Titration of galenic preparations of cannabis oil: the experience of the Levante Ligure reference laboratory

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ABSTRACT

Introduction: the oily galenic preparations are produced in Liguria by the hospital pharmacies of the ASL 2, ASL 3, ASL 4 and by the IRCCS Istituto Giannina Gaslini. In Italy, the titration of the active ingredients is required for each batch of cannabis oil produced because the pharmacological activity is based on the content of carboxylated and decarboxylated cannabinoids in the final product. Different methods for galenic preparations are available; the absence of standardization seems to be at the basis of the important variability in the concentration of the active ingredients. This retrospective study is aimed to evaluate the variability of the concentrations of the active ingredients in the galenic preparations prepared in Liguria.
Methods: the study is based on the evaluation of four preparations for a total of 225 samples. For the titration liquid chromatography coupled to triple quadrupole mass spectrometry was used, to allow the determination of the decarboxylated and acid forms of the active ingredients. An indicative value of the decarboxylation yield was then calculated.
Results: the variability of the concentrations of the four active ingredients was similar within the different batches; the variability was rather linked to the hospital pharmacy from which they came. A clear difference emerged in the decarboxylation yield between ASL 2 and the pharmacies of ASL 3 and ASL 4.
Discussion: the verified differences remain constant in all the samples, demonstrating a systematic error due to the extraction procedure implemented by the pharmacies. These issues could be overcome through better adherence to standard extraction procedures or by the use of known cannabis-based products commercially available.

Key words: *cannabis oil, galenic preparation, medical cannabis*

INTRODUCTION

Cannabinoids or cannabinols are chemical substances that share the ability to interact with cannabinoid receptors and can be presented in three forms: endocannabinoids (lipids derived from arachidonic acid), synthetic cannabinoids (synthesized within chemical-pharmaceutical laboratories) and phytocannabinoids, i.e. the chemical compounds present in the *Cannabis sativa* plant (1). Phytocannabinoids include hundreds of compounds capable of interacting to different extents with the endogenous cannabinoid receptors CB1 and CB2 (2). The two most relevant molecules for their therapeutic effects are tetrahydrocannabinol (THC) and cannabidiol (CBD). These two molecules are present inside the inflorescence of *Cannabis sativa* in the form of their acid precursors: tetrahydrocannabinolic acid (THC-A) and cannabidiolic acid (CBD-A) which however do not show psychotropic and therapeutic effects (3-4). THC has a predominantly psychotropic activity while CBD has analgesic and antioxidant activity, and is able to modulate the action of THC on a brain level, prolonging its duration and mitigating its side effects (5-7). Compared to synthetic drugs with the same therapeutic indication, the synergy between THC and CBD with all the other components of the phytocomplex is at the basis of the greater effectiveness and lower incidence of side effects of these medicinal extracts (8-9).

By Ministerial Decree of 9 November 2015, patients who suffer from symptoms linked to certain pathologies, for which benefits have been demonstrated, can have access to therapeutic cannabis, including chronic pain, spasticity, nausea and vomiting and lack of appetite caused by anorexia or by cancer (10-12). The dosage of these extracts, however, is quite variable, as it depends on numerous factors, especially related to patient being treated (e.g. age, weight, nature of the pathology, severity of the symptoms, concomitant therapies).

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Last but not least, the route of administration must be considered; in fact these preparations can be administered orally through oils or decoctions, or by inhalation with the use of specific vaporizers (13-14). The titration of the active ingredients is required for each batch of product because the pharmacological activity is based on the content of carboxylated and decarboxylated cannabinoids present in the final product, the decoctions and cannabis oil have shown different pharmacokinetic properties and clinical consequences on the subject under treatment (15). In Italy some types of preparations from Holland are available with standardized concentrations of the active ingredients such as Bedrocan (THC 22% and CBD <1%), Bedrobinol (THC 13.5% and CBD <1%), Bediol (THC 6% and CBD 8%), Bedrolite (THC <0.4% and CBD 9%) and Bedica (THC 14% and CBD <1%). Since the end of 2016, cannabis produced in Italy by the Military Pharmaceutical Institute of Florence has been made available (it is called FM2 with THC 5-8% and CBD 7.5-12%), and from 2018 it can also be obtained from products imported from the Canadian company PEDANIOS/AURORA which won the ministerial tender. In Liguria Region for galenic preparations Bedrocan, Bediol and the national product Cannabis Flos FM2 are mainly used (16).

In the Liguria region, the number of patients being treated with cannabis has been estimated at over 1 000, mainly with oily galenic preparations. These preparations are produced by the hospital pharmacies of the ASL2, ASL3, ASL4 and IRCCS Gaslini in compliance with the -Technical standards approved by the Ligurian Health Agency (A.Li.Sa) after being authorized (resolution n.78 of 2018) (16). Various methods for preparing galenic preparations are reported in the literature; however, the absence of standardization seems to be at the basis of the considerable variability in the concentration of the active ingredients (17-18). In Liguria, two reference laboratories are in charge of the titration procedures of galenic preparations: the toxicology laboratory of Sarzana - ASL 5 and the laboratory of the IRCCS Giannina Gaslini Institute (Genova). In 2019, the laboratories developed a management protocol to standardize the titration procedure and harmonize the produced results. In fact, comparative evaluations of the results obtained through statistical analysis were carried out and this made it possible to draw up standard operating procedures (19). This retrospective study is aimed to evaluate the variability of the concentrations of the active ingredients in the galenic preparations prepared by the various pharmacies in Liguria region.

METHODS

Out of 300 samples analyzed by our laboratory (from March 2020 to October 2023), the evaluation was focused on four preparations (Bedrocan, Pedanios 22/1, Bediol and FM2), for a total of 225 samples prepared by the three Ligurian hospital pharmacies identified for these preparation: ASL 2, ASL 3, ASL 4 (Table 1).

The choice of cannabis varieties to perform the evaluation is given by the percentage of the two active ingredients on which the titration is carried out (and on their acid forms). Actually, regarding the Bedrocan and Pedanios 22/1 varieties, the total THC content is estimated around 22%, while a total CBD content is estimated <1%. For the Bediol and FM2 varieties, however, the quantity of THC and CBD present in the inflorescence is similar and is around 8%. The three hospital pharmacies use the Romano Hazekamp extraction method (20). For the titration of the galenic preparations liquid chromatography coupled to triple quadrupole mass spectrometry have been used, to allow the determination of the decarboxylated and acid forms of the active ingredients (THC, CBD, THC-A and CBD-A respectively) (21). The validation of the analytical method was carried out following the European Medicines Agency (EMA) guidelines for the validation of bioanalytical methods (22). The instrument used is Agilent 6430 Triple Quad coupled to Agilent 1200 series HPLC. The two laboratories participate to the External Quality Assessment (EQA) of the Istituto Superiore di Sanità.

Table 1
Number of samples analyzed according to the type of inflorescence and sites of preparation

Inflorescence	ASL 2	ASL 3	ASL 4	Total
Pedanios 22/1 (18)	6	2	10	18
Bedocran (87)	23	14	50	87
Bediol (103)	63	15	25	103
FM2 (17)	4	4	9	17
Total	96	35	94	225

RESULTS

Assuming that the totality of the active ingredients extractable from the inflorescence is given by the sum of the active ingredients themselves found in the titration, we calculated an indicative value of the decarboxylation yield. The average concentration values of the four analytes (THC, CBD, THC-A, CBD-A) are presented in Tables 2-5, according to the type of inflorescence and to the site of preparation.

Table 2 shows the results about Pedanios 22/1. The results for the for the Bedrocan preparations are shown in Table 3, were the average concentrations of the active ingredients are presented. Table 4 shows the average concentration value obtained for the Bediol variety. Finally, the results obtained for the FM2 inflorescence are reported in Table 5. In Tables 2 to 5, the great variability of the measured concentrations between the different sites of the samples origin is evident.

Table 6 presents the percentage of the decarboxylated THC in Bedrocan and Pedanios 22/1, which contain a high percentage of the this active ingredient , according to the origin of the samples. The results show once again the important variability related to the site of sample production.

The same observation applies to the percentages of decarboxylation in Bediol and FM2 which contain both THC and CBD in relevant percentages. The results from ASL 3 ASL 4 are quite similar but very different from those of ASL 2.

Table 2
Average concentration values (SD) found for Pedanios 22/1 (18 samples)

	Pedanios 22/1			
	THC (g/L)	CBD (g/L)	THC-A (g/L)	CBD-A (g/L)
ASL 2 (n=6)	14.2 (4.26)	0.09 (0.08)	7.70 (6.49)	0.13 (0.12)
ASL 3 (n=2)	7.69 (4.62)	0.04 (0.04)	18.23 (7.04)	0.07 (0.06)
ASL 4 (n=10)	5.31 (1.49)	0.13 (0.07)	24.02 (9.96)	0.13 (0.09)

THC, tetrahydrocannabinol; CBD, cannabidiol; THC-A, tetrahydrocannabinolic acid; CBD-A, cannabidiolic acid.

Table 3
Average concentration values (SD) found for Bedrocan (87 samples)

	Bedrocan			
	THC (g/L)	CBD (g/L)	THC-A (g/L)	CBD-A (g/L)
ASL 2 (n=23)	12.10 (2.89)	0.11 (0.06)	6.26 (4.40)	0.11 (0.08)
ASL 3 (n=14)	7.78 (2.54)	0.09 (0.07)	11.52 (4.71)	0.10 (0.09)
ASL 4 (n=50)	8.09 (2.54)	0.06 (0.05)	23.17 (6.04)	0.08 (0.08)

THC, tetrahydrocannabinol; CBD, cannabidiol; THC-A, tetrahydrocannabinolic acid; CBD-A, cannabidiolic acid.

Table 4
Average concentration values (SD) found for Bediol (103 samples)

	Bediol			
	THC (g/L)	CBD (g/L)	THC-A (g/L)	CBD-A (g/L)
ASL2 (n=63)	3.79 (1.09)	3.07 (1.24)	1.51 (0.94)	4.39 (1.67)
ASL3 (n=15)	2.69 (0.67)	1.71 (0.59)	3.49 (1.54)	7.30 (1.59)
ASL4 (n=25)	3.19 (1.37)	1.76 (0.82)	3.93 (1.66)	8.12 (2.40)

THC, tetrahydrocannabinol; CBD, cannabidiol; THC-A, tetrahydrocannabinolic acid; CBD-A, cannabidiolic acid.

Table 5
Average concentration values (SD) found for FM2 (17 samples)

	FM2			
	THC (g/L)	CBD (g/L)	THC-A (g/L)	CBD-A (g/L)
ASL 2 (n=4)	6.12 (0.59)	6.69 (1.89)	0.30 (0.25)	2.17 (1.32)
ASL 3 (n=4)	3.08(0.98)	1.86 (0.49)	3.94 (0.89)	9.25 (1.62)
ASL 4 (n=9)	4.51 (1.79)	2.66 (1.56)	5.28 (2.08)	10.52 (3.34)

THC, tetrahydrocannabinol; CBD, cannabidiol; THC-A, tetrahydrocannabinolic acid; CBD-A, cannabidiolic acid.

DISCUSSION AND CONCLUSION

The data obtained in the study highlight that, for the same type of inflorescence, the variability of the concentrations of the four active ingredients is similar within the different batches, but rather dependent from the variation of the hospital pharmacy from which they came. A clear difference emerged in the decarboxylation yield between ASL2 and the pharmacies of ASL 3 and ASL 4.

Regarding the type of inflorescence with a high percentage of THC (Pedanios 22/1 and Bedrocan), the percentage difference in yield of titrated THC between Pharmacy ASL 2 and ASL 3 is equal to 31% while the difference between ASL 2 and ASL 4 is 42%. Even regarding the Bediol and FM2 inflorescences, where both THC and CBD are taken into consideration, the difference between the samples coming from the ASL 2 pharmacy and those coming from the other two hospital pharmacies remains evident, in this case the average difference it is comparable and is around 40%.

Table 6
Percentage (mean ad SD) of decarboxylated THC for Bedrocan and Pedanios 22/1.

	%THC decarboxylated	
	Bedrocan	Pedanios 22/1
ASL 2	67.8 (19.6)	67.3 (14.3)
ASL 3	43.1 (20.9)	30.6 (20.7)
ASL 4	26.3 (8.7)	24.7 (5.1)

Bedrocan (87 sample: 23 for ASL 2, 14 for ASL3, 50 for ASL 4)
Pedanios 22/1 (18 samples: 6 for ASL 2, 2 for ASL 3, 10 for ASL 4.
THC, tetrahydrocannabinol.

Table 7
Percentage (mean and SD) of decarboxylated THC and CBD for FM2 and Bediol.

	%THC decarboxylated	
	FM2	BEDIOL
ASL 2	95.2 (4.0)	71.4 (16.9)
ASL 3	43.6 (7.8)	45.1 (13.1)
ASL 4	45.7 (17.8)	45.8 (16.3)
	%CBD decarboxylated	
	FM2	Bediol
ASL 2	74.7 (16.1)	42.0 (17.5)
ASL 3	16.7 (3.4)	19.3 (6.9)
ASL 4	18.8 (7.8)	18.1 (7.9)

FM2 (17 samples: 4 for ASL 2 and ASL 3, 9 for ASL 4)
Bediol (103 samples: 63 for ASL 2, 15 for ASL 3 and 25 for ASL 4)
THC, tetrahydrocannabinol; CBD, cannabidiol;

From our results we can see how this difference remains constant in all the samples, demonstrating the presence of a systematic error due to the extraction procedure implemented by the pharmacies. The lack of standardization of galenic preparations, despite the same method applied, therefore remains one of the critical issues that emerged in the choice of this type of preparation. This makes difficult both the therapeutic dosage for the prescribing doctor and the clinical monitoring of the patient undergoing therapy, with risks linked to possible side effects that could appear in the event of dosage increases. These critical issues could be overcome through better adherence to standard extract preparation procedures (20) or by the use of known cannabis-based products marketed by the pharmaceutical industry (authorized by AIFA, the Italian Medicines Agency). Those products are available in already standardized concentrations of CBD and THC, and can significantly facilitate the work of the pharmacist who must prepare balanced and personalized oil preparations for the needs of individual patients.

CONFLCIT OF INTEREST

None

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