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Cannabinoids concentration variability in cannabis olive oil galenic preparations

Chiara Carcieri[a,c]*, Cristina Tomasello[a,b]*, Marco Simiele[a,e], Amedeo De Nicolò[a], Valeria Avataneo[a], Luca Canzoneri[d], Jessica Cusato[a], Giovanni Di Perri[c,e] and Antonio D'Avolio[a,e]

[a]Laboratory of Clinical Pharmacology and Pharmacogenetics, Department of Medical Sciences, University of Turin, Turin, Italy, [b]Hospital Pharmacy, Maria Vittoria Hospital, ASL CITTA' DI TORINO, Turin, Italy, [c]Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin, Italy, [d]Pain Management Center, San Luigi Hospital, Orbassano, Turin, Italy and [e]CoQua Lab s.r.l, Academic Spin-off of University of Turin, Turin, Italy

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Correspondence
Antonio D'Avolio, ASL Citta'di Torino, Corso Svizzera 164, 10149 Turin, Italy.
E-mail: antonio.davolio@unito.it

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*Chiara Carcieri and Cristina Tomasello are equally contributed to this work.

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Abstract

Objectives Knowledge of the exact concentration of active compounds in galenic preparations is crucial to be able to ensure their quality and to properly administer the prescribed dose. Currently, the need for titration of extracts is still debated. Considering this, together with the absence of a standard preparation method, the aim of this study was to evaluate cannabinoids concentrations variability in galenic olive oil extracts, to evaluate the interlot and interlaboratory variability in the extraction yield and in the preparation composition.

Methods Two hundred and one extracts (123 (61.2%) from Bedrocan®, 54 (26.9%) from Bediol®, 11 (5.5%) from Bedrolite®, and 13 (6.5%) from mixed preparations) were analysed by liquid chromatography coupled with tandem mass spectrometry, quantifying cannabinoids (THC, CBD, THCA, CBDA and CBN) concentrations.

Key findings The RSD% of THC and CBD concentrations resulted higher than 50%. Specifically for Bedrocan®, Bediol®, Bedrolite® (5 g/50 ml), these were THC 82%, THC 53% and CBD 91%, THC 58% and CBD 59%, respectively. The median extraction yields were greater than 75% for all preparations.

Conclusions Our results highlighted a wide variability in THC and CBD concentrations that justify the need for titration and opens further questions about other pharmaceutical preparations without regulatory indication for this procedure.

Introduction

Cannabis plant contains about 565 compounds, among which 120 are cannabinoids.[1] Cannabis-based medications represent an alternative therapeutic strategy for different diseases, and they were approved in 2011 in many European countries. The study of the active molecules in *Cannabis sativa*, in particular tetrahydrocannabinol (THC), unveiled the existence of endocannabinoid system three decades ago. Tetrahydrocannabinolic acid (THCA) is the main constituent in raw cannabis, and it converts to Δ9-THC when heated over a certain temperature. To date, two cannabinoid receptors have been identified: CB1 and CB2. THC, binding to both CB receptors, is responsible for the psychoactive effects (mediated by CB1),[2] on the other hand, it also affects other targets, such as ionic channels and enzymes with potential painkiller, antiemetic, antikinetosic properties, stimulating appetite and acting as intraocular hypotensive agent.[3] Another cannabinoid molecule, cannabidiol (CBD), is not psychoactive, as it does not bind CB1 and CB2 receptors with appreciable affinity, but retains other beneficial effects of THC, listed above.[4] Currently, the literature reported evidence on cannabis medical use[5,6] highlighted effectiveness for a wide range of

diseases. In detail, cannabinoids represent a reasonable therapeutic option in the analgesia for spasticity associated with pain diseases (e.g. multiple sclerosis),[7] for chronic pain (e.g. oncologic and neuropathic pain)[8–10] resistant to NSAIDs, corticosteroids or oppioids,[10] against chemotherapy-related nausea and vomiting,[11] cachexia and anorexia in patients with cancer or AIDS[12] in glaucoma resistant to conventional therapies,[13] to reduce facial and body movements in Gilles de la Tourette Syndrome[14] and many other clinical conditions.[15–19] Cannabinoids pharmacokinetics vary on the basis of drug dose and route of administration.[20] Following oral ingestion, only 10–20% of THC enters blood flow, due to extensive hepatic metabolism and to low water solubility. CBD shows a bioavailability and oral absorption similar to THC.[21]

To date, six different varieties are available on the market, with standardized THC and CBD concentrations: Bedrocan®, Bedrobinol®, Bediol® (*C. sativa* with mean amounts of 22%; 13.5% and 6.5% for THC and <1%, <1% and 8% for CBD, respectively), Bedica® (*Cannabis indica* with 14% THC and <1% CBD), Bedrolite® (*C. sativa*, with approximately 0.4% THC and 9% CBD) and Bedropuur® (high-THC *C. indica* variety, with <1% CBD).[22] In Italy, the medical use of cannabis started in April 2007, nevertheless no official guideline for medical use of cannabis is currently available, making its use difficult in the clinical practice. Sativex®, an oromucosal spray THC/CBD based, is the only approved drug in Italy just for adult patients with multiple sclerosis not responder to other medications. To date, several galenic products are available, according to European Pharmacopeia[23]: cannabis decoction filter bags, unit dose formulation for inhalation and cannabis extracts, mainly in olive oil.

There are different methods to prepare cannabis oil: they are relatively simple and do not require particular instruments, consisting in the simple extraction of cannabinoids in olive oil,[24] but their quali-quantitative composition has been poorly studied. Therefore, the titration of active principles with specific and sensitive methodologies, such as liquid or gas chromatography coupled with mass spectrometry, is strongly needed (in Italy, it is mandatory).

Recent studies reported that cannabinoids concentrations in oil preparations vary according to temperature and time of extraction, other than highlighting a better stability of cannabinoids in oil extracts rather than in alcohol or in decoction.[25,26]

As poor standardization is currently applied to the galenic preparation of cannabis oil extracts, the evaluation of their interlot variability from different laboratories could be really useful.

In fact, poor information is currently available in the literature about the expected variability in extraction yields and cannabinoids concentration in the 'real practice' galenic oil preparations.

Therefore, the main aim of this study was the quantification of cannabinoids in different galenic oil preparations from different laboratories, each one inspired to the same base procedure[24] to evaluate the overall interlot variability in different cannabis types.

Materials and Methods

Chemicals

Olive oil (pharmaceutical grade), cannabidiol (CBD), cannabinol (CBN), cannabidiolic acid (CBDA), cannabidiol-d3 (CBD-d3), (-)-delta9-tetrahydrocannabinol (THC), (-)-delta9-tetrahydrocannabinol-d3 (THC-d3) and isopropanol LC-MS grade were purchased from Sigma–Aldrich (Milan, Italy); Tetrahydrocannabiniolic acid (THCA) was purchased from LGC (Milan, Italy). Acetonitrile LC-MS grade was purchased from VWR (Milan, Italy). HPLC grade water was produced with Elix® coupled with Synergy® UV water purification system (Merck Millipore, Milan, Italy). The samples of cannabis oil were sent to the laboratory by several Italian pharmacies for the cannabinoid titration, required by Italian law.

Chromatographic and mass spectrometric conditions

Chromatographic analysis was performed on a Shimadzu Nexera X2® LC system coupled with a LCMS-8050® tandem mass spectrometer (Shimadzu, Kyoto, Japan). Chromatographic separation was performed on an Acquity® UPLC HSS T3 column, (2.1 × 30 mm, 1.8 µm; Waters, Milan, Italy) maintained at 30 °C through the column oven.

Briefly, chromatographic separation was obtained through a gradient of mobile phases A (ACN : water 75 : 25 + 0.05% formic acid) and B (isopropanol : ACN 80 : 20 + 0.05%) at 0.4 ml/min.

The initial condition was 0% solution B, which was linearly increased to 100% over 1.5 min, this condition was maintained for 1 min, then the column was re-equilibrated to initial conditions for 1 min (total runtime 3.5 min). Autosampler was maintained at 10 °C and the injection volume was 5 µl. Data processing and system control were managed through the LabSolution® 1.0 software (Shimadzu, Kyoto, Japan).

Tandem mass spectrometric detection was carried out through electrospray ionization source set in positive ionization mode (ESI+).

Ionization conditions were optimized by directly injecting solutions containing each single drug, bypassing

the column (Fast Injection Analysis, FIA): the optimization process was automatically performed using the 'optimization for method' function of the chromatographic system.

The optimized instrument parameters were as follows: capillary voltage 4 kV, nebulizing gas flow 3 l/min, drying gas flow 10 l/min, heating gas flow 10 l/min, interface temperature 300 °C, heating block temperature 400 °C, desolvation line temperature 250 °C.

The ion monitoring was performed in multiple reaction monitoring (MRM) mode, with the mass transitions and collision energies (CE) here reported as follows: CBD 315.10 → 192.90, CE 22; CBD-d3 318.50 → 196.10, CE 24; THC 315.25 → 193.20, CE 24; THC-d3 318.20 → 196.00, CE 26; CBDA 359.10 → 341.25, CE 16; THCA 359.15 → 341.05, CE 18; CBN 311.10 → 223.00, CE 22.

Stock solutions, standards and quality controls

All cannabinoids stock solutions were acquired at a concentration of 1 mg/ml in acetonitrile or methanol and stored at −80 °C or −20 °C, as indicated by datasheets.

CBD-d3 and THC-d3 were at concentration of 0.1 mg/ml in methanol and stored at −20 °C.

Internal standard working solution (IS) was made with CBD-d3 and THC-d3 (both at 0.2 µg/ml) in water : methanol (50 : 50 v : v) at the time of the analysis.

Matrix olive oil was obtained by mixing 5 µl of olive oil in 99.995 ml of isopropanol [1 : 20 000 diluition].

The highest standard sample (STD 6) and the three quality controls, high (QC-H), medium (QC-M) and low (QC-L), were prepared by spiking matrix olive oil with stock solutions and then stored at −20 °C. The calibration ranges for all compounds were from 1250 ng/ml (STD 6) to 5 ng/ml (STD 1) and QCs concentrations were 1000 ng/ml (QC-H), 500 ng/ml (QC-M), 50 ng/ml (QC-L) for all analytes.

Sample preparation

Cannabis oil samples were stored at −20 °C protected from light: all samples were analysed within 1 week.

The vast majority of pharmacies reported to follow the protocol described by Romano and Hazekamp[24] for preparing cannabis oil. Briefly, this protocol consists in the addition of cannabis to olive oil (1 g in 10 ml) and then the heating in a water bath at 100 °C for 2 h. Before filtration, the oil was left to cool off.

Nevertheless, this procedure does not represent a real standard, so some changes have been allowed in different laboratories, based on the technical experience and the available instrumentation.

Specifically, the vast majority of samples underwent a preheating step, before extraction of APIs in olive oil, at a range of temperature between 120–140 °C in a oven for 30 min, to achieve a nearly complete decarboxylation of THCA and CBDA to THC and CBD, respectively.[27]

Concerning titration procedure, the STD 6, QC and cannabis oil samples were thawed at the time of the analysis at room temperature for 15 min. Lower STDs were freshly prepared by serial 1 : 2 dilution from STD 6 to STD 1 with blank matrix olive oil, to obtain six different spiked concentrations plus a blank sample (STD 0). At every analytical session, QC samples were analysed in double replicate.

Fifty µl of cannabis olive oil samples were 20 000-fold diluted in eppendorf with isopropanol in two steps, vortex mixing each time for 10 s. One hundred µl of diluted samples, STDs and QCs were added to 100 µl of internal standard working solution directly in glass vials, vortex mixed for 10 s and injected in the LC system.

Statistical analysis

Differences in extraction yields between different cannabis types have been tested with Kruskal–Wallis test (for the overall comparison) and a *post hoc* Dunn's test to identify individual groups with higher or lower means. Differences in the variability in extraction yields between different cannabis types have been tested through the Levene's test for homogeneity of variances. Comparisons have been performed on groups with enough sample size to allow a correct testing (only Bedrocan®, Bediol® and Bedrolite® based preparations at 5 g/50 ml have been included).

Results

Two hundred and one cannabis oil samples from 10 different pharmacies were collected and their main cannabinoids levels quantified by LC-MS system. Considering the dilution factor of 20 000, the chromatographic method covered the range for all cannabinoids in olive oil extracts from 25 to 0.1 mg/ml. The lower limit of quantification (LLOQ) was 5 ng/ml for each compound, corresponding to 0.1 mg/ml in cannabis olive oil preparation. One hundred and twenty-three samples (61.2%) were prepared with 5 g Bedrocan® in 50 ml of olive oil, 54 samples (26.9%) with 5 g Bediol® in 50 ml of olive oil and 11 samples (5.5%) with 5 g Bedrolite® in 50 ml of olive oil. The remaining 13 samples (6.5%) are different preparations made with lower or higher amount of cannabis (Bedrocan®, Bediol®, Bedrolite®) in 50 ml of olive oil. This latter kind of preparations was not taken into account for statistical analysis. A summary of the distribution of cannabis olive oil extracts, classified by type of cannabis and pharmacy, is reported in Table 1.

Table 1 Distribution of analysed extracts classified by pharmacy and cannabis strain

Pharm.	No. samples	No. Bedrocan 5 g/50 ml	No. Bediol 5 g/50 ml	No. Bedrolite 5 g/50 ml	No. other
Pharm-A	71 (35.3%)	39	16	8	8
Pharm-B	2 (1.0%)	2	0	0	0
Pharm-C	85 (42.3%)	59	24	2	0
Pharm-D	17 (8.5%)	12	5	0	0
Pharm-E	8 (4.0%)	5	2	0	1
Pharm-F	5 (2.5%)	0	4	0	1
Pharm-G	6 (3.0%)	4	1	0	1
Pharm-H	1 (0.5%)	1	0	0	0
Pharm-J	5 (2.5%)	1	1	1	2
Pharm-K	1 (0.5%)	0	1	0	0
Total	201	123 (61.2%)	54 (26.9%)	11 (5.5%)	13 (6.5%)

Table 2 Mean, standard deviation, minimum, maximum, median and 1°–3° quartiles of CBD, THC, CBDA, THCA and CBN concentrations in cannabis olive oil extracts from Bedrocan, Bediol, Bedrolite at 5 g/50 ml

	Mean (mg/ml)	SD (mg/ml)	Min (mg/ml)	Max (mg/ml)	Median (mg/ml)	1°Q (mg/ml)	3°Q (mg/ml)
Bedrocan 5 g/50 ml (No 123)							
CBD	<LOQ	0.032	<LOQ	0.171	<LOQ	<LOQ	<LOQ
THC	9.376	7.684	1.401	23.850	4.784	3.466	18.280
CBDA	<LOQ	0.047	<LOQ	0.497	<LOQ	<LOQ	<LOQ
THCA	13.222	9.845	<LOQ	32.017	15.471	1.598	21.296
CBN	<LOQ	0.224	<LOQ	2.457	<LOQ	<LOQ	<LOQ
Bediol 5 g/50 ml (No 54)							
CBD	3.030	2.753	0.660	8.297	1.428	1.047	6.263
THC	3.300	1.763	1.358	6.596	2.415	2.010	5.244
CBDA	5.989	3.526	<LOQ	10.837	6.885	1.636	8.833
THCA	4.029	3.017	<LOQ	9.383	4.490	0.091	6.615
CBN	<LOQ	0.074	<LOQ	0.529	<LOQ	<LOQ	<LOQ
Bedrolite 5 g/50 ml (No 11)							
CBD	4.901	2.911	0.339	7.540	6.171	3.041	6.829
THC	0.457	0.266	0.016	0.837	0.490	0.310	0.628
CBDA	2.919	3.492	0.606	9.742	0.917	0.754	3.715
THCA	0.103	0.194	<LOQ	0.637	<LOQ	<LOQ	0.114
CBN	<LOQ	–	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Cannabinoids concentration in olive oil

Mean and median concentrations of cannabinoids (THC, CBD, THCA, CBDA and CBN), together with their standard deviations and interquartile ranges, divided on the basis of the cannabis strain (Bedrocan®, Bediol®, Bedrolite® 5 g/50 ml), are reported in Table 2. THC and CBD concentrations distribution in Bedrocan®, Bediol®, Bedrolite® preparations is further represented in Figure 1.

The RSD% of THC and CBD concentrations for Bedrocan®, Bediol®, Bedrolite® 5 g/50 ml were THC 82%, THC 53% and CBD 91%, THC 58% and CBD 59%, respectively.

The extraction yields for total THC and CBD were calculated considering the sum of THC plus 0.877*THCA concentrations (0.877 is the mass ratio of the two compounds) and CBD plus 0.877*CBDA and comparing the results to nominal concentrations of total THC and CBD declared by the manufacturer (ratio between the sum and nominal value expressed as percentage).[22]

The median extraction rates for Bedrocan®, Bediol® and Bedrolite® 5 g/ml were 90% THC/THCA, ND CBD/CBDA; 102% THC/THCA, 100% CBD/CBDA; 108% THC/THCA, 75% CBD/CBDA, respectively. These differences resulted statistically significant, with a higher THC extraction yield for Bediol than Bedrocan ($P < 0.001$) and higher extraction yield of CBD for Bediol than Bedrolite ($P < 0.001$).

Finally, other than differences in the median extraction yields, statistically significant dishomogeneity in the variance of extraction yield of THC was observed between different cannabis types ($P = 0.025$), highlighting a significantly wider variability of THC extraction yield in Bedrocan-® based preparations.

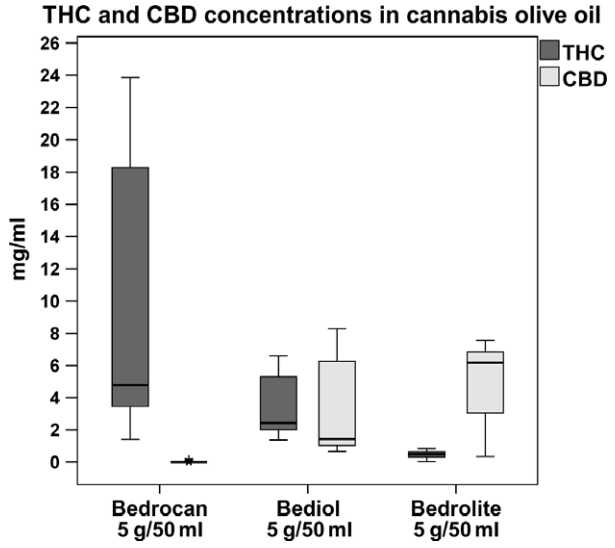

Figure 1 Distribution of tetrahydrocannabinol (THC) and cannabidiol (CBD) concentrations among 188 cannabis olive oil extracts.

Discussion

Different varieties of *medicinal-grade* cannabis are available from the Dutch Ministry of Health for many international pharmacies as a pharmaceutical raw material. Each medicinal cannabis variety, cultivated indoors according to guidelines from Good Agricultural Practice (GAP),[22] has a standardized profile of Active Pharmaceutical Ingredients (APIs) and levels of contaminants safe for human use.

In our samples, Bedrocan® results the most widely used variety for galenic preparation of medicinal cannabis-based drugs (62.1% of total samples). Cannabis oil concentration of 0.1 g/ml resulted the most commonly used in our sample, following the extraction method reported by Romano and Hazekamp.[24] Only pharmaceutical grade olive oil was used, according to Official Italian Pharmacopoeia.[28]

This work highlighted very variable cannabinoids concentrations, both inter- and intralaboratory (and therefore the same operating procedure), also using the same cannabis strains and inflorescences-oil ratio (1 : 10).

As observed in this paper, the interlot variability in the extraction yields resulted higher in Bedrocan-® based preparations, and, on the other hand, that Bediol-® based preparations show significantly higher extraction yields both for THC (compared to Bedrocan®) and CBD (compared to Bedrolite®). This phenomenon could be associated with the lower particle size of the pieces of Bediol® inflorescences, which could result in a slightly higher extraction yield.

Preparation protocol for APIs extraction sometimes included a preheating step, to enhance the yield of decarboxylation of acid-APIs (THCA, CBDA): this optional step, and the lack of a standardized temperature and time, greatly contributes to a possibly clinically relevant interlot variability in APIs concentrations (Figure 1), as well as to the variability in THCA/THC/CBN or CBDA/CBD ratios.[29,30]

The only report about cannabinoids concentrations in olive oil extracts was recently published by Citti *et al.*[26]: this study reported the concentrations of five cannabinoids (CBD, THC, THCA, CBDA and CBN) in four samples of Bediol® oil extracts. The extraction rates reported for total CBD and THC (intended as the sum of neutral and acid form for each cannabinoid) were slightly lower than our data. Nevertheless, the data from that study were obtained on samples prepared with a slightly different protocol. In fact, there is not a general agreement on which is the best temperature and time for activation of acid-API to THC and CBD and pharmacies applied their own methods following own experiences (anyway remaining in a range between 120–140 °C). On the other hand, the avoidance of the preheating step could be beneficial to ensure the integrity of the phytocomplex, preserving most of volatile compounds such as terpenes.

Going further, despite the efforts in standardizing cannabinoids concentrations in inflorescence, their nominal values are approximate, as admitted by the manufacturer.[22] In fact, these concentrations can change during the years (e.g. THC in Bedrocan® from 19% to 22%), thus contributing to fluctuation in cannabinoids concentration within the extracts.

Nevertheless, these fluctuations in cannabinoid concentrations in the inflorescences (between 5–10%) are not capable to explain alone the observed wide variability in our study.

Taken together, these critical issues in standardizing cannabis oil preparations highlight the need of a postpreparative titration of APIs. On the other hand, these suggest the future need for development standardized medicinal products, with known amount of APIs.

Conclusion

Our study proves the variability in galenic preparations of medicinal cannabis oil, justifying the need to provide concentrations data for each preparation. The exact knowledge of composition of the prepared medicinal products is crucial for physicians, to be able to properly adapt the prescribed dose to the available preparation, and for pharmacists, to be able to evaluate and ensure the quality of galenic preparations; besides, it is widely believed that other components (as terpenes) might play an important role in the medicinal properties of cannabis.[29] Further studies of correlation between APIs ratio

and outcomes/toxic effects using therapeutic drug monitoring could be useful to improve the clinical management of patients, to ensure safety and efficacy of galenic formulation and, in the perspective of personalized therapy, to be able to choose the best preparation for each patient. Limitations of this study are represented by the small sample size and by the limited possibility to compare different protocols/manuality, due to the retrospective approach (the majority of samples were from only two pharmacies).

In conclusion, our data justify the need to obtain concentrations data for each oil preparation and opens further questions about other pharmaceutical preparations without regulatory indication for titration, other than giving a rationale for the development of new preparations containing standard amounts of cannabinoids.

Further studies are needed to evaluate the impact of differences in galenic preparations on cannabinoids pharmacokinetics and clinical outcomes.

Declarations

Conflict of interest

Dr. Marco Simiele, Prof. Antonio D'Avolio and Prof. Giovanni Di Perri are co-founders of the academic spin-off of University of Turin CoQua Lab. The spin-off is a R&D laboratory and contributed to developed the chromatographic method for quantify cannabinoids in cannabis olive oil respecting the Italian law in this field (DGR no. 11, 15th June 2015. 'Therapeutic use of cannabis and cannabinoids active ingredients'). CoQua Lab manages the private analysis required from pharmacies for the titration of cannabis olive oil extracts.

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