Poster Reprint

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Does Your Dog Have Anxiety After a Rough Day at the Lake: Analysis of CBD Extracts for Dog Treats

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Introduction

Recent legalization of cannabis, in some US states, has led to the promotion of cannabidiols (CBDs) as a supplement for various ailments. CBDs are usually extracted from the flowers and buds of cannabis or hemp, without the intoxicating or psychoactive chemical tetrahydrocannabinol (THC). Many of the implied benefits: reducing anxiety, anti-inflammatory, anti-depressant and sleep-aid, have been extensively tested only in animal trials. Over $14.4 billion was spent on pet supplies/OTC medicine and pet owners are looking for natural alternatives. Many pet owners are supplementing their pet's diet with products derived from legal hemp extractions. Due to the differences in extraction techniques and phenotypical difference between hemp strains, an untargeted chemical analysis approach is needed to know the composition of the oil supplements.

The data presented in this poster illustrates the analytical capability of an accurate mass high resolution GC/Q-TOF with low energy EI and chemical ionization functionality (Figure 1) to help with unknown compound detection and increased identification confidence to provide detailed information on specific CBD extracts.

Figure 1: Agilent 7250 GC/Q-TOF

Experimental

Sample Preparation:

Six commercially available CBD oil pet supplements were obtained for this analysis. Five replicate samples, for each individual oil, was provided for a statistical significant analysis approach. The oils were diluted 500x in dichloromethane, d-PAHs were spiked, at 400pg μL^{-1}, to provide an internal standard for normalization. In addition to the samples, a n-alkane standard was injected to provide retention indices information for a higher degree of confirmation to supplement the library hit result of the unknown identification workflow. The sample injection sequence was randomized with 21 blank DCM vials, and four pooled samples used as QCs.

Analytical conditions for the GC/Q-TOF platform are listed in Table 1

Low eV Optimization:

A survey of multiple eV settings was acquired, then reviewed to determine the amount of spectral tilt necessary for high confidence in the detection of molecular ions.

Software:

All-data analysis was performed with the MassHunter Suite. This included MassHunter Qualitative Analysis B08, MassHunter Quantitative Analysis B08, MassHunter Unknowns Analysis, and Mass Profiler Professional

Table 1: Agilent 7250 GC/Q-TOF; 7890B GC Parameters[2]

GC and MS Conditions:		
Column	2x DB-35ms UI, 15 m, 0.25 mm ID, 0.25 µm film; purged union	
Injection volume and liner	1µL	Single-taper w/wool Ultra Inert
Split	10:1 split	
Inlet temperature	280 °C	
Oven temperature program	60 °C for 1.5 min	
	30 °C/min to 180 °C	
	15 °C/min to 255 °C	
	10 °C/min to 320 °C; hold 6.5min	
Carrier gas	Helium Col 1 - 1.2. mL min^{-1} Col 2 – 1.4mL min^{-1} const. flow	
Transfer line temperature	300 °C	
Source temperature	300°C	
Quadrupole temperature	150°C	
Spectral range	45 to 650 m/z	
Spectral acquisition rate	5 Hz, both centroid and profile	
Electron Energy	70 eV and 14 eV	
Emission	5µA and 0.8µA, respectively	

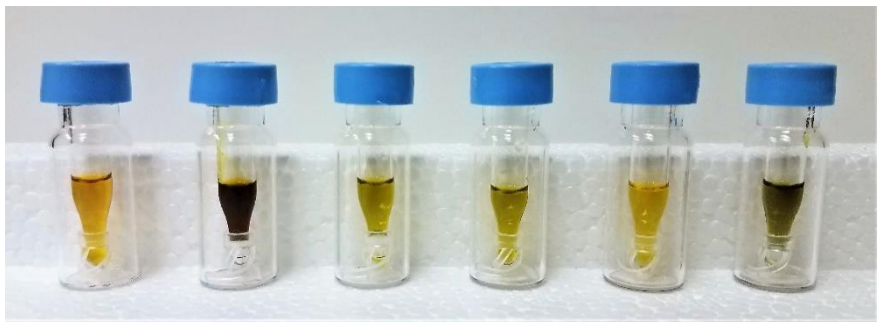

Figure 2: Each sample had a different color and viscosity; 3 and 4 appeared very similar.

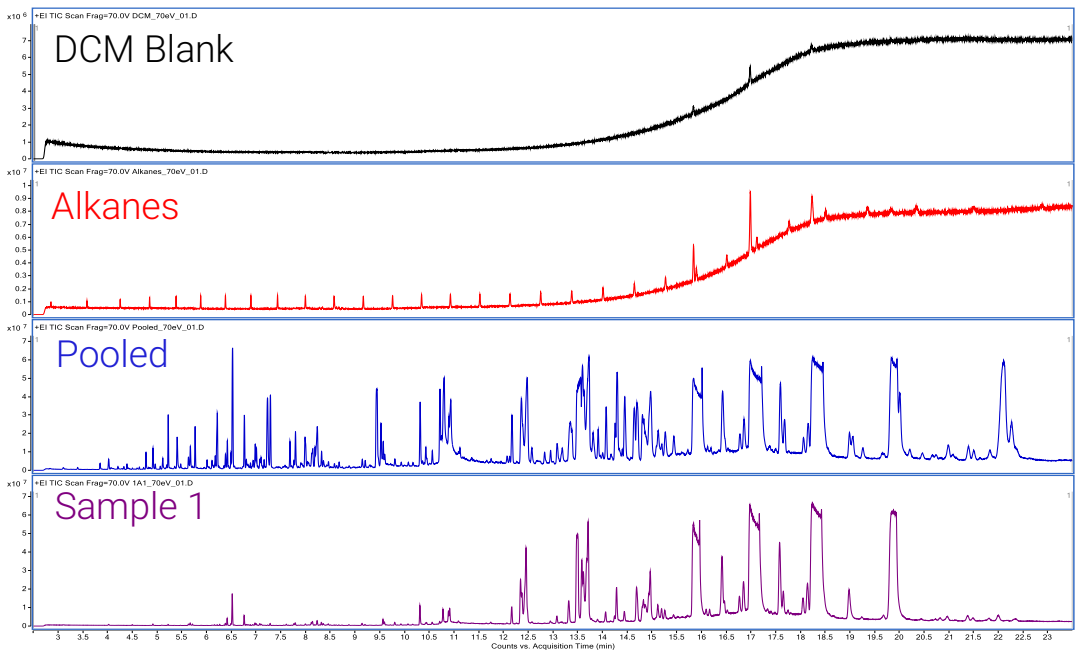

Figure 3: RTICs for the blank, n-alkane standard, pooled QC and 1 sample (A).

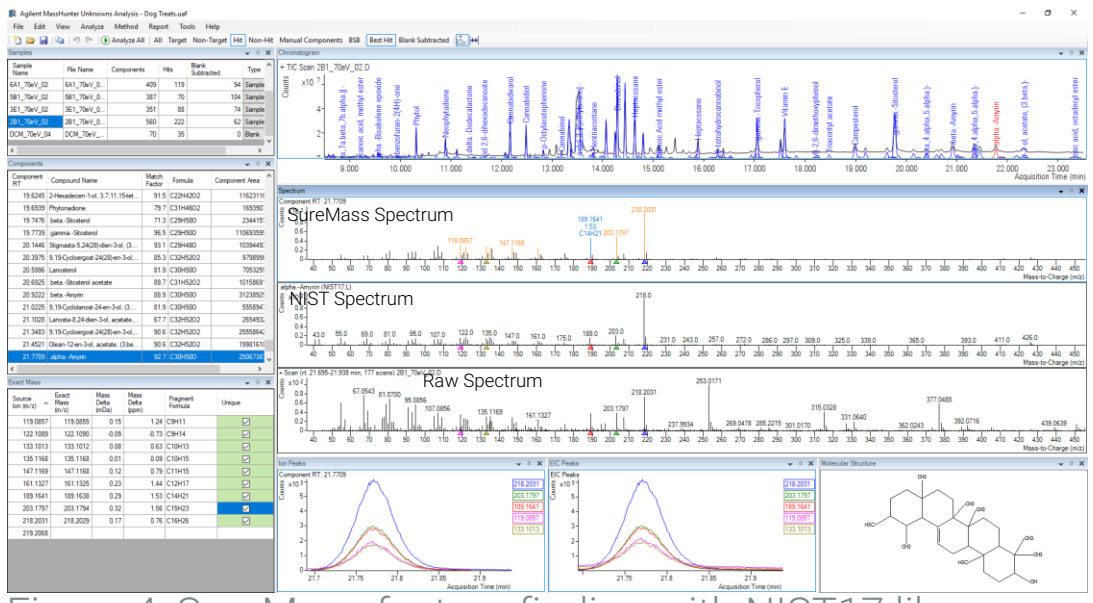

Figure 4: SureMass feature finding with NIST17 library search for identification. The formula from the NIST entry is used for accurate mass and fragment confirmation.

Figure 5: Comparison of 70eV and 14eV for α-Amyrin with elemental composition calculation.

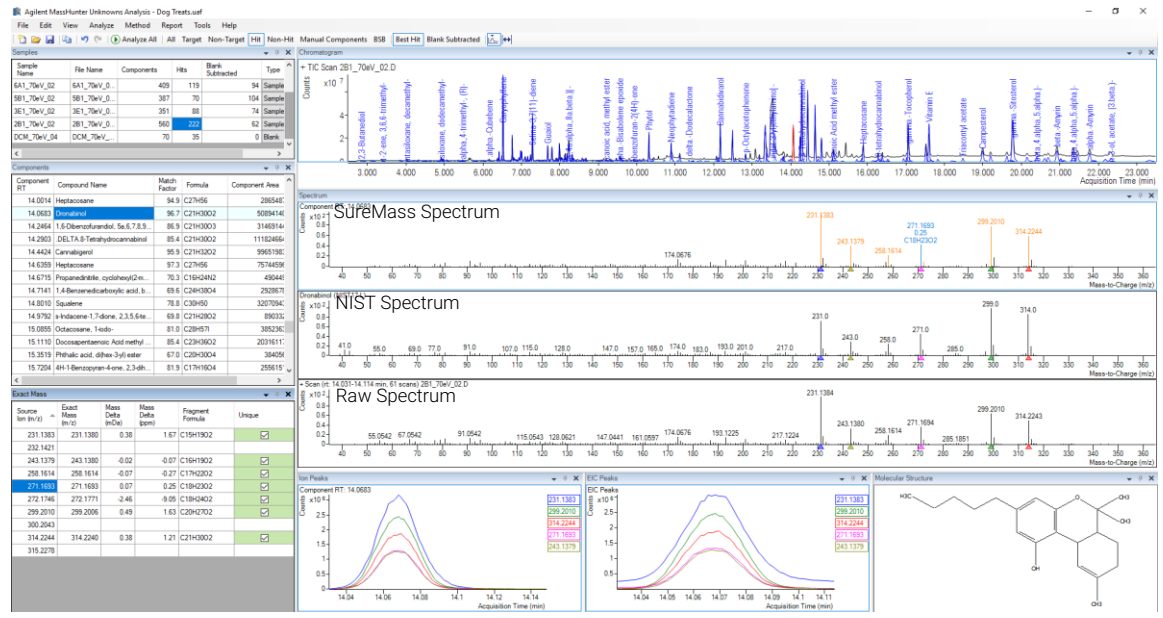

Figure 6: SureMass feature finding and NIST17 library match for dronabinol.

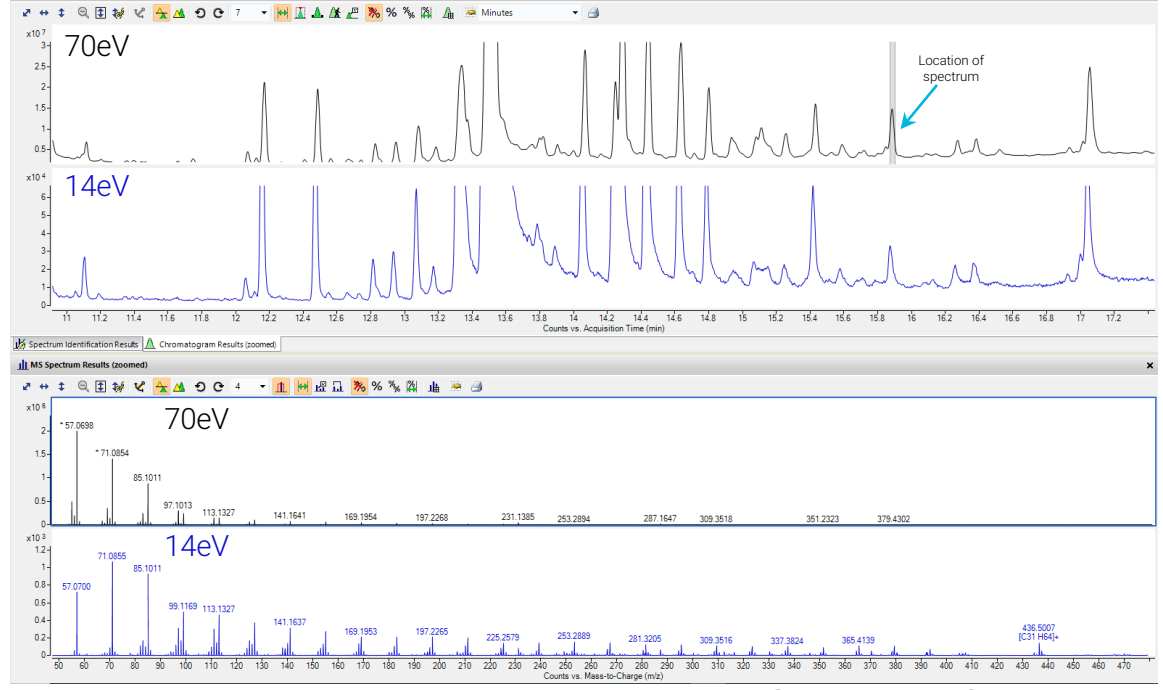

Figure 7: 14eV provided molecular ion information for the correct identification of a hydrocarbon. The 70eV library match was heptacosane ($C_{27}H_{56}$) but the compound is hentriacontane ($C_{31}H_{64}$).

Figure 8: Sample 3 had one of the higher amounts of cannabindiol, 14eV provided enhanced molecular ion and accurate elemental composition calculations.

Results and Discussion

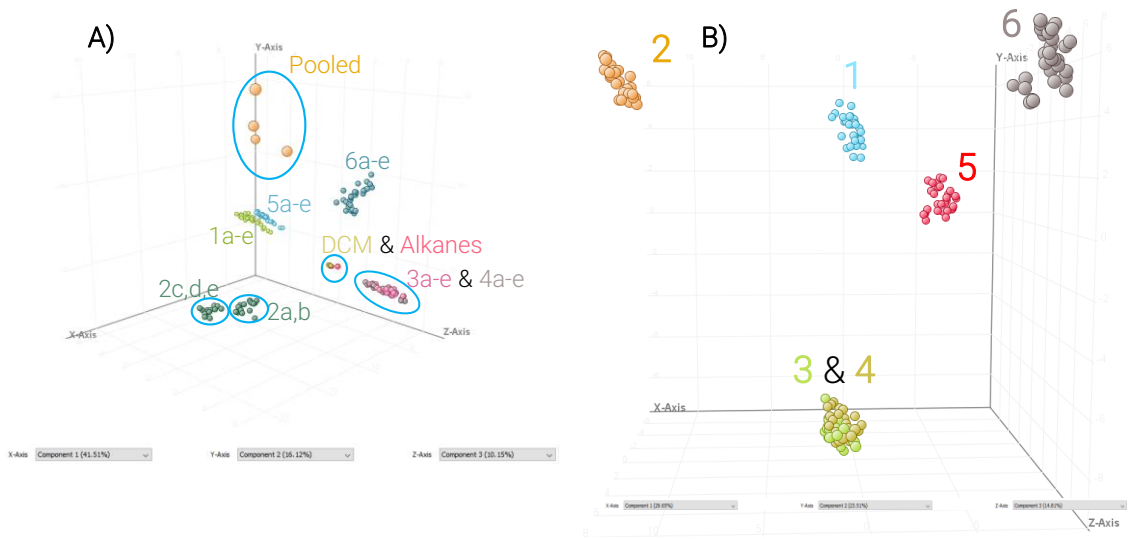

Figure 9: PCA plot A (left) performed for QC of the samples. PCA plot B (right) produced from a Oneway ANOVA and a 2.0 fold change, 30% of the variance is explained by component 1.

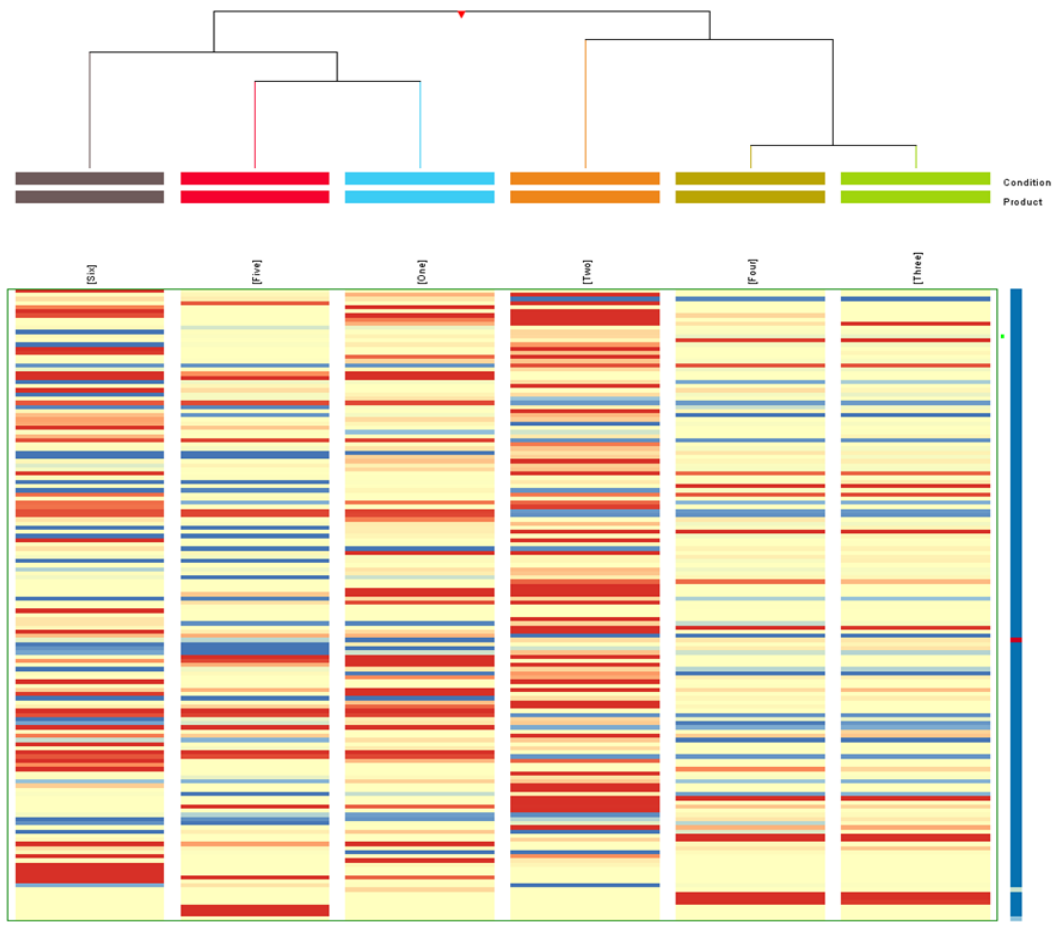

Figure 10: Unsupervised hierarchal clustering; 152/843 Entities. QC = Filter by Flags & Frequency; ANOVA ($p<0.05$), Fold-Change >= 2

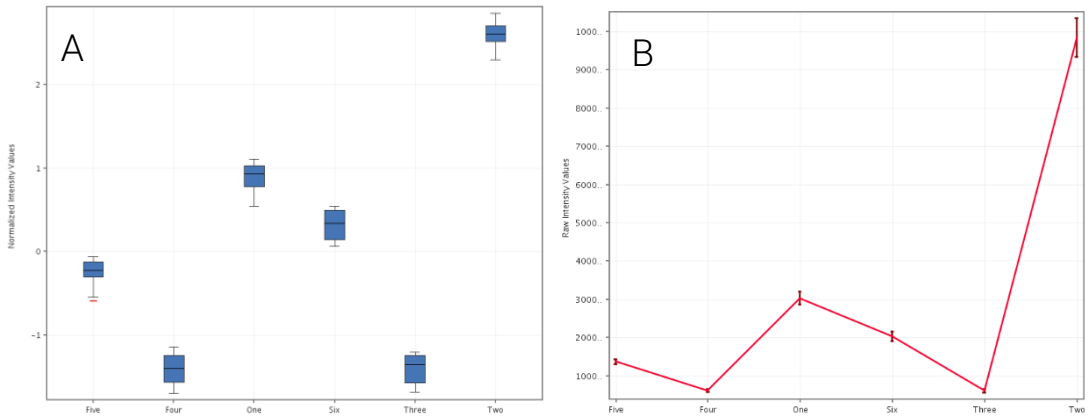

Figure 11: Dronabinol (psychoactive isomer of Δ^9-THC) amount differences between samples. A) box and whisker plot showing variations between samples when baselining to the mean of samples. B) Raw intensities for Dronabinol.

Table 2: Low eV relative results for CBD content between samples do not correlate to labelled amounts. *manufacture did not disclose CBD amount.

Product	CBD (mg mL^{-1})	Average Peak Area (n=2)	% Relative Ratio
1	3.4	16296765	24
2	None provided*	67816499	100
3	1.0	7280377	11
4	6.7	5936324	9
5	3.3	2610632	4
6	8.8	6014898	9

The Purged Ultimate Union between the columns allowed for quick and efficient backflushing to provide consistent retention times even in heavy matrix with over 230 injections. Consistent retention times was a requirement due to the differential analysis and the alignment of the features throughout the sequence of samples and replicates. Identified chemotypes included: fatty acids, fatty acid esters, di and triglycerides, tocopherols, fragrances, essential oils, sterols, and steroids. α,β-amyrin (figure 4&5) have been shown to work as an anti-inflammatory by activating the cannabinoid receptors CB1 and CB2[1]. Dronabinol, a psychoactive compound extracted from the resin of Cannabis was found at a significant level in one of the extracts. Of the six extracts analyzed, only one exhibited a significant amount of cannabidiol with a signal 3-9x increased over the other samples.

Conclusions

High resolving power, accurate mass and low eV provided additional information to a difficult untargeted analysis

- Feature finding reproducibility provided reliable differential analysis
- Most oils were significantly different based of unique components and concentration of specific CBDs.
  - The exception was sample 3 &4
- Low eV provided additional information and confirmation for fragile molecules.
- High resolving power quantitation provided excellent results for complex samples.

Reference

[1]Simão da Silva, Kathryn A.B., et.al. 2011. Activation of cannabinoid receptors by the pentacyclic triterpene α,β-amyrin inhibits inflammatory and neuropathic persistent pain in mice. Pain. 152(8):1872-1887.

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