

# $\Delta^9$ -tetrahydrocannabinolic acid A: a reliable marker for differentiating between the consumption of illegal cannabis products and legal, medical $\Delta^9$ -THC

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**ABSTRACT:**  $\Delta^9$ -Tetrahydrocannabinolic acid A ( $\Delta^9$ -THCA-A) is the biosynthetic precursor of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) in plant material of *cannabis sativa*, without having psychoactive effects.  $\Delta^9$ -THCA-A is not contained in pharmaceutical  $\Delta^9$ -THC formulations. Thus,  $\Delta^9$ -THCA-A can serve as a marker for differentiating between the consumption of illegal cannabis products and legal fully synthetic  $\Delta^9$ -THC.

*Key Words:* Cannabis,  $\Delta^9$ -tetrahydrocannabinol,  $\Delta^9$ -tetrahydrocannabinolic acid

Cannabis is the most popular recreational drug globally<sup>1</sup>. The resin of Cannabis plant contains more than 84 chemical compounds with psychoactive effects, known as cannabinoids, the most efficacious of which is  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC)<sup>2</sup>. The biogenesis of  $\Delta^9$ -THC starts with olivetolic acid, that is produced in the plant from the condensation of hexanoyl-CoA with three molecules of malonyl-CoA. In the next step, olivetolic acid is alkylated to cannabigerolic acid, which is then converted to cannabinolic acid. Cannabinolic acid is the precursor of  $\Delta^9$ -tetrahydrocannabinolic acid A ( $\Delta^9$ -THCA-A or  $\Delta^9$ -THCA).  $\Delta^9$ -THCA-A is a non-psychoactive substance that is decarboxylated to  $\Delta^9$ -THC via heating, when cannabis products are smoked<sup>3</sup>.

The therapeutic effects of  $\Delta^9$ -THC have been known since ancient times, when cannabis was used as a medicine for many different pathological conditions such as insomnia, headaches, gastrointestinal disorders and pain<sup>4</sup>. Nowadays the cultivation,

possession and transfer of cannabis is illegal in most of the European countries and is convicted by law. On the other hand, medical  $\Delta^9$ -THC is provided, mainly in the U.S, to treat a range of diseases and the ensuing symptoms. Marinol<sup>®</sup> (U.S, Canada) is an oral drug containing fully synthetic  $\Delta^9$ -THC, known as dronabinol and is used to treat anorexia in people suffering from AIDS, and also to minimize nausea and vomiting associated with cancer therapy<sup>5</sup>. Another popular pharmaceutical compound, used to treat spasticity and neuropathic pain associated with multiple sclerosis, is Sativex<sup>®</sup> (U.S, Europe, New Zealand). Sativex<sup>®</sup> is an oromucosal spray which contains nabiximols, a mixture of compounds derived from cannabis plant. The principal cannabinoid components of the drug are synthetic  $\Delta^9$ -THC and cannabidiol<sup>6</sup>.

The high popularity of illegal cannabis and the use of legal fully synthetic  $\Delta^9$ -THC in recent years, demonstrated the need to find reliable markers in biological samples for differentiating between the consumption of illegal cannabis products and legal medication containing fully synthetic  $\Delta^9$ -THC.

$\Delta^9$ -THCA-A could serve as a marker of illegal consumption of cannabis products if the substance is detected in biological samples of cannabis users.  $\Delta^9$ -THCA-A attracted

scientists' attention in recent years, as it is the precursor substance of natural  $\Delta^9$ -THC in plant material and is not contained in Marinol<sup>®</sup> or Sativex<sup>®</sup>. In 2005, it was shown that during smoking cannabis joint,  $\Delta^9$ -THCA-A is only partially converted to  $\Delta^9$ -THC and therefore it can be taken up by the consumer<sup>7</sup>.

In 2007, Jung et al. detected  $\Delta^9$ -THCA-A in the urine and blood serum samples collected from police controls of drivers suspected for driving under the influence of drugs (DUID). Liquid chromatography tandem-mass spectrometry (LC-MS/MS) was used for  $\Delta^9$ -THCA-A determination.  $\Delta^9$ -THCA-A was detected in the urine and blood serum samples of cannabis users in concentrations of up to 10.8 ng/ml in urine and 14.8 ng/ml in serum. The concentration of  $\Delta^9$ -THC was higher than the concentration of  $\Delta^9$ -THCA-A in most serum samples<sup>8</sup>. In the above paper,  $\Delta^9$ -THCCOOH-D3 was used as internal standard (IS) because the isotopically labeled  $\Delta^9$ -THCA-A was not commercially available.

In 2012, Roth et al. succeeded to synthesize deuterated  $\Delta^9$ -THCA-A<sup>9</sup>. Metabolism studies of  $\Delta^9$ -THCA-A in rats showed that the main metabolites of THCA-A were formed in close analogy to  $\Delta^9$ -THC metabolism and twelve metabolites were identified<sup>10</sup>.

In 2013, Brabanter N. et al. developed a fast method for quantification of  $\Delta^9$ -THCA-A in urine using microwave-accelerated derivatization and gas chromatography-triple quadrupole mass spectrometry. Their method could quantify  $\Delta^9$ -THCA-A in 30 min, using only 1 mL of urine<sup>11</sup>.

In 2013, Raikos N. et al. analyzed fifty eight authentic whole blood and the respective plasma samples collected from drivers suspected for driving under the influence of cannabis, using LC-MS/MS.  $\Delta^9$ -THCA-A concentrations ranged from 1.0 to 496 ng/mL in blood samples and from 1.4 to 824 ng/mL in plasma samples.  $\Delta^9$ -THC was present in all blood samples with levels ranging from 0.7 to 51 ng/mL. The plasma:blood partition coefficient had a mean value of 1.7<sup>12</sup>.

In 2010, Auwarter et al. concluded that  $\Delta^9$ -THCA-A can be incorporated in hair only by external contamination e.g. by condensate of smoked cannabis material and not by blood

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or sweat and  $\Delta^9$ -THCA-A could be a valuable marker for external cannabinoid contamination of hair<sup>13</sup>. In conclusion,  $\Delta^9$ -THCA-A can be detected in blood and urine samples of cannabis consumers and this detection in biological fluids may serve as a marker for differentiating

between the intake of legally prescribed  $\Delta^9$ -THC medication and illegal cannabis products. However, the knowledge about its usefulness in forensic cases is very limited and further research is needed.

## $\Delta^9$ -τετραϋδροκανναβινολικό οξύ A: ένας αξιόπιστος δείκτης για τη διάκριση μεταξύ της χρήσης παράνομων προϊόντων της κάνναβης και της λήψης φαρμακευτικής $\Delta^9$ -THC.

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**ΠΕΡΙΛΗΨΗ:** Το  $\Delta^9$ -τετραϋδροκανναβινολικό οξύ A ( $\Delta^9$ -THCA-A) αποτελεί την πρόδρομη ουσία της  $\Delta^9$ -τετραϋδροκανναβινόλης ( $\Delta^9$ -THC) στο φυτό *cannabis sativa*, χωρίς ψυχοδραστικές ιδιότητες. Το  $\Delta^9$ -THCA-A δεν εμπεριέχεται στα φαρμακευτικά προϊόντα που περιέχουν συνθετική  $\Delta^9$ -THC. Συνεπώς το  $\Delta^9$ -THCA-A μπορεί να χρησιμεύσει ως ένας δείκτης για τη διάκριση μεταξύ της χρήσης παράνομων προϊόντων της κάνναβης και της λήψης φαρμακευτικής  $\Delta^9$ -THC.

Λέξεις Κλειδιά: Κάνναβη,  $\Delta^9$ -τετραϋδροκανναβινόλη,  $\Delta^9$ -τετραϋδροκανναβινολικό οξύ A

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