Letter to the Editor

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Is “light cannabis” really light? Determination of cannabinoids content in commercial products

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To the Editor,

In the recent past, both the European Regulations No. 1307/2013 [1] and No. 2017/1155 [2] and the Italian law No. 242/2016 [3] have allowed the cultivation of cannabis plant varieties containing up to 0.2% (with a tolerance of up to 0.6%) of the psychotropic cannabinoid Δ -9 tetrahydrocannabinol (THC) with no indication on permitted percentage of the other compounds, first of all being non-addictive psychoactive cannabidiol (CBD). The free cultivation of this cannabis variety has been intended only for: food and cosmetics; fiber, hemp, powders, wood chips, oils or fuels, material intended for the practice of green manure for bioengineering, for phytoremediation of polluted sites; crops dedicated to teaching and research by public or private institutions, and finally crops for nursery gardening [3].

Nevertheless, web forums for cannabis consumers explain how to consume this so-called “light cannabis” buying it in hemp shops which sell the product as “perfume for the environment” or “collector’s inflorescences”. Actually, the product is usually smoked for the expected subjective effects mainly due to the presence of the anxiolytic and myorelaxant CBD [4–7]. Indeed, while

the THC content in light cannabis has to be within 0.2%, it has been shown that CBD content is highly variable ranging from around 2 or 3% up to 40% [4].

The detection of THC, CBD and other minor cannabinoids in light cannabis has become crucial to associate their presence in the products with eventual side effects and health risks of these products as the possibility of a positivity to cannabis screening test for legal purposes [4].

In this concern, it has to be remembered that THC and CBD are present in the cannabis plant together with their carboxylated precursors tetrahydrocannabinolic acid A (THCA-A) and cannabidiolic acid (CBDA), whose percentage with respect to the decarboxylated substances can vary as a function of the plant tissue-type, age, variety, growth conditions (nutrition, humidity and light levels), harvest time and storage conditions [8]. Although acidic precursors do not show psychotropic effects, when cannabis is smoked they are immediately converted into decarboxylated THC and CBD, increasing the amount of these latter compounds in the ingested product with consequent intensified pharmacological effects.

While liquid chromatography coupled to mass spectrometry is able to distinguish and individually measure THC, CBD and their acid precursors, THCA and CBDA [9], this is not entirely possible when employing gas chromatography-mass spectrometry [10]. Indeed, in this last case, the high temperature of both the injector and detector lead to decarboxylation of acidic precursors, especially when not previously derivatized but, in any case, a complete derivatization yield is difficult to obtain [10] (a maximum of 70% is reported) and thus quantification is less reliable.

For this reason, it has been questioned that, as the determination of the cannabinoid acidic precursors is not mandatory nor often performed for cannabis products, the total consumed THC and CBD content could be underestimated, with consequences on subjective effects on the one hand and legality of their sale on the other.

To demonstrate that light cannabis products really contain total THC (including the portion of acidic precursor THCA-A) at 0.2% and eventually up to 0.6% and to monitor the variable content of total CBD, we determined THC,

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CBD and their acid precursors in 12 different light cannabis products consisting of dried flowering tops purchased from hemp stores in five different Italian cities using, for the chromatographic separation and condition, previously validated by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) [4, 9]. Only in five out of the 12 products (all cannabis *Sativa L.* flowering tops), THC and CBD percentages were reported on the label.

In this way, it has been possible to compare UHPLC/MS-MS quantification of THC, CBD and acidic precursors with GC-MS measurement of total THC and CBD coming also from decarboxylation of acidic precursors.

For both analytical methodologies, cannabinoids were extracted in triplicates with the same extraction procedure. Briefly, to 6-mg amount of each product was added 2 mL of cyclohexane, and the tubes were then sonicated for 15 min and centrifuged for 5 min at 3500 *g*. The organic layer was evaporated to dryness at 40 °C under a nitrogen stream. In case of GC-MS analysis, the dried samples were re-suspended in 10 mL of ethyl acetate before 1 µL injection. In case of UHPLC-MS/MS analysis, the dried samples were re-suspended in 10 mL 0.1% formic acid in water before 5 µL injection.

Cannabinoid concentrations by UHPLC-MS/MS and GC-MS are presented in Table 1 as mean and standard deviation of three replicates for each methodology.

The obtained results indicate that:

- Total concentration of THC and CBD by UHPLC-MS/MS obtained by summing decarboxylated analytes and

acidic precursors (%THC tot: %THC + [0.877 × %THCA-A] and %CBD tot: %CBD + [0.877 × %CBDA]) was not statistically different (by Student's *t* test for paired data, *p* = 0.39) from values obtained by GC-MS.

- In the examined products, total THC content was under 0.2% in four cases and under 0.4% in the remaining eight, while CBD content ranged from 2.00 to 8.63% when examined by GC-MS and from 2.20 to 10.00% in case of UHPLC-MS/MS analysis. Concerning the latter observation on CBD content, light cannabis is gaining importance especially for the presence of the non-addictive psychoactive CBD acting as a myorelaxant, as an alleged sleep inducer and for its calming effects, but it has to be reminded that CBD is not health risk-free and it causes serious drowsiness [11].
- In case of light cannabis products, which reported cannabinoid percentage in the label, obtained results were always higher than those reported by the label in case of THC and for the majority of cases lower than those reported for CBD. Nevertheless, in case of THC, legality was anyway respected as THC never exceeded the value of 0.4%.

In conclusion, our study showed that all the examined samples from light cannabis products being sold in Italy complied with the national law: THC content was less than 0.2% (0.6% is tolerated), and THCA-A content was marginal. As previously demonstrated [4–6], CBD content was variable and unrelated to that of THC. UHPLC-MS/MS and GC-MS methods have proven suitable for accurately and precisely measuring cannabinoid

Table 1: THC, CBD and acidic precursors concentration (mean ± SD of triplicates for both methodologies) in “light cannabis” products by UHPLC-MS/MS and GC-MS.

Product	THC declared in the label (% W/W)	UHPLC-MS/MS			GC-MS	CBD declared in the label (% W/W) ^a	UHPLC-MS/MS			GC-MS
		THC (% W/W)	THCA-A (% W/W)	THC _{tot} (% W/W)			CBD (% W/W)	CBDA (% W/W) ^a	CBD _{tot} (% W/W) ^a	
1	ND	0.02 ± 0.01	0.03 ± 0.03	0.04 ± 0.02	0.10 ± 0.04	>3.8	1.18 ± 0.14	0.94 ± 0.06	2.00 ± 0.10	2.2 ± 0.12
2	ND	0.05 ± 0.00	0.01 ± 0.01	0.06 ± 0.01	0.10 ± 0.03	>4.0	1.76 ± 0.14	0.60 ± 0.07	2.29 ± 0.10	2.75 ± 0.15
3	ND	0.05 ± 0.02	0.06 ± 0.02	0.10 ± 0.02	0.16 ± 0.04	ND	1.28 ± 0.14	2.48 ± 0.04	3.45 ± 0.09	3.10 ± 0.20
4	ND	0.05 ± 0.06	0.22 ± 0.05	0.24 ± 0.06	0.17 ± 0.04	ND	1.47 ± 0.02	1.35 ± 0.06	2.65 ± 0.04	3.20 ± 0.10
5	ND	0.18 ± 0.05	0.13 ± 0.09	0.30 ± 0.07	0.25 ± 0.06	ND	2.06 ± 0.06	1.94 ± 0.09	3.76 ± 0.08	4.75 ± 0.14
6	ND	0.13 ± 0.03	0.11 ± 0.03	0.22 ± 0.03	0.23 ± 0.03	ND	2.44 ± 0.02	2.06 ± 0.12	4.25 ± 0.07	5.05 ± 0.12
7	ND	0.13 ± 0.03	0.08 ± 0.12	0.20 ± 0.08	0.16 ± 0.04	ND	1.97 ± 0.03	1.28 ± 0.05	3.09 ± 0.04	4.50 ± 0.12
8	0.1	0.12 ± 0.07	0.26 ± 0.13	0.34 ± 0.10	0.32 ± 0.08	2.2	1.17 ± 0.05	0.98 ± 0.04	2.03 ± 0.05	2.20 ± 0.07
9	ND	0.20 ± 0.09	0.11 ± 0.09	0.29 ± 0.02	0.28 ± 0.04	ND	2.18 ± 0.19	4.40 ± 0.20	6.04 ± 0.20	6.20 ± 0.05
10	0.2	0.20 ± 0.09	0.13 ± 0.03	0.32 ± 0.06	0.39 ± 0.05	9.0	2.84 ± 0.20	5.47 ± 0.14	7.63 ± 0.17	8.20 ± 0.12
11	0.4	0.11 ± 0.16	0.14 ± 0.08	0.24 ± 0.09	0.28 ± 0.04	13.0	1.51 ± 0.17	4.25 ± 0.08	5.24 ± 0.12	5.80 ± 0.15
12	ND	0.14 ± 0.03	0.03 ± 0.05	0.16 ± 0.04	0.19 ± 0.04	ND	2.59 ± 0.13	2.23 ± 0.09	4.55 ± 0.11	4.30 ± 0.14

^a(% W/W), percentage weight/weight. CBD, cannabidiol; GC-MS, gas chromatography-mass spectrometry; THC, tetrahydrocannabinol; UHPLC-MS/MS, ultra-high performance liquid chromatography tandem mass spectrometry. Bold values are UHPLC-MS/MS and GC-MS values of THC and CBD compared between each other.

contents in cannabis products even if there is a need for each analytical laboratory to evaluate the conversion yield [10], and to consider that THC value determined after decarboxylation gives a minimal content, rather than an exact value.

A limitation of the study resides in the fact that the analysis was only performed on 12 samples, although products containing “light cannabis” are extremely common in Italy and in many other EU countries, and results could not be considered representative of all the products on the “light cannabis” market.

Nevertheless, as the most common products sold on international websites have been analyzed, we proved, at least in these products, that “light cannabis” is indeed light.

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References

1. Regulation (EU) no 1307/2013 of the European parliament and of the council. Establishing rules for direct payments to farmers under support schemes within the framework of the common agricultural policy and repealing Council Regulation (EC) No 637/2008 and Council Regulation (EC) No 73/2009. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:347:0608:0670:EN:PDF>. Accessed: 2020-12-01.
2. Commission Delegated Regulation (EU) 2017/1155 of 15 February 2017. <https://eur-lex.europa.eu/legal-content/IT/TXT/PDF/?uri=CELEX:32017R1155&from=EN>. Accessed: 2019-30-12.
3. Disposizioni per la promozione della coltivazione e della filiera agroindustriale della canapa. LEGGE 2 dicembre 2016, n. 242. <https://www.gazzettaufficiale.it/eli/id/2016/12/30/16G00258/sg>. Accessed: 2020-12-01.
4. Pacifici R, Pichini S, Pellegrini M, Tittarelli R, Pantano F, Mannocchi G, et al. Determination of cannabinoids in oral fluid and urine of “light cannabis” consumers: a pilot study. *Clin Chem Lab Med* 2018;57:238–43.
5. Pacifici R, Pichini S, Pellegrini M, Rotolo MC, Giorgetti R, Tagliabracci A, et al. THC and CBD concentrations in blood, oral fluid and urine following a single and repeated administration of “light cannabis”. *Clin Chem Lab Med* 2019. DOI: 10.1515/cclm-2019-0119. [Epub ahead of print].
6. Pichini S, Mannocchi G, Berretta P, Zaami S, Pirani F, Pacifici R, et al. Δ^9 -tetrahydrocannabinol and cannabidiol time courses in the sera of “light cannabis” smokers: discriminating light cannabis use from illegal and medical cannabis use. *Ther Drug Monit* 2020;42:151–56.
7. Mannucci C, Navarra M, Calapai F, Spagnolo EV, Busardò FP, Cas RD, et al. Neurological aspects of medical use of cannabidiol. *CNS Neurol Disord Drug Targets* 2017;16:541–53.
8. Berman P, Futoran K, Lewitus GM, Mukha D, Benami M, Shlomi T, et al. A new ESI-LC/MS approach for comprehensive metabolic profiling of phytocannabinoids in Cannabis. *Sci Rep* 2018;8:14280.
9. Pacifici R, Marchei E, Salvatore F, Guandalini L, Busardò FP, Pichini S. Evaluation of cannabinoids concentration and stability in standardized preparations of cannabis tea and cannabis oil by ultra-high performance liquid chromatography tandem mass spectrometry. *Clin Chem Lab Med* 2017;55:1555–63.
10. Dussy FE, Hamberg C, Luginbühl M, Schwerzmann T, Briellmann TA. Isolation of Δ^9 -THCA-A from hemp and analytical aspects concerning the determination of Δ^9 -THC in cannabis products. *Forensic Sci Int* 2005;149:3–10.
11. Huestis MA, Solimini R, Pichini S, Pacifici R, Carlier J, Busardò FP. Cannabidiol adverse effects and toxicity. *Curr Neuropharmacol* 2019;17:974–89.