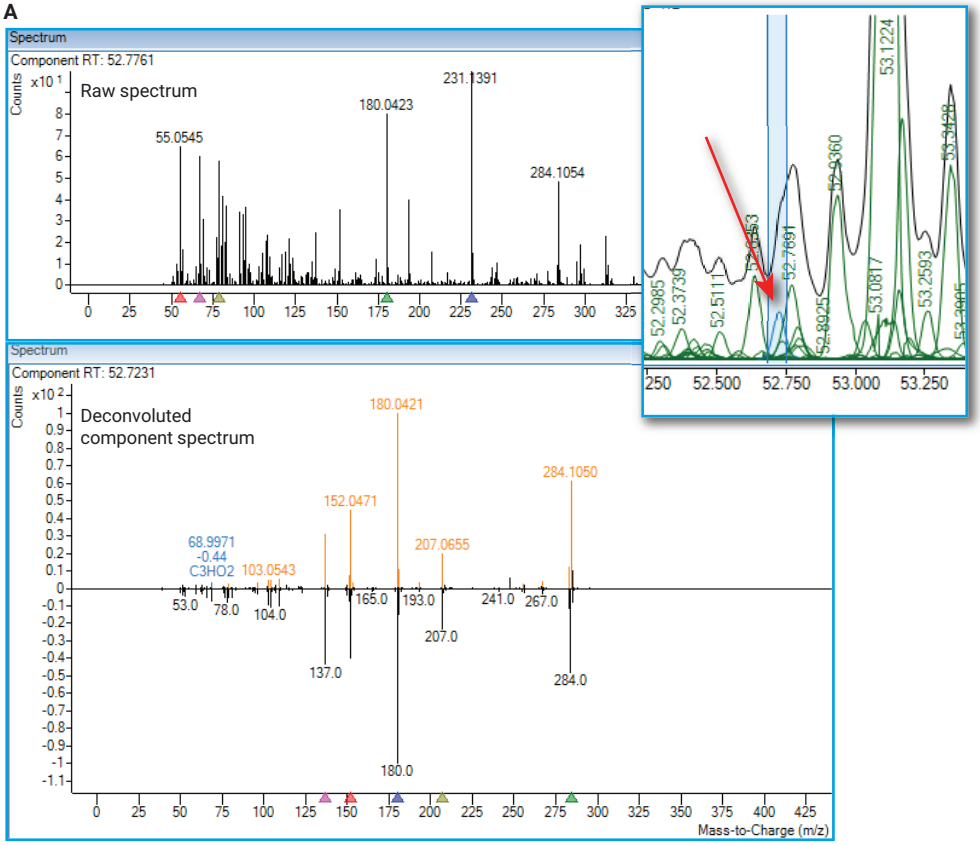
Figure 4. Compound classes in the PCDL.

Target and nontarget workflows using the accurate mass library

Both target and nontarget workflows were evaluated using the Natural Products PCDL and 1D GC/Q-TOF data acquired from hemp CBD oils samples. Nontargeted analysis was performed in the Unknowns Analysis software (Figure 5). The deconvolution algorithm for accurate mass (SureMass) was used to isolate individual components from matrix, and the new Natural Products PCDL was used for the compound identification. Remarkably, the observed difference in RIs between components and hits was small (Figure 5B), despite the difference in the oven program between 1D and 2D GC methods.

Targeted screening was performed in MassHunter Quantitative Analysis software using the GC screener algorithm (Figure 6). This workflow facilitates suspect screening and targeted quantitation simultaneously if standards are available. Another advantage of this approach is that a user can set parameters on a compound-by-compound basis, thus providing a high degree of flexibility. The screening conditions have been optimized for each workflow separately to minimize false positives and false negatives.

A



B

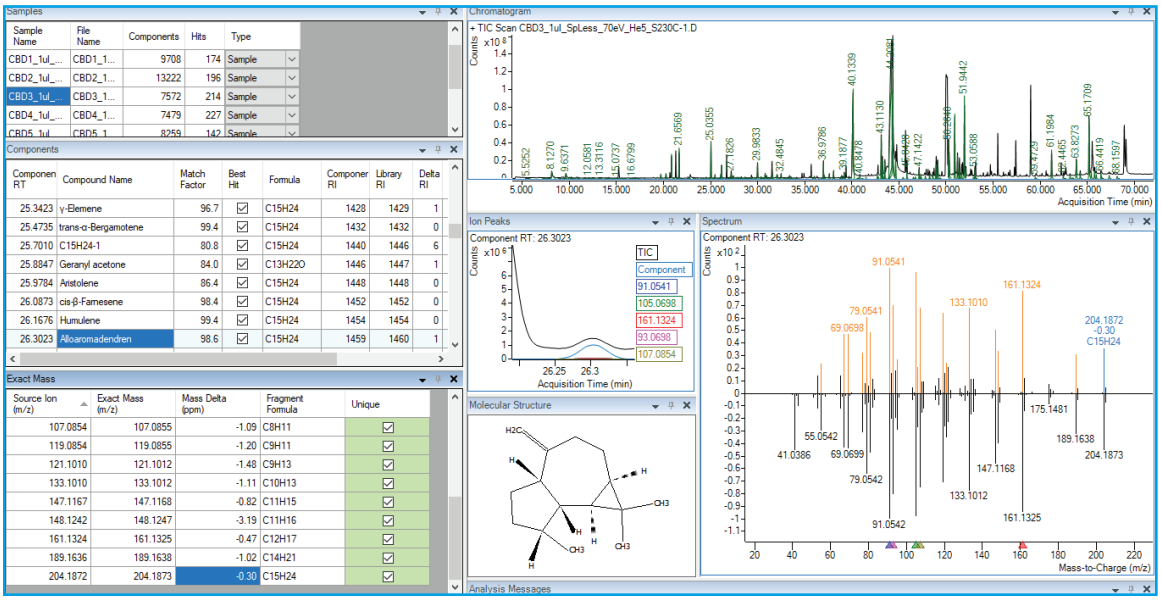


Figure 5. Nontarget screening in Agilent MassHunter Unknowns Analysis software. (A) SureMass is an accurate mass deconvolution algorithm that is efficient in extracting a single component spectrum. (B) The ExactMass feature helps eliminate false positives by examining if the accurate mass ions in a spectrum fit the subset of the molecular formula of the hit.

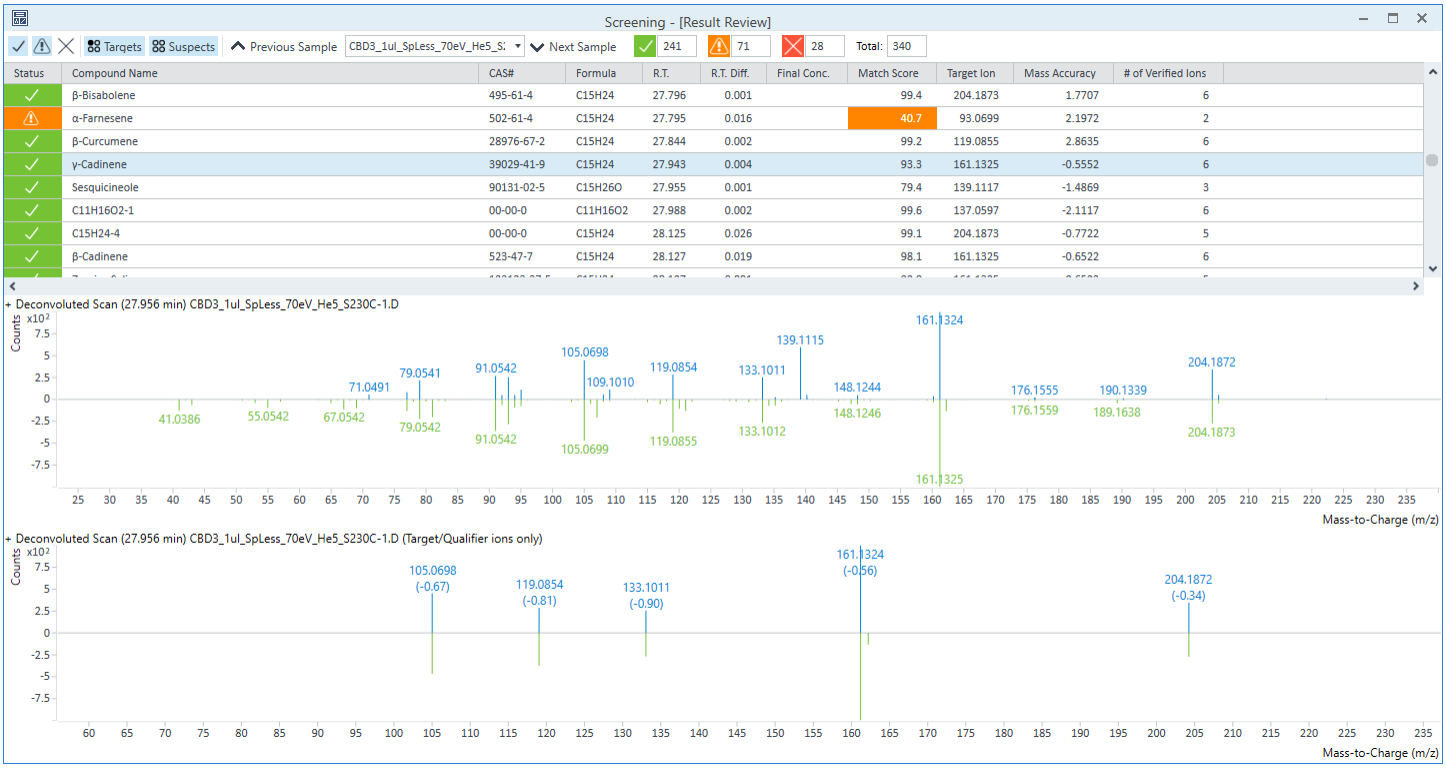


Figure 6. Target screening summary window in Agilent MassHunter Quantitative Analysis software.

In both cases, most of the true hits (confirmed with manual verification) were detected with a high library match score (LMS) of >80 (Tables 2 and 3). When using the nontargeted approach, the number of the true hits with an LMS of <80 was significantly higher compared to the target screening. However, when using the target screening, most of the true hits had an LMS of >90. The LMS threshold is one of the key parameters in the screening method; therefore, it might be helpful to keep in mind this difference between the two approaches and optimize it separately for each application.

Overall, the targeted and nontargeted screening workflows using the accurate mass natural products PCDL yielded a similar number of identified compounds (Table 4), although target screening identified a slightly higher number of the true hits in all samples.

Table 2. Percentage of confirmed compounds observed via nontarget screening by LMS across CBD oil and cannabis samples.

Match Score	CBD1	CBD2	CBD3	CBD4	CBD5	CBD6	Cannabis Extract
>90	45.4	48.5	42.5	39.2	41.5	49.3	45.2
80 to 90	20.1	20.4	24.8	24.2	24.6	23.3	18.3
<80	34.5	31.1	32.7	36.6	33.8	27.4	36.5

Table 3. Percentage of confirmed compounds observed via target screening by LMS across CBD oil and cannabis samples.

Match Score	CBD1	CBD2	CBD3	CBD4	CBD5	CBD6	Cannabis Extract
>90	69.0	63.8	71.4	76.3	64.4	63.1	50.4
80 to 90	20.5	19.1	16.9	12.1	16.7	20.9	23.9
<80	10.5	17.0	11.7	11.6	19.0	16.0	25.6

Table 4. Number of the true hits identified in target versus nontarget screening approaches.

Workflow/ Sample Name	CBD1	CBD2	CBD3	CBD4	CBD5	CBD6	Cannabis Extract
Target Screening	187	201	230	233	169	172	112
Nontarget Screening	174	196	214	227	142	146	104

Conclusion

This application note describes the creation of new accurate mass library for cannabis materials and other natural products using a hemp CBD oil sample analyzed using the GC × GC/Q-TOF MS. This library is designed to be used with 1D GC/Q-TOF data and available for free upon request. Both target and nontarget workflows are compatible with the natural products PCDL with the target screening approach being slightly more sensitive.

Disclaimer

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

References

1. Andre, C. M.; Hausman, J.; Guerriero, G. Cannabis Sativa: The Plant of the Thousand and One Molecules. *Front. Plant Sci.* **2016**, *7*, 19. DOI: 10.3389/fpls.2016.00019
2. Gallily, R.; Yekhtin, Z.; Hanus, L.O. The Anti-Inflammatory Properties of Terpenoids from Cannabis. *Cannabis Cannabinoid Res.* **2018**, *3(1)*, 282–290. DOI: 10.1089/can.2018.0014
3. Flores-Sanchez, I. J.; Verpoorte, R. Secondary Metabolism in Cannabis. *Phytochem*. Rev. **2008**, *7*, 615–639.

www.agilent.com

DE00519179

This information is subject to change without notice.

Printed in the USA, November 2, 2022
5994-5468EN

Agilent

Trusted Answers