Development of a Cannabinoid Analysis within a Regulated Environment

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Interest in the therapeutic properties of organic compounds from cannabis such as cannabinoids has exploded in recent years. This has led to a significant increase in the number of products hitting the market focussed on what the industry terms nutraceuticals. These nutraceuticals are food or fortified food products that are purported to supplement the diet but, also potentially assist in treating or preventing disease. Examples include cannabidiol (CBD) fortified oils which must adhere to the lower legal limits of tetrahydrocannabinol (THC) [1]. Since nutraceuticals are not as rigorously tested and regulated to the extent of pharmaceutical drugs, in recent times there has been a strong movement within the nutraceutical industry towards improving standards and regulation. On 1st November 2018, the United Kingdom legalised medicinal cannabis, allowing the pharmaceutical industry to provide medicinal cannabis extracts approved through clinical trials under pharmaceutical regulations. Unlike the common nutraceuticals these medicinal cannabis extracts can include THC. This article describes the development of a robust analytical method for the analysis of eleven primary cannabinoids within an FDA 21 CFR Part 11 ready chromatography data system (CDS), supporting laboratories seeking to follow the FDA fundamental elements of electronic data quality: ALCOA+.

Introduction

Cannabis contains a number of chemical alkaloids known as cannabinoids. Primary cannabinoids of interest to most laboratories are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). In extracts from the plant, THC and CBD exist as the native acid forms, tetrahydrocannabinolic acid (THCA) and cannabinolic acid (CBDA). These gradually decarboxylate to THC and CBD through exposure to heat and light [2].

Cannabis may be analysed for different purposes, the most common of which is the potency, characterised by the quantitation of THC, CBD and CBN. The Analytical Monograph Cannabis Flos (Version 7.1, November 28 2014) released by the Dutch Office for Medicinal Cannabis describes a methodology for analysis of cannabinoids for release testing of Cannabis Flos (flowers / granulated) [3]. Furthermore, based on this monograph method the typical solvents used to extract cannabinoids are typically 'strong' organic solvents due to their lipophilicity, however; early eluting compounds can suffer from poor peak asymmetry using this monograph method due to the strength of the extraction solvent. This can be solved by using the co-solvent injection mechanism previously published [4]. This article highlights the use of a high-resolution UHPLC method to determine the potency of cannabis extracts with the Shimadzu Cannabis Analyser for Potency within an FDA 21 CFR Part 11 ready CDS environment and the use of intelligent Peak Deconvolution Analysis (i-PDeA) for challenging separations.

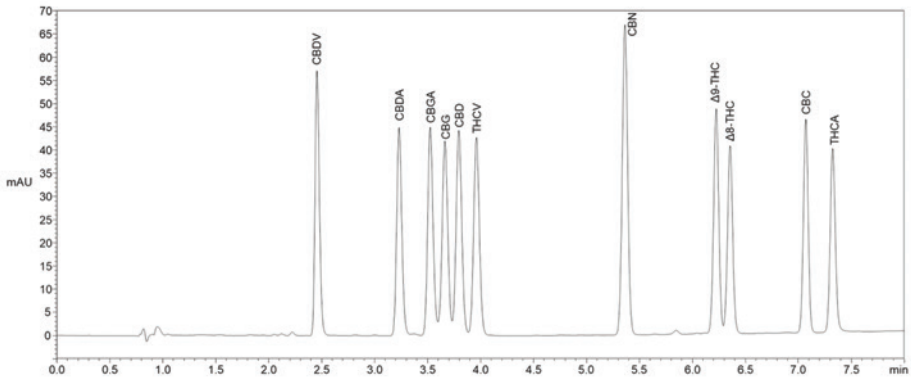

Figure 1: HPLC chromatographic analyses of 11 common cannabinoids. Chromatographic conditions are included in section Method Details.

Cannabinoid methods carried out on HPLC instruments analysed all 11 common cannabinoids in under 8 minutes with a low-pressure maximum of 193 bar / 2,800 psi as depicted in Figure 1. [5]

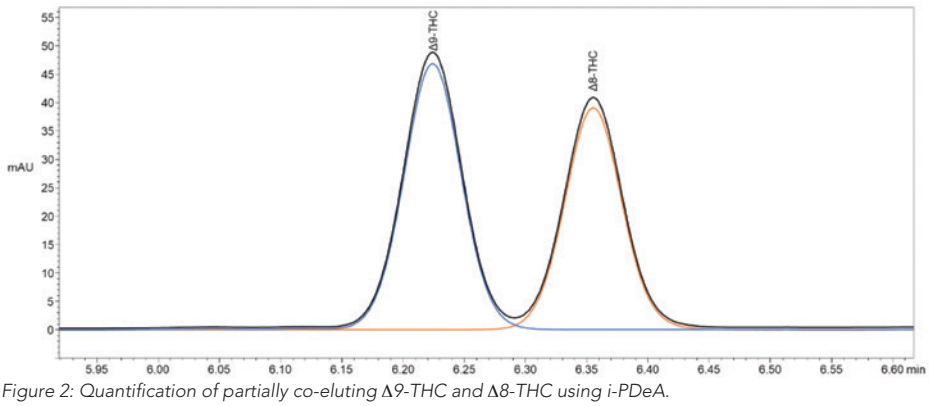

Figure 2: Quantification of partially co-eluting Δ9-THC and Δ8-THC using i-PDeA.

Fully resolving cannabinoids with similar structural properties can prove challenging, the result in Figure 1 demonstrated several components with a resolution factor of <1.5. It is possible to use an intelligent algorithm previously reported for such challenging separations, in order to successfully quantify these partially co-eluting compounds more readily. This is called the intelligent Peak Deconvolution Algorithm (i-PDeA) [6]. Using this technique, it has been reported to deconvolute even positional isomers of o-methyl acetophenone, m-methyl acetophenone and p-methyl acetophenone [7]. As depicted in Figure 2 the same technique can be used for the deconvolution of the Δ9-THC and Δ8-THC co-eluted peak.

It was the objective of this study to further improve the resolution, whilst maintaining faster analysis of these cannabinoids. This was paramount where baseline separation was sought in order to quantify each component successfully, such as within medicinal cannabis analysis.

Retention modelling

Although retention modelling has been successfully employed in optimising analytical separations of small molecules for over 30 years, it is still not universal. Published chromatographic methods using trial and error approaches continue to be prevalent. Retention modelling software packages provide a fast and efficient means to optimise analytical separations whilst selecting conditions that provide the most robust methods. This type of Quality by Design (QbD) approach has become popular within the pharmaceutical industry and the FDA has cited a risk-based approach to drug development as a desirable state for the near future [7]. These reasons lead to the method described in this article being optimised using ACD/LC Simulator, Advanced Chemistry Development, Inc, Toronto, Canada. In Figure 3, regions of colours depicting resolution >1.5 correspond to LC conditions fully [baseline] resolving all 11 cannabinoids. The higher the resolution (Rs) number the greater the resolution of the critical pair. With this information it is possible to choose the analytical conditions which provide the optimal separation within a desired run time. Furthermore, it is also possible to select a region which offers robustness by simulating potential variance in temperature or tG. In addition, other variables such as pH and ternary mobile phase compositions can also be investigated using this strategy.

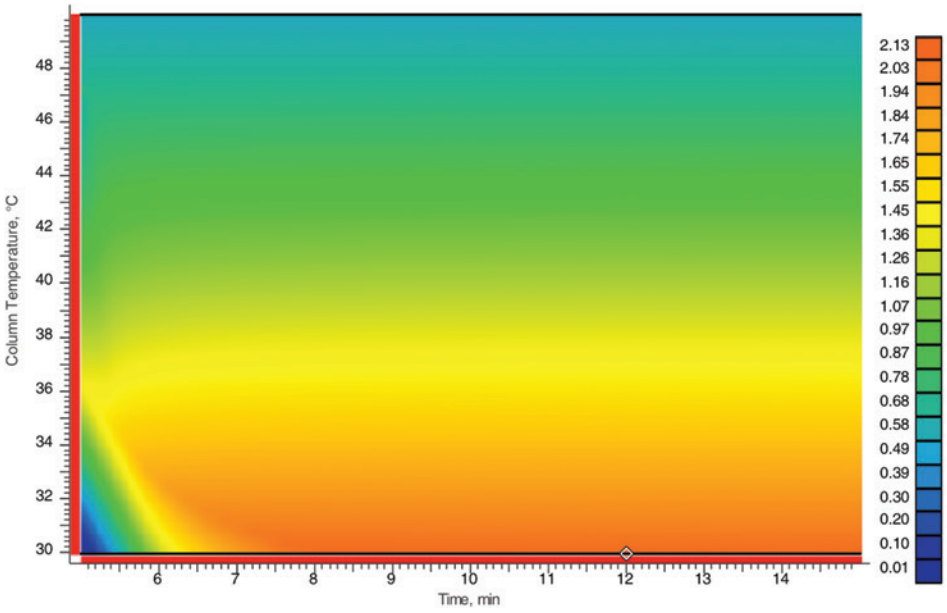

Figure 3: Simulated analytical conditions of 200 experiments.

Method Details

The Shimadzu Cannabis analyser equipped with a photodiode array detector was used for the analysis. Accurate and reproducible resolution for all 11 common cannabinoids were achieved using the NexLeaf CBX column over a 12-minute analytical gradient. The analytical conditions are shown in Table 1.

Table 1. LC Method Parameters.

LC System	Shimadzu Cannabis Analyser
Column	NexLeaf CBX uHPLC column, 2.7 µm, 150 x 4.6mm
Mobile phase A	Water + 0.1% formic acid
Mobile phase B	Methanol + 0.1% formic acid
Rinse solution	Methanol + 0.1% formic acid
Flow rate	1.5 mL/min
Gradient program	0 - 12 min, 70-95%B
Column temperature	30 °C
Injection volume	10 µL
Detector	3D-PDA
Co-injection	Water

The accompanying FDA 21 CFR Part 11 ready CDS used was the Shimadzu LabSolutions DB software. This analysis data system provides ER/ES compliance in regulated environments and included multi-data report functionality.

Materials

All solvents and diluents used were HPLC grade and pre-filtered via 0.45 µm filters from Romil Ltd. All diluents were isopropyl alcohol and methanol. Standards listed in Table 2 were obtained from Sigma-Aldrich® at a concentration of 1 mg/mL (in methanol). Formic acid (puriss p.a.) was purchased from Sigma-Aldrich®.

Table 2: Analysed compounds.

Abbreviation	Item Description
CBC	Cannabichromene
CBD	Cannabidol
CBDA	Cannabidiolic acid
CBDV	Cannabidivarin
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBN	Cannabinol
Δ8-THC	Δ8-Tetrahydrocannabinol
Δ9-THC	Δ9-Tetrahydrocannabinol
THCA	Δ9-Tetrahydrocannabinolic acid
THCV	Tetrahydrocannabivarin

Sample preparation

Varying sample matrix within the nutraceutical industry, has led to a plethora of dilution methods being reported. The most common on the market are oils containing CBD, these can be manufactured with varying types of oil such as hemp, olive or medium chain triglycerides (MCT) which are derived from coconuts. The next common sample type is vape/e-liquids, which have seen growth in general over the past few years, these have now been produced to include CBD within the e-liquids. Those companies that are producing these products from the raw materials, need to also test the flower and/or bud they are using.

Due to oil-based products being used in both nutraceuticals and pharmaceutical production we tested a single MCT based product multiple times. Previous

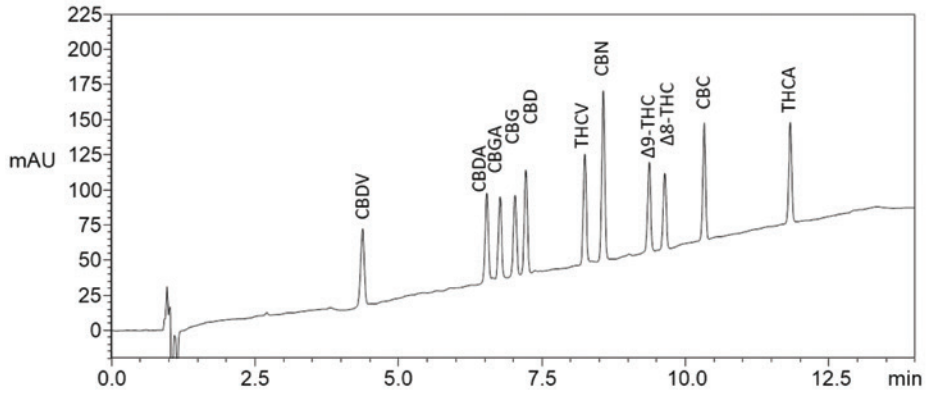

Figure 4: Resolution of 11 common cannabinoids, 10-ppm mix at 220 nm.

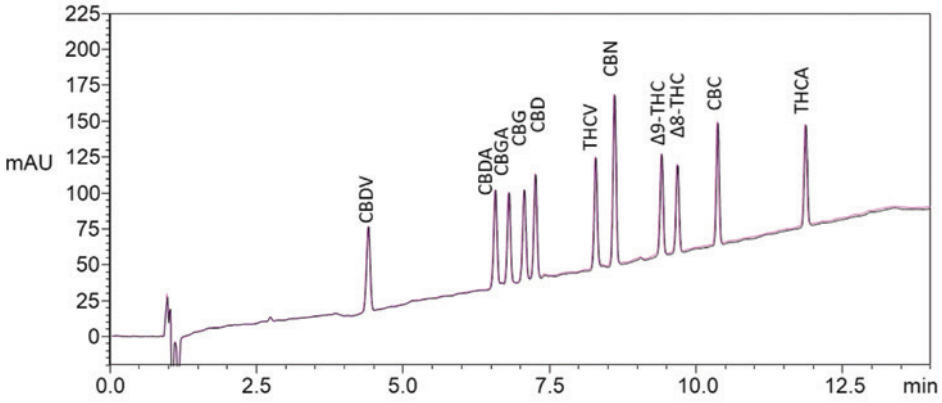

Figure 5: Six replicate injections of 10-ppm cannabinoid mixture, %RSD of 0.6%.

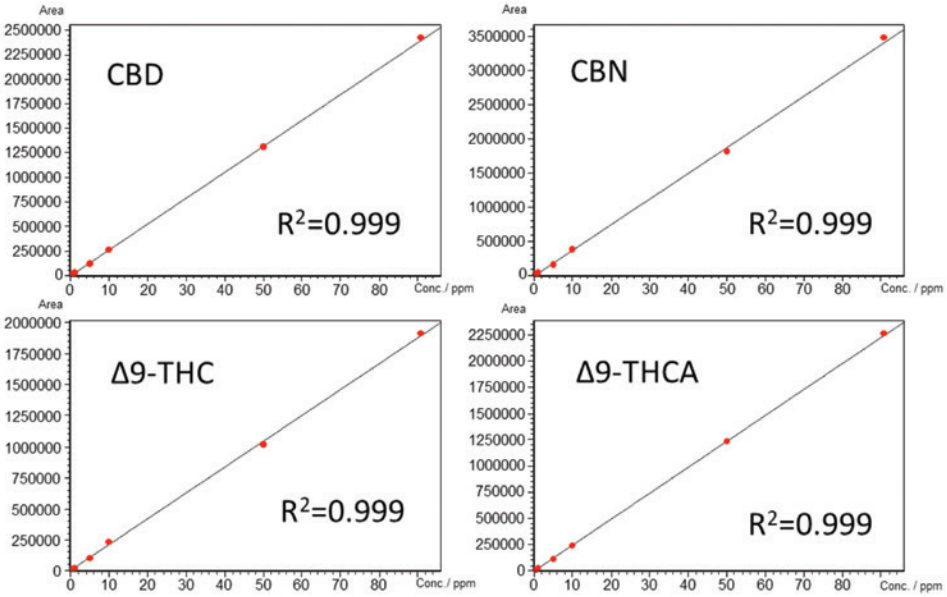

Figure 6: Linearity plots for CBD, CBN, Δ9-THC and Δ9-THCA, 0.5, 1, 5, 10, 50 and 90.9 ppm.

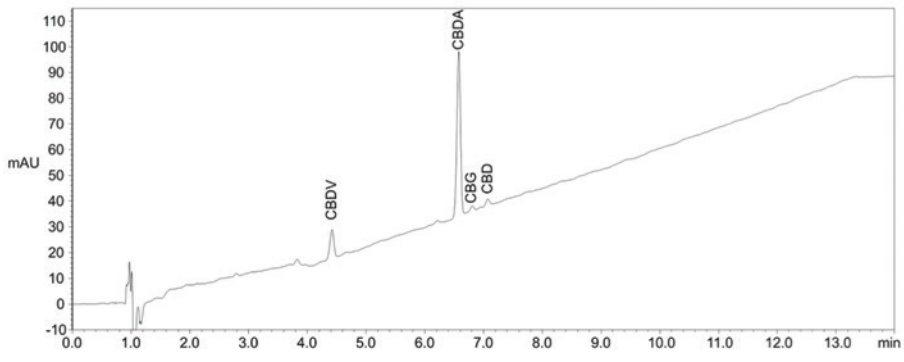

Figure 7: Overlay of 5 prepared CBD oil samples, CBD was found with a %RSD Rt 0.116%, Area 1.178%.

experience of the oil used had shown that only olive oil required any additional dilution procedure. We also tested an e-liquid sample at a higher concentration of CBD.

The sample was diluted in isopropyl alcohol (IPA) and methanol. The solution was filtered and further diluted with methanol to obtain a sample with a CBD concentration within the linear range.

Results and Discussion

As simulated by the retention modelling software; baseline resolution of all 11 cannabinoids was achieved in Figure 4 with good peak asymmetry for all cannabinoids including early eluting compounds, which can be affected by strong diluent sample injections. The maximum observed back pressure was registered at 486 bar / 7,050 psi (column dependent).

As shown in Figure 5, chromatographic repeatability was demonstrated via 6 replicate injections of a 10-ppm cannabinoid mixture with a %RSD of 0.6%.

A 6 level linearity plot was generated for each cannabinoid standard from 0.5 ppm through to 90.9 ppm. With a 10 µL injection this means the low standard equates to only 0.005 µg of cannabinoid on column can be routinely detected, see Figure 6.

Sample analysis

A CBD Oil sample was prepared five times, employing the methodology described in the sample preparation section, to ensure both the analytical method and sample preparation were robust. The chromatogram below demonstrates the robustness of the methods.

A vape sample was then prepared following the same procedure and tested using the same analytical method, the chromatogram can be seen in Figure 8.

Reporting

Compliant industries have seen a push towards validated computer systems over the last decade. This move towards automated processes within analytical laboratories has seen an increase in compliant laboratories dedicating time and resources to complex reports.

As part of this study we also worked with data to build a custom report based on two injections of the same sample at differing concentrations, the final report not only reports all relevant details, but amalgamates the two injections data. This data allows for

either an average result if both sets of data are within the calibration curve, or selection of data if only one is within the calibration curve. It is also possible to add a detection limit if no peak is detected or lower than the bottom standard used.

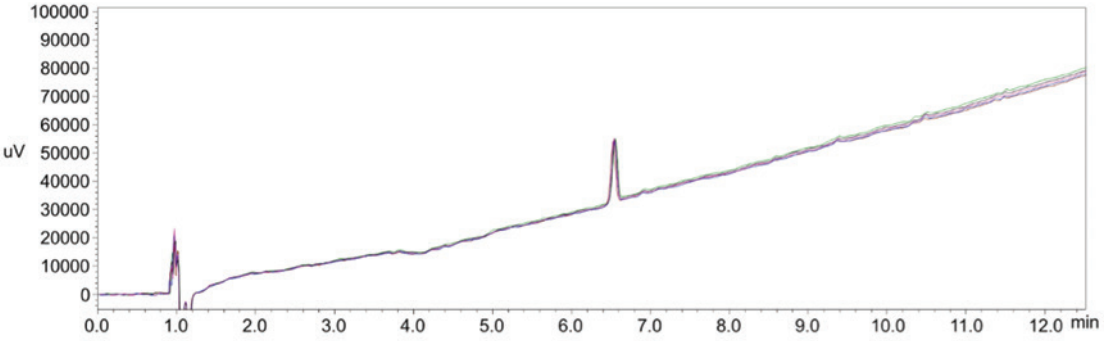

Figure 8: A single e-liquid vape sample

This type of report would usually involve an analyst manually creating a document from the various sample injections, here we can see that the report shows which results are outside the calibration data set, and the amalgamated results, including use of a limit value.

Conclusion

This study demonstrated the development of a new robust liquid chromatography method successfully resolving 11 common cannabinoids using 3D-photodiode array detection. Furthermore, the ability to use intelligent tools such as i-PDeA to help quantify challenging separations or co-eluting peaks caused by impurities or matrices from real samples, will improve laboratory productivity.

Shimadzu has the use of both Labsolutions DB and CS, which are fully compliant. The two options both enable full data integrity, including all data being encrypted to ensure security. The full compliance (if needed) has transparency, legitimacy and validity of the data generated within a regulated environment.

The additional option, Multidata Report, as shown in the reporting section, is another intelligent tool that can be used to customise the sample report generated to highlight results (pass/fail can be coloured or labelled), making report summaries easily understood.

Acknowledgements

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Cells highlighted are within calibration range

Sample Information

Sample Name	Sample ID	CBDV Area	CBDA Area	CBGA Area	CBG Area	CBD Area	THCV Area	CBN Area	d9-THC Area	d8-THC Area	CBC Area	THCA Area	Dil. Factor
Vape 1	A	118018	13719.76	1287	19548	595168	25601	527	1161	0	201	503	281
Vape 1	B	11333	1480.84	0	1773	57741	2421	0	0	0	0	0	2810
Vape 2	A	117797	13629.28	1542	19606	594045	25704	589	1178	0	197	207	281
Vape 2	B	11398	1394.68	0	1820	58032	2430	0	0	0	0	0	2810
Vape 3	A	115923	13488.08	1520	19286	584638	25221	574	1162	0	184	0	281
Vape 3	B	11348	1461.80073	0	1783	57741	2434	0	0	0	0	0	2810
Oil 1	A	21980	3953.64	361	10371	1039441	3607	27899	50609	2246	26192	4037	81
Oil 1	B	440	0	0	0	20877	0	579	989	0	482	671	4050
Oil 2	A	21505	3973.92	365	10145	1019966	3462	27534	49590	2116	25699	4550	81
Oil 2	B	384	0	0	0	19204	0	514	882	0	447	608	4050
Oil 3	A	21430	3964.64	354	9884	1013527	3399	27255	49182	2118	25356	4220	81
Oil 3	B	394	0	0		19166	0	511	905	0	450	616	4050

Table below generated from sample areas above, utilising data only within calibration range. If both dilutions are within range an average is represented. A limit of detection can be displayed if all data is lower than the calibration curve.

Sample Information

Sample Name	Sample ID	CBDV Conc. ppm	CBDA Conc. ppm	CBGA Conc. ppm	CBG Conc. ppm	CBD Conc. ppm	THCV Conc. ppm	CBN Conc. ppm	d9-THC Conc. ppm	d8-THC Conc. ppm	CBC Conc. ppm	THCA Conc. ppm
Vape 1		6444.79	825.58	LIMIT	1317.22	33573.65	1467.30	LIMIT	LIMIT	LIMIT	LIMIT	LIMIT
Vape 2		6433.15	821.03	LIMIT	1320.48	33728.38	1472.91	LIMIT	LIMIT	LIMIT	LIMIT	LIMIT
Vape 3		6334.49	813.93	LIMIT	1302.53	33573.65	1446.57	LIMIT	LIMIT	LIMIT	LIMIT	LIMIT
Oil 1		400.20	96.41	LIMIT	231.30	20136.77	77.19	339.68	871.59	126.39	491.59	95.52
Oil 2		392.99	96.70	LIMIT	227.65	18854.60	74.91	336.27	855.62	123.92	483.81	104.01
Oil 3		391.85	96.57	LIMIT	223.43	18825.48	73.92	333.67	849.22	123.96	478.39	98.55

Figure 9: Selected section of the generated report