Screening CBD Oil Pet Supplements for Mycotoxins using LC-MS Quadrupole System with Accurate Mass Calibration

Sue D'Antonio[1]; Yongdong Wang[2]; Don Kuehl[2]; Anthony Macherone[1,3]

[1]Agilent Technologies, Santa Clara, CA; [2]Cerno Bioscience, Norwalk, CT; [3]Johns Hopkins University School of Medicine, Baltimore, MD

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Introduction

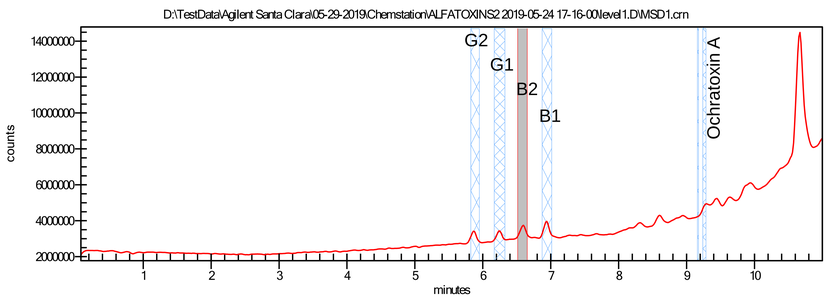
Cannabidiol (CBD) oils manufactured specifically for pets is a rapidly growing market. Many pet owners are supplementing their pets' diet with products derived legally from hemp to ameliorate maladies ranging from anxiety to tumors. In this study, we obtained actual CBD oil pet supplement samples to perform untargeted analyses using liquid chromatography-mass spectrometry (LC-MS) to screen these products for the presence of mycotoxins. The single quadrupole, unit resolution LC/MS data was calibrated into spectrally accurate and mass-accurate data, using known standard reference ions covering the m/z range of interest. The resulting data provides up to 100-times improvement in mass accuracy for spectral matching and identification of mycotoxins.

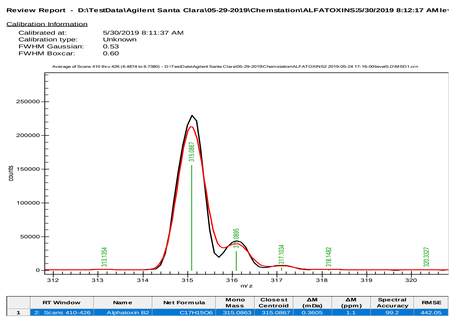
Experimental Setup

CBD oil pet supplement samples were diluted 500-fold with dichloromethane. A 50-microliter aliquot of the diluted sample was then added to 950-microliters of methanol. The LC/MS was an Agilent 1260 Prime / 6125 LC/MS XT system fitted with a 2.1 mm x 100 mm Poroshell C18, 1.9-micron column. The mobile phase was HPLC grade water modified with 0.1% (v/v) formic acid in the A channel, and HPLC grade methanol modified with 0.1% (v/v) formic acid in the B channel. The mobile phase gradient ranged from 72% A to 95% A. The run time was 15 minutes and the post-run time was 2 minutes. For simplicity, the mass and spectral accuracy was achieved using MassWorks software[1] and one of five mycotoxin standards injected at several different concentration levels. The determination and differentiation of various mycotoxins and THC/CBD/CBC are performed on pure mycotoxin standards, pure Hemp standards, and spiked Hemp oil samples and real world samples collected from testing labs.

MS Calibration and Accurate Mass Compound Identification

A mixture standard containing potentially toxic aflatoxin G2, G1, B2, B1, and ochratoxin A found in CBD oil pet supplements were purchased and analyzed with LC/MS with the following TIC chromatogram:



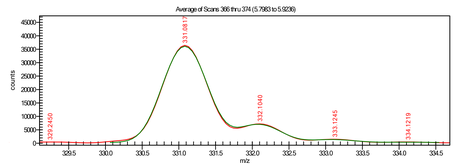


	RT Window	Name	Net Formula	Mono Mass	Closest Centroid	ΔM (mDa)	ΔM (ppm)	Spectral Accuracy	RMSE
1	2 Scans 410-426	Aflatoxin B2	C17H15O6	315.0863	315.0867	0.3906	1.13	99.2	442.05

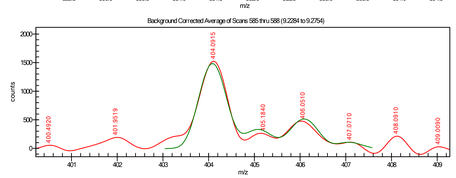
A single ion calibration using aflatoxin B2 (m/z 315) from the highest of 5 concentration levels was performed with MassWorks software through the full mass spectral calibration process published elsewhere[2], resulting in the calibration review/report shown on the left and indicating excellent mass accuracy error of less than 1mDa and Spectral Accuracy of better than 99%.

In order to validate the accurate mass measurement and the unknown mycotoxin identification capability of this approach, the MassWorks calibration was first applied to the same 5 standards including the calibrant (Aflatoxin B2) at the lowest of 5 concentration levels, with the following accurate mass measurement and CLIPS (Calibrated Lineshape Isotope Profile Search) unknown ID results, using a ±20mDa mass tolerance window and C/H/N/O/Cl as possible elements. Confident identification could be made for 4 out of the 5 mycotoxins with a combination of mass accuracy and spectral accuracy. Very similar results were obtained when these mycotoxins were spiked into the Hemp Seed oil sample.

Ochratoxin A	[M+H]+	Exact Mass (Da)	Accurate Mass (Da)	Mass Error (mDa)	Spectral Accuracy (%)	Rank (Total Hits)
Aflatoxin G2	C17H15O7+	331.0812	331.0817	0.5	98.1	1 (89)
Aflatoxin G1	C17H13O7+	329.0656	329.0625	-3.1	98.9	1 (90)
Aflatoxin B2	C17H15O6+	315.0863	315.0902	3.9	98.3	2 (69)
Aflatoxin B1	C17H13O6+	313.0707	313.0834	12.7	98.3	1 (68)
Ochratoxin A	C20H19ClNO6+	404.0895	404.0915	2.0	86.0	32 (136)

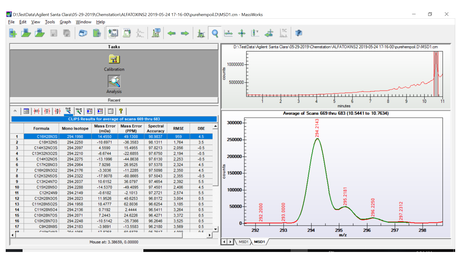


CLIPS unknown elemental composition search correctly identified the aflatoxin G2 as the top hit out of a total of 89 formula candidates at 98.1% spectral accuracy and 0.5mDa mass error.

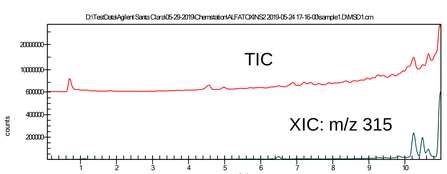


The mass spectral interference and the low signal level from ochratoxin A compromised its identification confidence with 86.0% spectral accuracy, indicating that the higher sensitivity and resolving power of a TOF system would add value here.

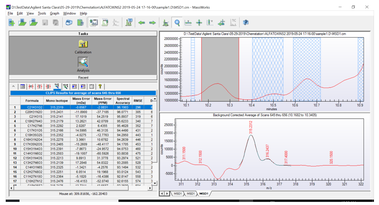
Results and Discussion



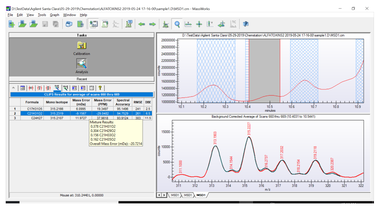
When applied to the identification of the major peak in pure Hemp Seed oil sample, we found that CLIPS identification with C/H/N/O/Cl would not account for the A+2 signal and only the addition of S element would allow for high spectral accuracy identification of this most likely compound.



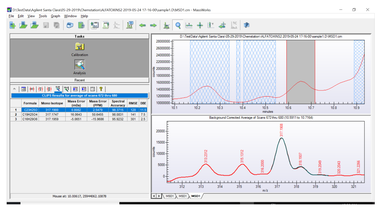
For a real pet supplement Sample #1, we are interested in finding out whether this approach would be able to distinguish the active CBD compounds and one of the 5 mycotoxins, aflatoxin B2, both at m/z 315. The XIC shown on the left reveals the presence of major peaks after RT 10min.



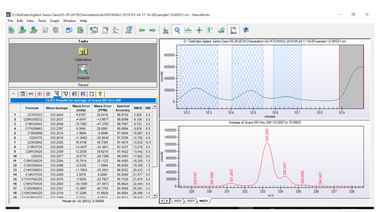
CLIPS unknown elemental composition search correctly confirmed one form of CBD found in this real sample #1, with mass accuracy of 1mDa and spectral accuracy of 98.2%.



CLIPS mixture ion series analysis capability correctly confirmed another form of CBD found in this real sample #1, along with possible other forms of minor postulated modifications and respective relative concentrations, with -10 mDa mass error and 94.8% spectral accuracy.



CLIPS unknown elemental composition search indicates this may be from a different substance, different from either CBD or aflatoxin B2, a compound worth further investigation.



CLIPS unknown elemental composition search correctly confirmed yet another form of CBD found in this real sample #1, with mass accuracy of 7mDa and spectral accuracy of 98.9%, in the form of the more prominent hydration product.

Conclusion

Accurate mass and spectral accuracy obtained from single quadrupole LC/MS system can be a useful and economical tool for pet supplement analysis, either for the active CBD or possible mycotoxin contaminant screening in meaningful concentration ranges and with typical matrices and backgrounds. The LC/MS system is reasonably stable to allow for an external calibration to be used for batch analysis.

The most interesting part of this application demonstrated that it is possible to between differentiate CBD and aflatoxin B2 (both at nominal m/z 315) with confidence.

For weak signals with significant spectral interferences, the compromised spectral accuracy can help alert the user of the need for higher resolution and more sensitive MS systems such as TOF.

References

1. MassWorks, Cerno Bioscience, Norwalk, CT USA.
2. Y. Wang, M. Gu., Anal. Chem., **2010**, *82*, 7055.