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ORIGINAL ARTICLE

Cannabidiol in urine is not a proof of CBD consumption—lesson learned from urine sample analysis in routine caseworks

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Abstract

Purpose Cannabidiol (CBD) has been gaining popularity in recent years. Knowing that CBD products can contain more tetrahydrocannabinol (THC) than expected, interpretation of cannabinoids concentration in urine can be tricky, especially when low amounts of THC and CBD are found. Moreover, interpretation can also be difficult due to interindividual variation in pharmacokinetics. The objective of this work was to take a critical look at the data from our daily practice as a toxicology laboratory.

Methods We have collected results obtained in a first batch of 1074 urine samples submitted to cannabinoids analysis, and results of cannabinoids content of a second batch of 719 seized materials.

Results CBD was detected in 163 urine specimens (15%). Its concentration was higher than the limit of quantification of 5 ng/mL in 108 samples only (10% of the sampling population). Most of CBD-positive samples were associated with a high THC-COOH concentration (> 500 ng/mL in 63.8% of CBD-positive samples) suggesting only a few CBD consumers in our population. Cannabinoids composition of seized plant materials (drug type at first glance) revealed CBD in 110 of them (15% of the sampling population), with a concentration mostly below 1%. All of the resin samples were CBD positive, and contained more THC compared to flowers.

Conclusions We can conclude that urine samples from drug-type cannabis users contained a low amount of CBD, what was not described previously. These findings are useful for the interpretation of cannabinoids results in daily practice.

Keywords Cannabidiol · THC · Urine · Seized material

Introduction

It has been decades that cannabidiol (CBD) is at the forefront of the scene, considering its potential benefit for a wide variety of health problems [1, 2]. Sativex® is the only drug containing cannabinoids which is approved in Belgium. This drug is prescribed as antispastic in multiple sclerosis, and contains a mix of CBD and tetrahydrocannabinol (THC). Besides that, in the USA, Epidiolex® contains only CBD and can be used for the treatment of 3 specific types of seizures observed in children (Dravet syndrome, Lennox–Gastaut syndrome and tuberous sclerosis complex) [3]. However, widespread use of non-medical cannabidiol—unapproved on the drug market—is also described for a variety of conditions such as anxiety, pain and depression [4].

On one hand, in Belgium, cannabis containing more than 0.2% of total THC (sum of neutral and acid forms) is illegal. Seeds are not regulated, as the law only mentions flowers, resins, extracts and tinctures. The national law regulating illicit substances is one hundred years old, and clarifications were added in the 2017 Royal Decree [5]. People who are now claiming a personal use of cannabis and are arrested with less than 3 g, risk only light legal proceedings, unless there are aggravating circumstances (for example at school or prison). Other countries can apply other thresholds: for example, legal cannabis can contain up to 0.3% of total THC in Luxembourg and France, whereas a limit of 1% is applicable in Switzerland.

Since the end of 2018 while taking advantage of a lack of clarity in the Belgian law, lots of shops selling CBD products (unclassified in the law) appeared in the country. Since April 2019, CBD was then classified as "another

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smoked product" and duties were considerably increased. Trader's profit margins were consequently reduced, and many shops had to close. Products were then transferred to unspecialized stores, such as those who sold tobacco products.

On the other hand, still in Belgium, professional drivers are submitted to a urine drug test to assess their ability to drive. Based on the 1998 Royal Decree concerning driving license, if it is positive, offenders have to demonstrate a 6-month abstinence period. After this period, they can be submitted to a new drug test [6]. They regularly mention CBD consumption to explain a positive cannabis test; the main challenge being to determine whether this is true.

There is still a need of data to help with the interpretation of biological results in various cases, such as driving license, but also workplace drug testing, legal proceedings or medical care.

Urine is the matrix of choice for drug testing because it is easy to collect, non-invasive, and reflects an extended detection window compared to blood. At first glance, CBD consumption alone gives rise to unambiguous results, without THC-COOH (11-nor-9-carboxy-THC, the main metabolite of THC which is usually found in urine). Difficulties can arise when a previous cannabis consumption is involved. Our objective was to take a critical look at the numerous results obtained in our daily practice and highlight a potential trend. It will be illustrated by 2 cases in which assistance was specifically requested by the physician for interpretation. We also checked the CBD content of cannabis samples circulating in the same area and during the same time period, to explain CBD detected in urine. Urine samples and plant materials were analyzed by two separate and unrelated methods.

Materials and methods

Chemicals and reagents

All the analytical standards are from LGC Standards (Teddington, UK).

n-Hexane and LCMS-grade methanol from J.T. Baker were purchased by Filter Service (Eupen, Belgium). KH_2PO_4 and K_2HPO_4 used to prepare the incubation buffer, NaOH to adjust the buffer pH and KOH, as well as ethyl acetate and acetic acid 96% were purchased from V.W.R Belgium (Leuven, Belgium). β-Glucuronidase (from Helix pomatia, type HP-2, ≥ 100 000 units/mL) and ammonium bicarbonate used to prepare the mobile phase were purchased from Merck (Darmstadt, Germany). Ammonia water 25% used to adjust the mobile phase pH and hydrochloric acid came from ThermoFisher Scientific (Waltham, MA, USA).

Plant analysis

Method used to quantify main cannabinoids in plant material was described in detail elsewhere [7]. Briefly, a liquid–liquid extraction was performed on dried plant materials, followed by dilutions. Extracts were analyzed by ultra-high-performance liquid chromatography combined with a photodiode array detector (UPLC-DAD from Waters, Milford, MA, USA). Mobile phase consisting of methanol and 0.1% formic acid in water was applied during a 16-min gradient mode.

Urine analysis

Sample preparation

One hundred microliter of internal standard solution (mixture of THC-d_3 and THC-COOH-d_3, both at 1 mg/L; CBD-d_3 and CBN-d were not easily available when developing the method) was added to 1 mL of urine before a first hydrolysis at 40 °C in the presence of 40 µL of β-glucuronidase in an acidic environment (2 mL of phosphate buffer 0.1 M pH 6.8). After a night of incubation (16 h), 50 µL of KOH 12 M was added to the sample and the second incubation took place for half an hour at 60 °C. The sample was then acidified, with 400 µL of hydrochloric acid 1 M and 200 µL of acetic acid 10% before a liquid liquid extraction with 5 mL of hexane/ethyl acetate (9/1:v/v). After agitation and centrifugation, the organic layer was evaporated to dryness and the extract was dissolved in a mixture of methanol/water (80/20:v/v) before chromatographic analysis.

Preparation of standards

The internal standard solution was prepared by mixing 100 µL of THC-d_3 and THC-COOH-d_3 (100 mg/L each) in 9800 µL of methanol. For the calibration curve, a working solution with a concentration of 10 mg/L of THC-COOH, CBD and cannabinol (CBN) was prepared by diluting the compounds in methanol. A tenfold dilution was done in the same solvent to obtain a diluted working solution. The calibration curve used for quantification was prepared by spiking the appropriate volume of working solutions in blank urine.

Chromatographic analysis

Chromatographic analysis was performed on an UPLC Acquity® coupled to a Quattro Premier XE® mass spectrometer, both from Waters. The chromatographic separation was achieved at 40 °C on an Acquity® UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm, Waters) using 10 mM ammonium bicarbonate pH 10 as mobile phase A and methanol as

mobile phase B. After injection of 10 µL of sample, gradient elution began with 50% of both phases maintained during 0.3 min, using a flow rate of 0.45 mL/min. Gradient linearly decreased to 5% mobile phase A which was obtained at 2 min and kept during 0.9 min before going back to initial conditions, for a total runtime of 3 min.

Compounds were detected by the mass spectrometer working in the positive electrospray mode at 1.0 kV. The source temperature was 120 °C and the desolvatation temperature was 350 °C. The cone gas flow was set at 50 L/h, whereas the desolvatation gas flow was 800 L/h. Both gas were nitrogen. Transitions are available in supplementary materials (Table S1).

Data collection

During our routine work, this validated method was used to screen a batch of 1074 urine samples for THC-COOH, cannabinol and cannabidiol. These samples were analyzed for different reasons: patients admitted at emergency room with suspected intoxication, psychiatric evaluation, forensic expertise (rape, driving license, postmortem, etc.). Samples submitted to analysis at our laboratory are coming from Liege and surrounding area (Walloon region).

To check if urinary cannabidiol could be derived from drug-type cannabis use, cannabinoids concentration of a batch of 719 unrelated seized materials (seized during years 2018 and 2019, in the same time period and geographic area as the urine samples) was determined by UPLC-DAD. Most of the samples were entire plants seized by the police in illicit indoor cannabis cultures, and rarely samples seized from people in possession of a small packaging. Samples seized in CBD shops were excluded from this data analysis, as they were the main topic of another publication [7].

Illustrative case reports

These cases were selected because the physician that was in charge of the patient requested support to interpret the results of analysis.

The first case was a 29-year-old man with unknown history who was tested for his fitness to drive. The urinary CBD level was 208 ng/mL together with a THC-COOH concentration of 85.9 ng/mL. The man denied a drug-type cannabis consumption, which was stopped for 6 weeks (he previously consumed 1 or 2 joints a day for 6 months, and 1 or 2 g a day before that) but he declared a daily CBD consumption, without more details.

The second case was a 32-year-old woman locked up in a psychiatric hospital for a sudden psychotic decompensation. She was treated with antidepressants, and was a regular patient of the emergency room, mainly for orthopedic troubles or gastrointestinal disorders. THC-COOH was 131 ng/mL whereas the CBD concentration was 42.8 ng/mL. Considering that she was under high surveillance for 3 weeks, with no access to drugs, the physician needed help to understand these results.

Results

Data collection: urine samples

CBD was detected in 163 urine samples only (15% of the population). Among them, a concentration higher than the lower limit of quantification of 5 ng/mL was observed in 108 samples (66.3%). Mean CBD concentration observed in these 108 samples was 21.3 ng/mL (median: 10.0 ng/mL) meaning that most of the positive samples contained a really low amount of CBD. Graphical representation illustrating the number of urine samples in each category of CBD concentration can be found in supplementary material (Figure S1).

These 108 samples are represented in Fig. 1, illustrating the absence of correlation between CBD and THC levels.

Eighteen outliers could be identified using boxplots (11 samples containing a CBD level higher than 38.4 ng/mL and 7 samples containing a THC-COOH level higher than 4620 ng/mL). After rejecting them, correlation was recalculated. Nevertheless, nonparametric correlation test was still non significant ($R^2 = 0.1065$, $p = 0.1376$).

When detected, CBD was always going along with THC-COOH. A high THC-COOH concentration was associated with an increased probability to detect CBD: among the 163 CBD-positive samples, mean THC-COOH concentration was 1387 ng/mL (median: 765 ng/mL) and 104 samples (63.8%) exhibited a THC-COOH concentration higher than 500 ng/mL. The repartition of CBD-positive samples in each THC-COOH range is shown in supplementary materials (Figure S2).

By taking a closer look to samples containing a relatively low THC-COOH concentration (< 15 ng/mL which is the cut-off value of the Mandatory Guidelines for federal workplace drug testing), listed in Table 1, some samples could be difficult to interpret.

On the other hand, THC-COOH was detected in 657 samples (61%) and their concentrations were ranging from 3 to 10,300 ng/mL. The repartition of CBD-positive samples in each THC-COOH concentration class is depicted in Table 2.

A high THC-COOH concentration (> 500 ng/mL and obviously > 1000 ng/mL) is not associated with a high CBD concentration, but with a high probability to detect CBD. A logistic model replacing the CBD value by a binary variable (0 if CBD was not detected, 1 if CBD was detected) fitted to the data shown positive statistical link between THC-COOH

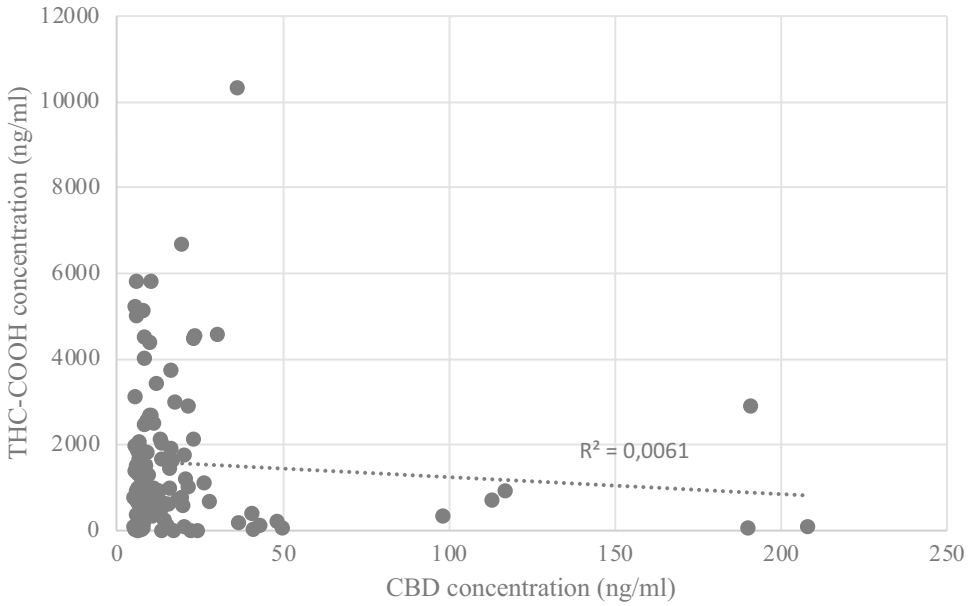

Fig. 1 THC-COOH concentration in function of the CBD concentration. Plotted line is the fitted linear regression and R^2 is the coefficient of determination

Table 1 Urine samples exhibiting a low THC-COOH amount. "Trace" indicates a result below the limit of quantification (5 ng/mL)

	THC-COOH (ng/mL)	CBD (ng/mL)
Urine 1	3	6.00
Urine 2	3	22.3
Urine 3	4	Trace
Urine 4	8	6.14
Urine 5	9	Trace
Urine 6	9	16.8
Urine 7	10	Trace
Urine 8	10	13.3
Urine 9	11	24.2
Urine 10	13	Trace
Urine 11	13	Trace

Table 2 Number of positive samples in each THC-COOH concentration range

THC-COOH concentration (ng/mL)	Number of samples	Number of CBD-positive samples (%)
THC-COOH ≤ 20	85	15 (17,6)
20 < THC-COOH ≤ 100	197	11 (5,58)
100 < THC-COOH ≤ 500	207	32 (15,5)
500 < THC-COOH ≤ 1000	74	33 (44,6)
> 1000	94	72 (76,6)

concentration and probability to detect CBD. Graphical representation of the estimated model is represented in supplementary materials (Figure S3).

Finally, cannabinol was never detected.

Data collection: plant material

Total THC content (THC + 0.877 × THC-A) results obtained in this study are summarized in Fig. 2. In each dataset:

- The box extends from the first quartile (Q1) to the third quartile (Q3),
- The line in the box shows the median of the dataset, while the cross is its mean,
- The lower whisker's end is given by max (min (THC), Q1-1.5*(Q3-Q1)),
- The upper whisker's end is given by min (max (THC), Q3 + 1.5*(Q3-Q1))
- Dots outside the whiskers are extreme data observations ("outliers").

Next to a vast majority of flower samples (541 samples) and leaves (99 samples), 44 of the 719 samples were resin samples, and all of the latter contained cannabidiol and/or cannabidiolic acid. Thirty-five samples were considered as "other" kinds of samples, such as powder, vegetal residues and branches. Total THC content in resins ranged from 0.419 to 40.6% (mean: 19.1%; median: 19.4%). This was high compared to flowers in which it ranged from 0.543 to 31.2% (mean: 13.3%; median:13.7%). Obviously, resins also contained more CBD than flowers (mean: 3.48% versus 0.062% for flowers or 0.530% if we considered only the 63 flowers in which CBD was detected).

Only 110 out of the 719 samples (15.2% of the population) submitted to analysis showed a detectable amount of total CBD. When positive, total CBD amount ranged from 0.077 to 7.78% (mean: 1.70; median: 0.186%). Most of the samples contained a total CBD content lower than

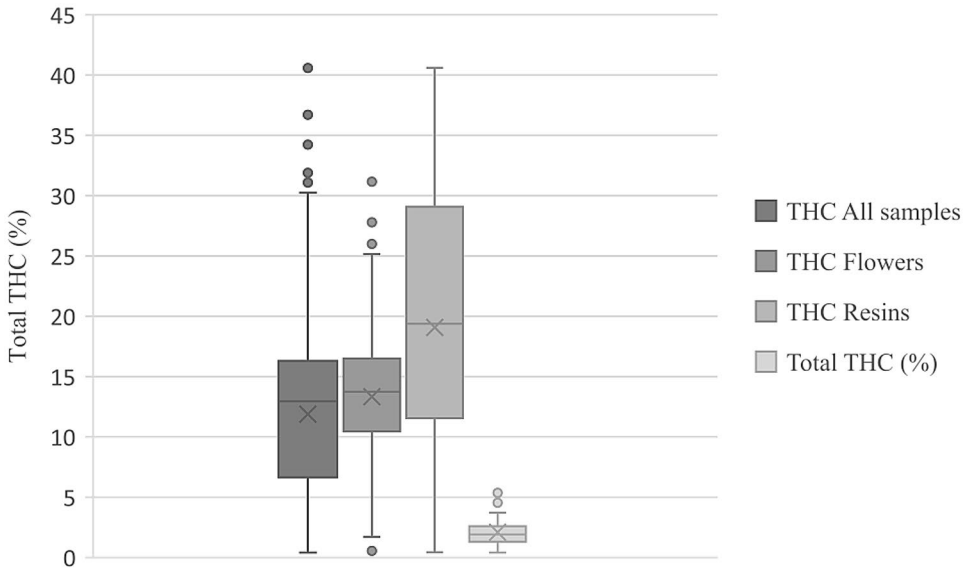

Fig. 2 Total THC content in all samples, flowers, resins and leaves

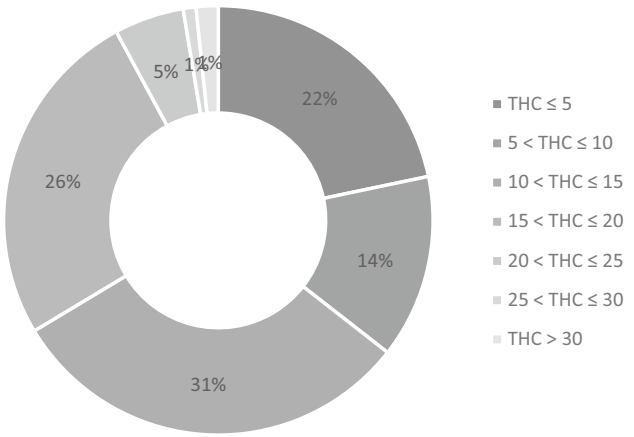

Fig. 3 Classification of samples according to their total THC content (sum of neutral and 0.877 × acidic forms, %)

1%. Classification of samples according to their total CBD content can be found in supplementary materials (Figure S4).

No correlation was observed with the total THC content. The highest CBD level was observed in a flower sample containing 5.08% of THC. Mean THC content was 11.9% when including all the samples (minimum: 0.401%; maximum: 40.6%), and it was 17.1% when only CBD-positive samples were considered. The total THC amount was rarely higher than 25%, as it can be seen in Fig. 3.

Low amount of cannabinol was detected in only 112 samples (min: 0.066%; max: 4.75%; mean: 0.442%) whereas cannabigerol (sum of neutral and 0.877 × acid form) was detected in 604 samples (min: 0.071%; max: 2.86%; mean: 0.804%).

Discussion

Urine samples analyzed in our routine work are probably not those of CBD shops clients, whatever the popularity of CBD in our region. Indeed, only a small number of samples were positive for CBD, and it was almost always detected next to a high THC-COOH level which is a proof of marijuana consumption. However, we have to keep in mind our previous publication in which half of the CBD products contained more THC than allowed [7]. The consumer is then exposed to THC even if he does not realize it, because blood concentration will be too low to lead to psychotropic effects.

The main finding of this data collection is that urine samples coming from consumers of THC-rich products will, of course, exhibit a high THC-COOH level (obviously depending on the time between consumption and sampling) but it will also be associated with a positive CBD result. Even if the presence of CBD in urine of marijuana consumers could be suspected, it was not described elsewhere. Probably linked to a lower limit of quantification (0.78 ng/mL), Gilman and colleagues have detected cannabidiol in almost half of their 256 urine samples (median value < 0.78 ng/mL), with significant interindividual variation [8]. On one hand, these samples were coming from study participants reporting consumption of various types of medical products (CBD or THC dominant, or equal amount, but analysis of the products was not performed). On the other hand, in their recent study including 20 cannabis smokers, Huestis et al. never detected cannabidiol in 2252 urine samples after controlled administration of cannabis by various routes [9]. A common finding between these 2 studies and the present one is the non-detection of cannabinol.

However, some exceptions to this rule (high THC-COOH level with low CBD concentration in urine) were observed

in our routine work, represented by the 11 samples containing low THC-COOH concentrations. These results have to be interpreted with great caution: either a concomitant use of drug-type cannabis and cannabidiol or the consumption of drug-type cannabis a long time ago? The consumption of cannabidiol highly contaminated with THC or, inversely, the use of THC with a high amount of CBD? Moreover, interpretation can be more difficult due to interindividual variation in pharmacokinetics. So, a formal answer cannot be given. This kind of result would probably be more easily interpreted together with other new ones, obtained on a separate sample, if possible (not possible with deceased people, for example) and relevant (not relevant in rape cases, for example).

At this point, a short reminder concerning THC pharmacokinetics can be useful. Smoking is the most common route of cannabis consumption. Peak plasma THC concentration is observed after a few minutes, psychotropic effects reach a maximum after 15–30 min and can last for about 2–3 h [10]. Due to significant first-pass metabolism, THC bioavailability after oral administration is low, and the same applies for CBD. Cannabis ingestion leads to a lower and later peak plasma concentration, but effects last longer by this route [11]. The main difference observed in chronic cannabis users concerns the prolonged detection window in biological matrices due to the accumulation of cannabinoids in the body. Consequently, THC-COOH can be detected for about 2 days in urine of unusual consumers, whereas chronic users can be tested positive during about a month.

Sample 9, with a relatively high CBD concentration, revealed most likely a CBD consumption. Spindle et al. conducted a useful pharmacokinetic study on 6 participants which will help us to make an unambiguous interpretation of the other samples [12]. In their study, urine samples of each subject were tested positive for THC-COOH (maximal concentration between 1.2 and 29.9 ng/mL) at least at one time point after inhalation or ingestion of CBD dominant cannabis (other conditions were also tested). In most samples, trace amounts were detected, but 2 participants gave urine specimens above 15 ng/mL which is the cut-off value of the Mandatory Guidelines for federal workplace drug testing. Indeed, a THC-COOH urinary concentration of 15 ng/mL is a recognized threshold used to demonstrate a cannabis consumption in this case [13]. So, according to the study of Spindle et al., our remaining 10 samples with low THC-COOH and CBD levels could potentially be explained by a cannabidiol consumption. Other teams have also shown that smoking CBD-rich product containing a small amount of THC could lead to the detection of THC-COOH in urine [14–16]. In the study of Spindle et al., peak urinary concentration was observed later when CBD was ingested compared to vaporization or inhalation, and it was also higher [12].

A THC/CBD ratio in blood and oral fluid was suggested by Pacifici in order to distinguish between the consumption of CBD- or THC-rich product [17]. In their recent study, Goggin and Janis developed another ratio in urine (including COOH metabolites for both compounds) to discriminate between a marijuana use and the consumption of CBD-rich products contaminated with THC [18]. Even if they had no idea of the time elapsed between the consumption and the sample collection, significant amount of carboxylated CBD was measured, which prevented us to apply their ratio. If doing so, without measuring CBD-COOH, all of our subjects would be classified as THC consumers. The lack of information on the CBD metabolites is a drawback of the presented work since their measurement would probably increase the number of positive samples by increasing the detection window.

Knowing that, we can now come back to our case reports. In the first case, CBD consumption was undeniable, as it was the highest CBD concentration ever observed. In the second case, the CBD concentration (42.8 ng/mL) was supposed to be too high to come from drug consumption. For both cases, THC-COOH was too high to be explained by a CBD contamination. So, we can confirm earlier drug consumption. It has to be emphasized that THC-COOH had already been detected in urine 3 months after smoking cessation [19] whereas cannabidiol shows a shorter elimination rate. However, to the best of our knowledge, cannabidiol pharmacokinetics in real chronic users has not been studied to date.

So, in this study, the low amount of CBD found in urine samples next to the high THC-COOH level comes from drug-type cannabis plant (even if a simultaneous consumption of a CBD product cannot be totally ruled out. The main question is always to see if there was a drug-type cannabis consumption. If there was an additional CBD consumption is of lesser importance). With 15% of detection, CBD in seized samples was as rare as it was in urine samples. It was found in all of the resins and 9.18% of other samples. From this observation, we can deduce that resin consumers are more likely to exhibit a CBD-positive urine sample. Resin samples, also called hashish, are a collection of plant secretions produced in the glandular trichomes of the plant. They do not look like a plant anymore, and are known to contain a higher THC content compared to herbal samples [20, 21]. Resin found in European markets seems to contain significant levels of CBD [22]. Niesink et al. also reported less than 0.5% CBD in most cannabis products, except for resins imported in the Netherland in which there was more CBD [23]. Our CBD level can also be compared with other European studies concerning resins seized in France [24] and Danemark [25] where levels were, respectively, 4% and 6%. Unlike THC levels, large differences in CBD levels were observed between flowers and resins, which contained about 50

times more CBD compared to flowers. It can be explained by the origin of the products: resins are mainly imported (mostly from Morocco, where THC-rich and CBD-rich chemotypes can be mixed [22]) whereas flowers are cultivated indoor in the country [26]. In our seized samples, resins are less common than flowers, which is also observed in other studies [27, 28].

Finally, potency observed in this study, i.e., 19.1 and 13.3% THC for resins and flowers, respectively, is totally in agreement with the data collected by the European Monitoring Centre for Drugs and Drug Addiction (EMCCDA) for Belgium, which indicates 20.7 and 13.7% THC, respectively, in 2018 [20]. However, they also describe a drop in potency for the year 2019 (to 15.3 and 7.88%, respectively) which was not observed in our study. The exponential increase in potency described in 2018 was apparently stopped after the publication of Freeman [22]. If we look at the 2018 data, Belgium ranks among the European countries where circulating cannabis is the most concentrated.

Conclusion

Interpretation of cannabinoids concentration in urine can be tricky, due to the presence of THC in CBD products and vice versa. As far as we know, this is the first publication concerning real routine samples. In our population, CBD detected in urine samples was mainly going along with a high THC-COOH level, revealing a drug-type cannabis consumption. As resins were always CBD positive, the probability to detect CBD was higher for these consumers. Our approach brings another data set adding some useful information which will help to interpret biological results as correctly as possible.

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Author contributions MD collected data, reviewed the literature and wrote the manuscript. CC helped with data interpretation, reviewed and approved the manuscript.

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Data availability statement The datasets generated during and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors did not receive support from any organization for the submitted work. The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval No ethical approval is required.

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