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Research Article

AN EFFICIENT HPLC-UV METHOD FOR DETERMINATION OF TETRAHYDROCANNABINOL IN OIL

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ABSTRACT

Objective: The purpose of this work was to develop and validate a high-performance liquid chromatography (HPLC) with ultra-violet (UV) detection method for the determination of Δ^9-tetrahydrocannabinol (THC) in oil.

Methods: HPLC analysis was carried out using an Agilent 1260 Infinity Series® II liquid chromatography system with ultra-violet detection. The stationary phase consisted of an ACE 5 µm C18 column 5 µm (250×4.6 mm) at a temperature of 40°C. The mobile phase consisted of acetonitrile and phosphate buffer (pH=2.5) (70:30, v/v) at a flow rate of 2 mL/min. The injection volume was 20 µL. The UV detector was set at 220 nm. Separation of THC from oil was carried outusing solvent extraction: 0.6 mL of methanol, 0.3 mL of THC in oil and two drops of internal standard were placed in a centrifuge tube, vortex mixed for 30 s, sonicated for 20 min, and centrifuged at 6000 rpm for 15 min. The organic layer was separated, stored at −20°C for 2 h, and centrifuged at 6000 rpm for 15 min. The organic layer was separated again, passed through syringe filters of 0.45 µm, and analyzed.

Results: The method was found to be linear in the range of 0.039–5.000 µg/mL, with acceptable inter- and intra-assay precision, accuracy, and stability. The limit of detection was 0.019 µg/mL and the limit of quantification was 0.039 µg/mL.

Conclusion: The developed method is quick and easy to use and can determine THC in oil with good peak shape and resolution. Application of the analytical method will help in the determination of THC in CBD oil for medicinal use.

Keywords: Tetrahydrocannabinol, Olive oil, High-performance liquid chromatography-ultra-violet.

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INTRODUCTION

Cannabis sativa is a flowering dioecious plant [1-3] and contains molecules with therapeutic properties, namely, flavonoids, terpenoids, alkaloids, and cannabinoids. Phytocannabinoids have different physiological effects [2,4] and can be classified according to the presence or absence of a carboxyl group in their structure; neutral cannabinoids do not have a carboxyl group and acidic cannabinoids have a carboxyl group [5]. Acidic cannabinoids are biosynthesized and accumulate in the plant and are decarboxylated to the neutral form of cannabinoids by non-enzymatic processes [6]. Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are neutral cannabinoids which have been often studied for their medicinal properties [7].

CBD is the main non-psychoactive cannabinoid [2,7,8] and has a good number of reported therapeutic applications [2,9]. CBD has been used in children for management of drug-resistant epilepsy [2,10] and has analgesic, antidepressant, antipsychotic, anxiolytic, antioxidant, anti-inflammatory, and neuroprotective effects [8,11,12].

THC exerts psychoactive effects [2,8,13] and was synthesized and isolated in 1964 [14]. THC was approved by the Food and Drug Administration (FDA) for the control of chemotherapy-induced nausea and vomiting, for appetite stimulation in acquired immunodeficiency syndrome patients [15] and for management of migraines [16].

Cannabis products are available in various dosage forms including oils. Cannabinoids can be found in commercial products containing different carrier oils such as sesame seed oil, hemp seed oil, medium chain triglyceride oils, and olive oil. Commercial oils usually contain low amounts of THC and high concentrations of CBD [17]. A number of products available in Europe claim to contain low concentrations of THC however information about the origin of the product is sometimes missing and quality of products might vary between batch to batch. Some studies have reported inconsistencies between the concentration of THC and CBD present in the oil and what is stated on the label of the product [18,19]. There is a need to develop and validate efficient, simple, and rapid analytical methods to determine THC in CBD products, in relation to what was discussed at the United Nation Narcotics Board with respect to the reclassification of CBD as a narcotic if concentration of THC present in CBD products does not exceed 0.2% (w/w) [20]. Analysis of cannabinoids can be carried out using different techniques with a commonly used technique being high-performance liquid chromatography (HPLC). HPLC can be coupled to different detectors such as an ultra-violet detector or mass spectrometry detector. UV detectors are suitable for analysis of cannabinoids as these absorb strongly in the UV region of the spectrum. UV detectors are simpler to operate and cheaper to run when compared to mass spectrometry detectors [2].

This paper describes the validation of an efficient HPLC-UV method for determination of THC in oil.

METHODS

Chemicals

Standards were procured as follows; THC (0.1 mg/mL in methanol) from LoGiCal® Wesel, Germany, HPLC-grade methanol (MeOH) and HPLC-grade acetonitrile (ACN) from Honeywell Riedel-de Haën, France, anyhdrous disodium hydrogen phosphate from Carlo Erba reagents, Val-de-Reuil, France, HPLC-grade ortophosphoric acid from Fisher Chemical, Leicestershire, UK and standard ibuprofen (purity 99.7%) from Sigma-Aldrich, Darmstadt, Germany. Water was purified using a Thermo Scientific Water Smart2Pure 3LPM.

Standard solution of THC and ibuprofen
Stock solutions of THC were prepared in extra virgin olive oil (EVOO). THC standard in MeOH (0.1 mg/mL) was diluted in EVOO to a concentration of 5 μg/mL. Samples were stored in the dark at 4°C.

Ibuprofen was the internal standard (IS) selected as it shows maximum ultraviolet (UV) of absorption at 220 nm, the wavelength used for this method [17]. The use of ibuprofen as an IS has been documented in other studies [17,18]. A standard solution of 30 μg/mL of ibuprofen in MeOH was prepared.

Sample preparation for HPLC
Sample preparation was as follows: 0.6 mL MeOH were added to 0.3 mL of THC in oil with two drops of IS and vortex-mixed for 30 s, sonicated for 20 min, and centrifugated for 15 min at 6000 rpm. The upper organic layer was transferred in a centrifuge tube and placed for 2 h at −20°C. A second centrifugation step of 15 min at 6000 rpm was carried out; the organic layer was separated again, passed through 0.45μm syringe filters, and analyzed.

HPLC system and conditions
Analysis was carried out using an Agilent 1260 Infinity Series® liquid chromatography system having a quaternary pump and UV-visible detector. Chromatographic separation was achieved on an ACE® RP C_{18} column (250 mm × 4.6 mm; 5 μm particle size). The temperature of the stationary phase was set at 40°C. The mobile phase consisted of ACN/phosphate buffer (70:30 v/v), at a flow rate of 2 mL/min. The UV detector was set at 220 nm and the injection volume was 20 μL.

Method validation
The method was validated according to the International Council of Harmonization Q2 guideline [21].

Linearity
To demonstrate the linearity of the method, six different sample concentrations were prepared for the calibration curve. The stock solution of THC in EVOO stored at 4°C was left at room temperature for 15 min before use. Concentrations of 5.00 μg/mL, 2.50 μg/mL 1.25 μg/mL, 0.63 μg/mL, 0.16 μg/mL, and 0.04 μg/mL of THC in EVOO were prepared. The solvent extraction procedure was carried out for each of the concentrations and the six concentrations were analyzed. Following every sample injection, an injection of MeOH alone was made onto the column to avoid any carry-over of THC. The concentrations were analyzed in triplicates.

A calibration curve of the ratio of the AUP of THC to that of ibuprofen (both in mAU.min) against concentration of THC (in μg/mL) was plotted. The correlation coefficient to measure the strength and the direction of the linear relationship between the concentration and the AUP was determined.

A line of best fit was plotted using the values attained. In the best-case scenario, a linear relationship – y=mx+b would be attained where y= Detector response (mAU); x=Concentration of THC (μg/ml); m=Slope of straight line; and b=Intercept.

Specificity
To demonstrate the specificity of the method, a blank sample consisting of EVOO was ran in triplicate. The chromatogram obtained was compared with the chromatogram obtained when analyzing THC in EVOO and by observing both chromatograms it could be confirmed that the peak obtained was attributed to THC.

Accuracy
The accuracy of the method was demonstrated by analyzing three concentrations of THC in EVOO: 0.04, 0.63, and 5.00 μg/mL. The concentration of the sample detected in μg/mL was divided by the true concentration of the sample that was injected in μg/mL. The results obtained were multiplied by 100 to express it as a percentage. Each concentration was run in triplicates to ensure reproducibility.

Precision
The precision of the method was demonstrated by the evaluation of the means of the percentage relative standard deviation (RSD). The calculation of the coefficient of variation was carried out by dividing the standard deviation with the mean and multiplying this by 100:

$$\text{Relative standard deviation } (\text{RSD}) = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Determination of inter-day precision was carried out by injecting THC in EVOO at six different concentrations: 0.04, 0.16, 0.63, 1.25, 2.5, and 5.00 μg/mL analyzed, on 3 consecutive days.

Determination of intra-day precision was carried out by injecting THC in EVOO at six different concentrations: 0.04, 0.16, 0.63, 1.25, 2.5, and 5.00 μg/mL in triplicates, on the same day.

Stability
Determination of the stability of the method was carried out by storing three different concentrations (0.04, 0.63, and 5.00 μg/mL) of THC in EVOO at −20°C. The three concentrations were analyzed after 1 and 3 weeks in triplicates.

Limit of detection (LOD)
The LOD was the lowest concentration of the analyte of interest that gave a detector signal. The concentration of THC in EVOO was diluted consecutively and injected. The lowest concentration which gave a signal on the detector was considered to be the LOD. Analysis was carried out in triplicate.

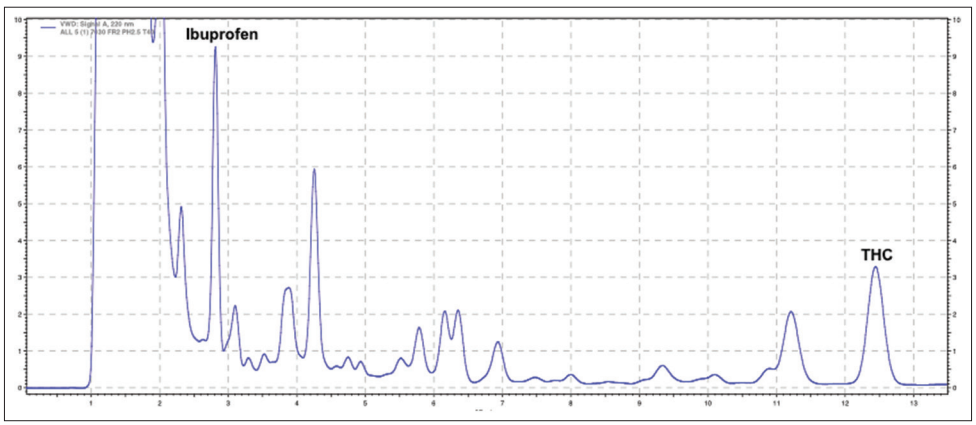

Fig. 1: Chromatogram produced using phosphate buffer (pH=2.5) and acetonitrile (30:70 v/v); detection wavelength 220 nm; flow rate 2 mL/min; and temperature at 40°C

Limit of quantification
The LOQ was the lowest concentration of the analyte of interest that gave a detector signal and could be quantified. The concentration of THC in EVOO was diluted consecutively and injected. The lowest concentration which gave a signal on the detector and could be quantified was considered to be the limit of quantification. Analysis was carried out in triplicates.

RESULTS

HPLC-UV determination of THC and ibuprofen
The HPLC-UV chromatogram for the analysis of THC and ibuprofen is shown in Fig. 1. Retention times were 2.847 and 12.707 min for ibuprofen and THC, respectively.

Linearity
The calibration curve of THC was linear ranging from 0.039 to 5.000 µg/mL. Linearity was assessed by comparing the AUP of the ratio THC/ibuprofen against six different sample concentrations of THC in EVOO.

Fig. 2 show the calibration curve obtained when solutions of 5.000 µg/mL, 2.500 µg/mL 1.250 µg/mL, 0.625 µg/mL, 0.156 µg/mL, and 0.039 µg/mL of THC were analyzed in EVOO.

Specificity
Specificity was confirmed by the absence of peaks or signals at the retention time of THC when a blank of EVOO was injected (Fig. 3).

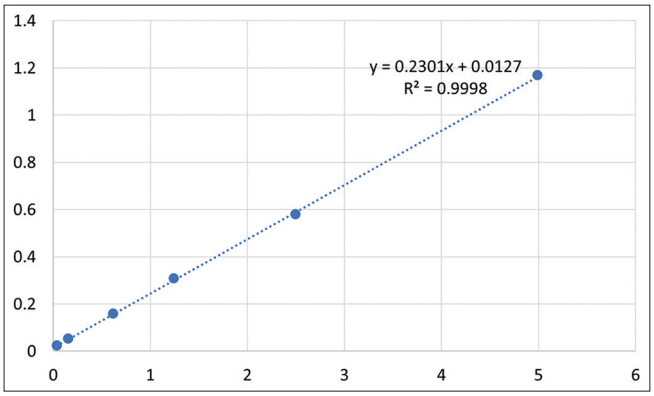

Fig. 2: Calibration curve for THC in EVOO. Plot of AUP versus concentration in µg/mL. When the six concentrations of THC in EVOO were analyzed, an r^2 = 0.9998 was obtained, indicating that the detector signal and AUP obtained is linearly proportional to the concentration of THC being analyzed in EVOO

Accuracy
Results of accuracy and recovery from THC in EVOO were acceptable (Table 1). All percentage recoveries calculated were between 93.408% and 99.959%.

Precision
The method was found to have an acceptable intraday and interday precision.

Intraday precision
The six concentrations of the THC in oil were analyzed in triplicates to calculate intraday precision of the method; the results of this are shown in Table 2.

The method shows acceptable intraday precision since all the RSD results obtained were below 4.500%.

Interday precision
The results obtained when the six concentrations of THC in oil were analyzed once a day every day on 3 different days are shown in Table 3. The method shows an acceptable interday precision since all the RSD results obtained were below 6.900%.

Stability
When three concentrations of THC in oil (5.000, 0.625, and 0.039 µg/mL) were analyzed after 1- and 3-weeks following storage at −20ºC, the chromatograms which resulted following analysis after 1 week and 3 weeks were not significantly different from the chromatograms of the analysis immediately after the sample was prepared in terms of quantities of THC.

Results for stability after 1 and 3 weeks using this method are shown in Table 4.

LOD
The lowest concentration of THC in EVOO that gave a signal but could not be quantified was 0.019 µg/ml (Fig. 4).

Limit of quantification
The lowest concentration of THC in EVOO that gave a signal and could be quantified was 0.039 µg/mL (Fig. 5).

DISCUSSION

An efficient, sensitive, and simple to use HPLC method for determination of THC in EVOO was developed and validated. EVOO was chosen as the sample matrix since it is used as a carrier oil in certain cannabis-based preparations, particularly CBD oils. An efficient extraction technique was developed for sample clean-up, effectively removing oil which is

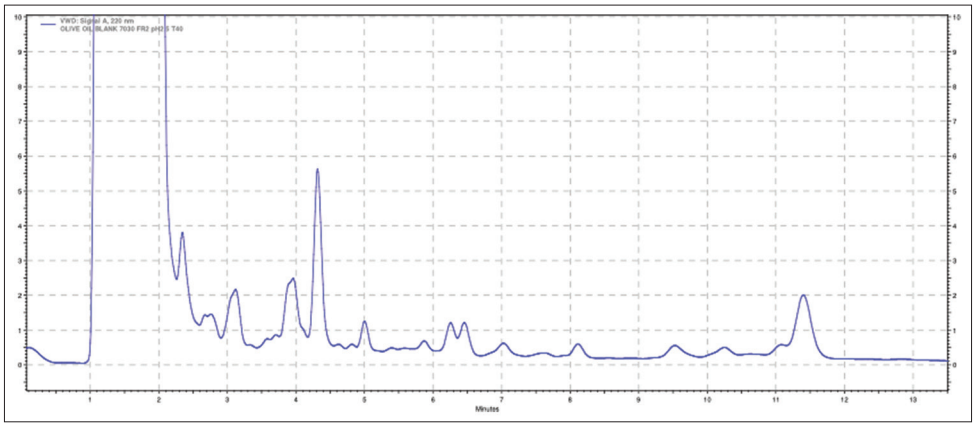

Fig. 3: Chromatogram of blank EVOO produced using phosphate buffer (pH=2.5) and acetonitrile (30:70 v/v); detection wavelength 220 nm; flow rate 2 mL/min; and temperature 40°C

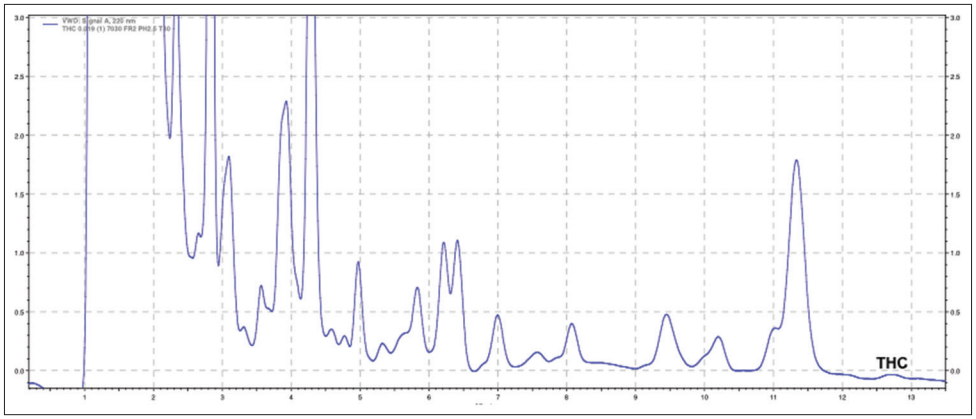

Fig. 4: Limit of detection of THC in EVOO

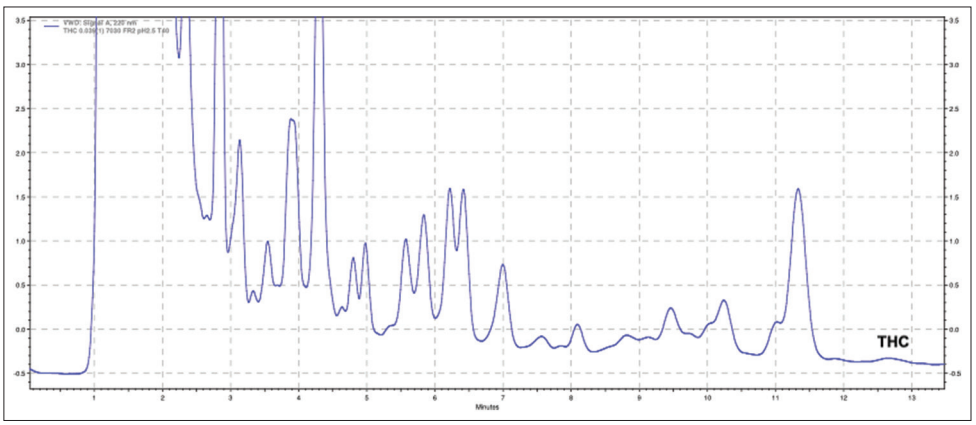

Fig. 5: Limit of quantification of THC in EVOO

Table 1: Accuracy of concentration of tetrahydrocannabinol in extra virgin olive oil

Standard concentration of THC (µg/mL)	Concentration of THC which was quantified in EVOO (µg/mL)	Percentage recovery
0.039	0.039	100.00
0.039	0.036	92.308
0.039	0.037	94.872
0.625	0.585	93.600
0.625	0.625	100.00
0.625	0.591	94.560
5.000	4.950	99.000
5.000	4.979	99.580
5.000	4.981	99.620

EVOO: Extra virgin olive oil, THC: Tetrahydrocannabinol

a viscous matrix and which can cause reproducibility problems [2]. MeOH is a commonly used extraction solvent and presents higher extraction efficacy for cannabinoids than other commonly used solvents [2]. Ethanol is also popularly used as an extraction solvent due to it having less of a negative impact on the environment but can present issues when performing ultrasonication [22]. Oil and MeOH are poorly miscible at room temperature and freezing at −20°C helps further separate oil from MeOH.

Ibuprofen has been used as an IS when analyzing cannabinoids [19]. Ibuprofen is a weak acid and has a pKa of 4.4 [23] with UV absorption maxima being in the region of 220 nm and 273 nm [19]. Ibuprofen eluted before THC and chromatographic run time was relatively short. The developed method achieved relatively low limits of

Table 2: Intra-day precision of tetrahydrocannabinol in extra virgin olive oil

Replicate number	Intra-day precision					
	Concentration THC in EVOO (µg/mL)					
	5.000	2.500	1.250	0.625	0.156	0.039
1	5.003	2.552	1.217	0.657	0.149	0.040
2	5.027	2.482	1.285	0.605	0.162	0.039
3	4.970	2.466	1.248	0.613	0.157	0.039
Mean	5.000	2.500	1.250	0.625	0.156	0.039
SD	0.029	0.045	0.034	0.028	0.007	0.001
RSD (%)	0.580	1.800	2.720	4.480	4.487	2.564

EVOO: Extra virgin olive oil, SD: Standard deviation, RSD: Relative standard deviation, THC: Tetrahydrocannabinol

Table 3: Inter-day precision of tetrahydrocannabinol in extra virgin olive oil

Day	Inter-day precision					
	Concentration THC in EVOO (µg/mL)					
	5.000	2.500	1.250	0.625	0.156	0.039
1	5.003	2.552	1.217	0.657	0.149	0.040
2	4.950	2.485	1.252	0.585	0.152	0.036
3	4.908	2.437	1.268	0.584	0.141	0.039
Mean	4.954	2.491	1.245	0.609	0.147	0.038
SD	0.047	0.058	0.026	0.042	0.005	0.002
RSD (%)	0.949	2.328	2.088	6.897	3.401	5.263

EVOO: Extra virgin olive oil, SD: Standard deviation, RSD: Relative standard deviation, THC: Tetrahydrocannabinol

Table 4: Stability of tetrahydrocannabinol in extra virgin olive oil after 1 week

Replicate number	Concentration THC in EVOO (µg/mL)		
	5.000	0.625	0.039
Stability of THC after 1 week			
1	4.967	0.563	0.035
2	4.966	0.574	0.037
3	4.973	0.576	0.037
Mean	4.969	0.571	0.036
SD	0.004	0.007	0.001
RSD	0.080	1.226	2.778
Stability of THC after 3 weeks			
1	4.958	0.575	0.036
2	5.004	0.597	0.031
3	4.973	0.576	0.032
Mean	4.978	0.583	0.033
SD	0.023	0.012	0.002
RSD	0.462	2.058	6.061

EVOO: Extra virgin olive oil, SD: Standard deviation, RSD: Relative standard deviation, THC: Tetrahydrocannabinol

quantification and detection compared to other published methods. A study carried out by Pichini *et al*., reported the limit of detection at 0.012 µg/mL, while, in this study, it was 0.019 µg/mL. Analysis carried out by Pichini *et al.*, made us of an MS/MS as a detector [24]. HPLC coupled to MS increases sensitivity of the analysis, but the use of equipment requires skilled expertise to operate and is more expensive to run [9,25,26].

CONCLUSION

The developed method for the determination and validation of THC in EVOO is reproducible, quick, and simple and has acceptable precision and accuracy. Developed and validated method for extraction and determination of THC from oil offers an option to evaluate compliance to meeting recommendations [27] of having concentrations of THC in CBD products not exceeding stated limits.

AUTHORS' CONTRIBUTIONS

Eva Tejada Rodriguez: Main researcher, author. Janis Vella Szijj: Supervisor, author, reviewer. Miriana Cachia: Researcher, laboratory support. Pauline Falzon: Laboratory support. Kersty Axisa: Researcher, laboratory support. Anthony Serracino-Inglott: Supervisor, reviewer. Lilian M. Azzopardi: Head of department, reviewer.

CONFLICTS OF INTEREST

The authors confirm that there was no conflicts of interest when conducting the study.

AUTHORS' FUNDING

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