THC And Cannabinoids: Chemistry, Methods Of Detection And Stability In Biological Samples

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Abstract

Introduction: The purpose of this article is to describe the chemical and pharmacological characteristics of THC and its metabolites, the methods used for detection for clinical and forensic purposes and their stability in reference biological matrices. Psychoactive aspects or aspects relating to the problem of abuse or addiction will not be explained.

Methodology: The article was written by integrating the authors' individual knowledge of pharmacology with printed material and online articles extracted from Google Scholar and PubMed. We used articles dealing with the chemical and toxicological analytical aspects of cannabis, selected from those published from 1990 to today.

Discussion and Conclusions: THC has interesting chemical-biological properties from high lipophilicity to mainly hepatic metabolization, CYP-mediated with the genesis of various metabolites, one of which is particularly active. Their long half-life and the prevalent distribution in the adipose tissue prolongs the effects in the chronic user, making the detection window up to over a month from the last intake. Blood, urine and hair are the reference matrices but saliva is also becoming increasingly used for this purpose.

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Introduction

The prevalence of cannabinoid consumption has reached high levels throughout the world in recent years and, in Italy, phytocannabinoids are by far the first illicit psychoactive substance consumed in the youth population, followed by cocaine and psychostimulants. Drug related to 2020, drug operations have made it possible to seize 19,868 kg of marijuana (there were over 23,000 in 2019 and over 39,000 in 2018) and over 9,000 of hashish\[1\]. The natural history of cannabis use usually begins in adolescence and continues until the age of 30-40, where most people stop or reduce consumption while a smaller percentage continues towards sporadic use. The percentage of cannabinoid addicts is not known but is estimated to be low, between 10 and 30% of total users\[2\]. It is certainly known that THC (tetrahydrocannabinol) is the main responsible for its psychoactive effects, but the phytocomplex includes over 80 cannabinoids, including CBD (cannabidiol), which seems to counterbalance its receptor effects by displacing THC from the binding with the CB1 receptor and presenting neuroprotective, anti-inflammatory, antiemetics and antiepileptic effects\[2\][3]. Other chemical compounds that are found in cannabis flos are sugars, hydrocarbons, terpenes and fatty acids. THC (or Δ⁹-THC, Figure 1) can be considered the progenitor of the phytocannabinoid family and is found in the plant’s flowers, leaves and resin\[4\]. Various isomers of THC are known, two of which occur naturally in cannabis. The most relevant is the (-) - trans isomer, known pharmacologically as dronabinol; it can be ingested or, commonly, smoked or inhaled with a vaporizer. In pure form, at low temperatures, it’s a crystalline solid, with a glass transition that makes it viscous and sticky when heated. Commercially THC occurs in solution as a yellow / brown oily liquid and has a very low solubility in water but good in most organic solvents\[2\][4].

\[Figure 1.\] THC chemical structure

In cannabis flos, THC is mainly present as a precursor tetrahydrocannabinolic acid (Δ⁹-THCA). The enzymatic condensation of geranyl pyrophosphate and olivetolic acid generates cannabigerolic acid, which in turn is cyclized by the enzyme THC acid synthase (Figure 2), thus resulting in the molecules of tetrahydrocannabinolic acid, cannabidiolic acid and cannabicromenic acid\[5\]. Exposed to heat (combustion or vaporization), the acids decarboxylate to form THC,
cannabidiol and cannabichromene. Cannabinol, on the other hand, is produced by the breakdown of THC.

![Figure 2. THCA biosynthesis](image)

The THC content in marijuana (Cannabis sativa) is in the order of 0.5-1% in large leaves, 1-3% in small leaves, variable in flowers from 10 to 27%, 5-10% in bracts, 10-60% in hashish, over 60% up to 99% in hashish oil. It’s present inside the trichomes, so the concentration is higher, as regards the live plant, in the inflorescence area and is even higher in the case of resin extraction products and curtains, to diminish over time, a process accelerated by heat and light.

**Objectives and Methodology**

In this mini-review article we want, after briefly describing the chemical characteristics of the THC molecule, to evaluate the metabolic steps in the body and the detectability of its metabolites in biological samples (also in relation to their physico-chemical stability in that matrices and briefly describe the analytical methods used in their detectability). The article aims to be a brief review of some literature data integrated with personal pharmaco-toxicological and analytical knowledge, so it was written by integrating with printed material and online articles extracted from Google Scholar and PubMed. We used articles dealing with the chemical and toxicological analytical aspects of cannabis, selected from those published from 1990 to today.

**Discussion**

Chemically, THC consists of a tricyclic 21-carbon structure, with no nitrogen and with two chiral centers in trans configuration. The pKa is 10.6 and two different numbering systems, dibenzopyran (Δ9) and monoterpen (Δ1) nomenclatures, are commonly used to describe THC. The major chemical characteristic compared to other substances of abuse (such as nicotine, cocaine or heroin) is the absence of alkaloid structures, where by alkaloids we mean basic chemical structure containing nitrogen atoms generally inside heterocyclic rings. Other cannabinoids include CBN (cannabinol, which is approximately 10% psychoactive like THC), cannabidiol (CBD), cannabigerol (CBG), cannabinodivarin and many others; THC decomposes when exposed to air, light, and exposure to acid can cause the compound to hydrolysis to CBN (which is essentially a breakdown product). The potency of a preparation is described based on the concentration of THC (nowadays specially selected varieties are grown with a concentration of up to 40% or more of THC and even less than 1% of CBD). The inhalation route, the main method of administration, causes a rapid and intense...
absorption of the active principle with high bioavailability in the central nervous system; it should in fact be remembered that exposure to heat activates the acid precursors present in the phytocomplex to pharmacologically active cannabinoids\[^9\]. However, pyrolysis destroys approximately 30% of the THC content. THC can be measured in Plasma and saliva just seconds after inhaling the first smoke cannabis puff. The concentration continues to grow rapidly with a peak at about 9 minutes albeit with intra and interindividual variability\[^10\]. THC metabolism is summarized in Figure 3: the THC hydroxylation on C9 from hepatic cytochrome p450 generates an equipotent metabolite, 11-OH-THC, considered a true psychoactive analyte. Other cytochrome p450 isoforms are involved in THC oxidation with over 100 metabolites identified, including di-and-tri hydroxylated compounds, ketones, aldehydes and carboxylic acids. 11-OH-THC predominates at the first oxidation product, with a peak concentration about 13 min after smoking\[^11\][^12\]. The oxidation of this metabolite produces the carboxylated form, THC-COOH, inactive which is glucuronate in phase 2 (to a lesser extent conjugated with glutathione, sulfate, amino and fatty acids).

\[\text{Figure 3. THC metabolism}\]

After oral administration, blood THC concentrations are much lower than after smoked assumption and the bioavailability is reduced to 6-20% because of degradation of the drug in the gastrointestinal tract, with a significant first pass metabolism in the liver. THCCOOH is the first metabolite which is misurate for toxicological analysis\[^12\][^13\]. In particular the acid-linked THCCOOH-glucuronide conjugate has an excretion life of 3-4 days; urinary levels generally decrease rapidly, until a concentration of 20-50 micrograms/L and, at this point, at a much slower rate. Also slow release of THC from lipid storage compartments and significant enterohepatic circulation contribute to its long terminal half-life in the plasma, which was reported as over 4 days in chronic users\[^14\]. Similarly, the terminal urinary excretion half life of THCCOOH was estimated as 3-4 days. Recent research on chronic, frequent cannabis users over the last 15 days showed quantifiable THC in the blood for over 7 days during abstinence\[^15\]. As for the biological samples, blood allows
(together with saliva) a detection of recent consumption while urine also allows a time window that can be extended up to 24 - 72 hours or more, based on the type of substance sought (such as in the case of cannabinoids)\[16\]. The keratin matrix, on the other hand, allows backward detection, bearing in mind that the growth rate of the hair is about one centimeter per month and through segmental analysis it is possible to detect the type and quantity of substances taken in the months preceding the collection\[17\]. Whole blood and plasma THC, 11-OH-THC and THCCOOH concentrations are stable after 4 weeks at -20°C and 4°C and for at least 1 week at room temperature. THC COOH-glucuronide is stable for 4 weeks at -20°C, unstable at 4°C over 4 weeks and very unstable at room temperature losing more than 40% over a week for whole blood\[18\][19]. When plasma is stored for a longer period, THC and THC COOH concentrations can decrease. Both substances have been examined in oral fluid, but the hydrophobic nature of them (also of THC -COOH) causes their reduced concentration in saliva, in the order of pg/ml. THC-COOH concentrations are stable in urine when the assay method hydrolyzes the thg.com glucuronide\[20\]. THC-COOH glucuronide is stable at -20 ° C for more than 10 days but at high temperatures and for longer storage periods it degrades to the carboxylated form. When urine specimens are stored for longer periods, total THCCOOH concentrations may decrease even at -20 ° C\[21\][22]. Regarding the detection methods, the initial screening analyzes include high immunoassay tests, GC-MS (gas chromatography coupled to mass spectrometry) and LC-MS / MS (liquid chromatography coupled to mass spectrometry). Urine is usually tested without sample preparation because of the high concentrations of drug and / or metabolites and the low level of other interfering compounds\[23][24][25\]. Most immunoassays contain antibodies directed against THC-COOH with a cross reactivity for THC, 11-OH THC and CBD and CBN\[26][27][28\]. Confirmation tests require a chemical technique based on a different scientific principle from the initial and routine confirmation methodologies including GC-MS, GC-MS / MS and HRMS\[29][30\]. (Figure 4)
Conclusions

The detection of THC and cannabinoids are frequently required in toxicological analytics as they are the main substances of abuse used. Compared with other drugs of abuse, the analysis of cannabinoids presents some difficulties: THC and some of its metabolites such as 11-OH-THC are very lipophilic and are present in low concentrations in biological fluids in the tissues. Complex samples, like blood and hair, require multistep extension to separate cannabinoids from endogenous lipids and proteins. THC has interesting chemical-biological properties from high lipophilicity to mainly hepatic metabolism, CYP-mediated, with the genesis of various metabolites, one of which is particularly active. Their long half-life and the prevalent distribution in the adipose tissue prolongs the effects in the chronic user, making the detection window up to over a month from the last intake. Blood, urine and hair are the reference matrices but saliva is also becoming increasingly used for this purpose.

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Conflict of Interest Statement

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