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Original Article

Detection of "11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol" in Pubic Hair of Cannabis Abuser: Target Ion GC-MS Method

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ABSTRACT

Cannabis substance abuse is widely causing addiction across the world. In the drug of abuse cases, hair plays an important role in identifying drug abuse and drug history consumption. However, scalp hair may not be available in every drug abuse cases; other body hairs play an essential role in forensic toxicological analysis. This study attempt has been made to use pubic hair as an alternative specimen for the detection of 11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol metabolite of cannabis abusers using the target ion by GC-MS method. Pubic hairs of 10 years cannabis abusers were collected and used for GC-MS analysis to detect THC-COOH metabolite. The study confirmed the presence of THC-COOH metabolite with the help of 371 target ions. This method will be useful in identifying the presence of cannabis in drug-facilitated crimes for forensic toxicological analysis. In further, this method would also be useful in identifying single drug exposure cases.

Keywords: Pubic hair, 11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol, Drug abuser, Target ion GC-MS, Forensic toxicology

INTRODUCTION

Some of the common illicit drugs are Hashish, Marijuana, Ganja, and Bhang, in which the psychoactive products are procured from *Cannabis sativa* plants [1]. Cannabis is the most widely produced and abused drug across the world. About 192 million people consume cannabis; its consumption increases in developing countries than in developed countries. In the world, more than half of drug offenses cases from 2014 to 2018 are due to cannabis [2]. India is a developing country, and about 2.8% of Indians aged 10-75 years (3.1 crore individuals) are currently using cannabis [3]. In India, the ratio of cannabis abusers is much more than the other drugs of abuse. The identification of cannabis in viscera or other biological fluid is a challenge to forensic science. Therefore, hair is a broader window for drug abuse cases to extract and confirm cannabis metabolites for a longer duration. In every drug abuse case, scalp hair is not available for forensic toxicological analysis. In this scenario, body hair plays an essential role in forensic analysis. The 371-target ion of11-hydroxy-Δ9-tetrahydrocannabinol was known to confirm the drug of abuse in urine [4]. The drug incorporation in hair through the passive diffusion model is proposed [5, 6], which opens the window for hair analysis in case of drug-related deaths. Hair plays an important role in identifying drug abuser history even after single-dose exposure. It is possible to detect hair samples with non-invasive techniques [7]. In hair, drug metabolites are

incorporated and deposited for which the detection is made through metabolite identification.

The cannabis plant consists of 421 chemicals, in which 61 are the compounds of cannabinoids. Among all other cannabinoids, delta^9-tetrahydrocannabinol (Δ^9-THC) is more vital in inducing behavioural toxicity. It is a tri-cyclic structure with 21 carbon chains and two chiral centers that have no nitrogen compound. Δ^9-THC has high lipid solubility, which is volatile viscous oil. It is made up of mono-carboxylic acids, which are converted into de-carboxylate on the process of heating [8]. In cannabis, Δ^9-THC is an active psychoactive compound that gets metabolized in the liver by microsomal hydroxylation to form11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) in the presence of catalyst P450 and further oxidized to form11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol (THC-COOH) metabolites (Figure 1) [9].

THC-OOH is the vital compound in detecting the presence of cannabis in abusers [8].This study aimed to use pubic hair for the detection of 11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol metabolite of cannabis abusers using the target ion through the GC-MS method.

MATERIALS AND METHODS

Chemicals and Reagents

11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol is used as a standard which was purchased from Cerilliant TX, USA. Methanol, Double distilled water of analytical reagent grade, and Dimethyl sulfoxide solvent was used.

Sample Collected

Pubic hair of cannabis abusers was collected from Gwalior Mansik Arogyashala Centre, Gwalior, Madhya Pradesh, India. The subject is a 10-year cannabis abuser who discontinued taking cannabis. The pubic hair sample was collected after 40 days of cannabis usage. The consent was taken from rehabilitation centre and subject before collecting samples.

Sample inclusion criteria

- Pubic hair: only cannabis abuser.

Sample exclusion criteria

- The subject has not taken cannabis along with other psychoactive drugs.
- If the subject has abnormality related to health.
- The subject has observed any psychotic disorder.

Sample Preparation

50 mg sample was taken using an electronic balance and washed using double distilled water twice for 5 minutes. The washed hair sample was dried using an oven at 60-70 degree for 2 minutes. The dried sample was cut into small pieces and dissolved in 2 ml of methanol in a beaker, sonicated for 3 hours, and the same procedure was repeated [11]. After sonication, the extract was filtrated and decanted. Then 200 microlitres of dimethyl sulfoxide were added and were concentrated to use for GC-MS analysis. Dimethyl sulfoxide is an aprotic solvent in which all organic and inorganic compounds can be solubilized and can be used for further analysis. 100 microlitres of the standard solution were dissolved in 900 microlitres of methanol + dimethyl sulfoxide, which is used as a stock solution. Then, 100 microlitres stock solutions were taken and dissolved in 900 microlitres of methanol + dimethyl sulfoxide for GC-MS analysis.

Instrumentation

The extraction was carried out using Bio Technique Ultrasonicator; the extract was analyzed using GC-MS TQ8040 model with a triple quadrupole mass detector with helium as a mobile phase. The CID mobile phase was used to increase the detection sensitivity of cannabis metabolites. The oven temperature was programmed from 50-60 degrees. Gas Chromatography specification; DB-WAX column, total 7.5 minutes run time, initial column temperature is 70 degrees for 4 minutes, 240 degrees for 3.5 minutes with 181.5 kPa pressure, the total flow 21.0 mL/min, column flow 3 mL/min. Mass Spectroscopy specification; ion source temperature is 230 degrees, and the scan mode was selected for this analysis.

RESULTS AND DISCUSSIONS

Detecting the presence of cannabinoids is essential for drug treatment analysis, drug tests in the workplace, investigation of drug driving impairment, and pharmacokinetic analysis. The increase in the usage of cannabis has made a way to find an effective detection method [12]. Different analytical techniques were used to identify the presence of cannabinoids in urine, nail, blood, and hair. The analytical techniques include Gas Chromatograph-Mass Spectrometry (GC-MS), High-Performance Thin Layer Chromatography (HPTLC),

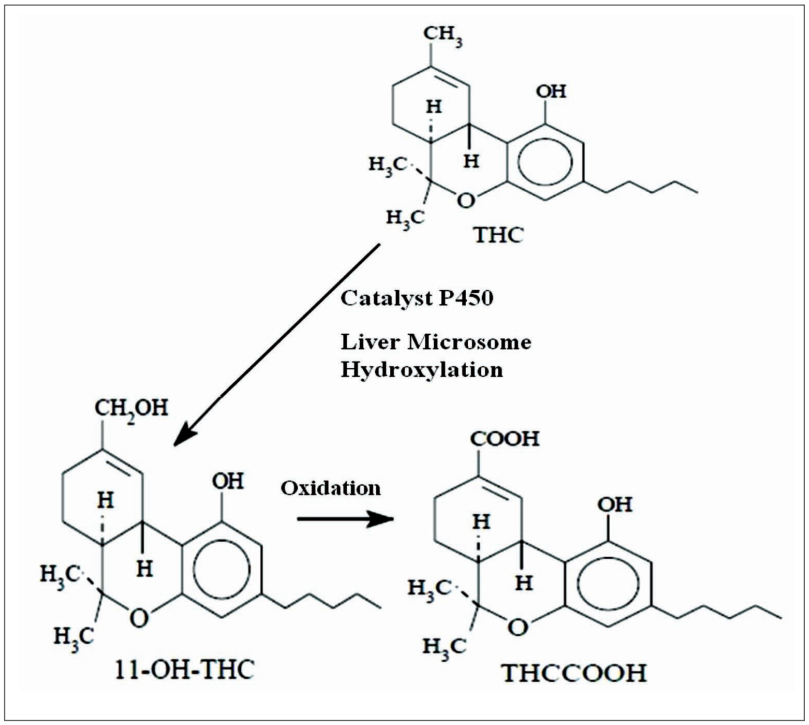

Figure 1: Mechanism of cannabis in forming THCCOH
Cannabis is converted into 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) by hydroxylation and further converted into 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH)

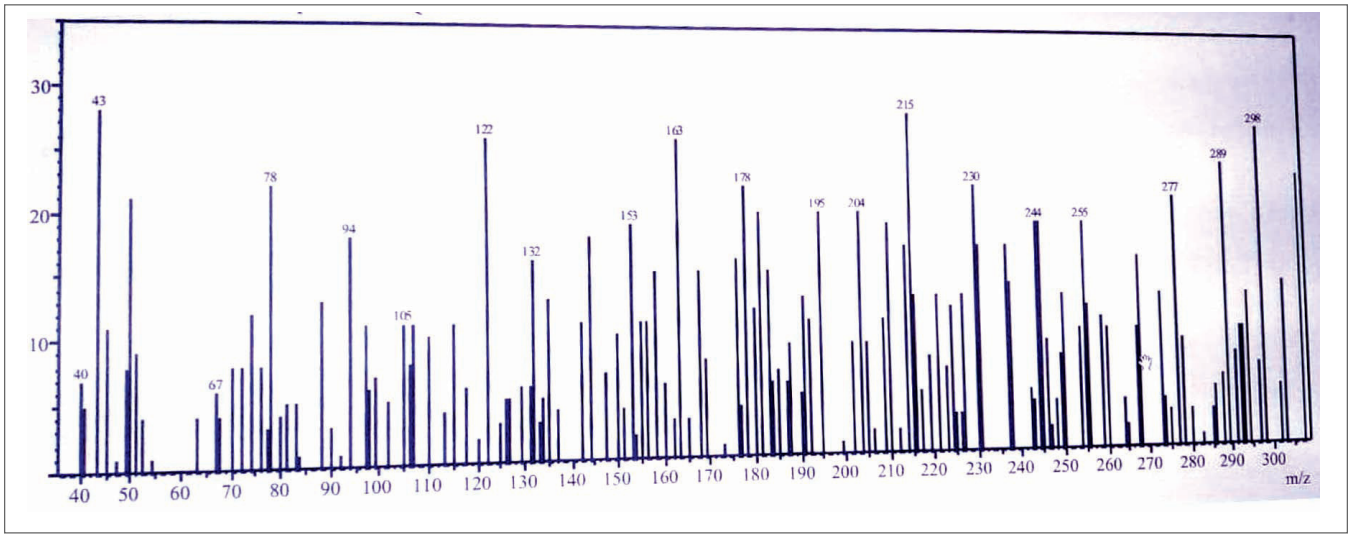

Figure 2: Blank mass spectra (dimethyl sulfoxide and methanol solvents)
The image represents the blank sample. X-axis: Retention time; Y-axis: the intensity of the signal. Each peak shows the presence of active compounds in which target ion 371 is absent.

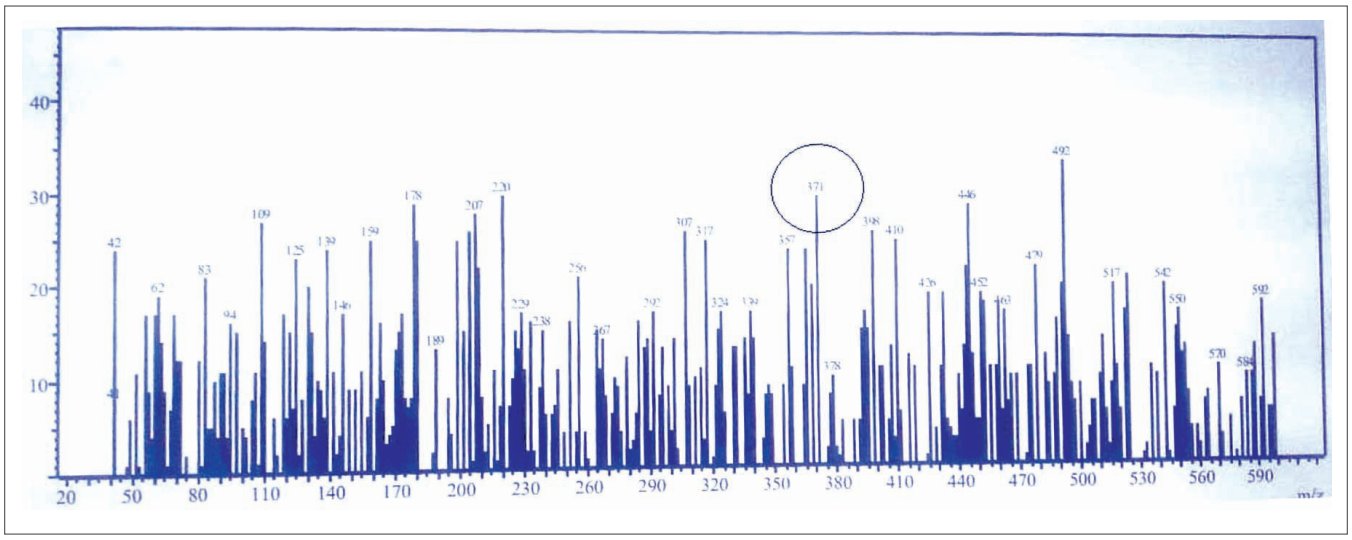

Figure 3: 11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol Standard Mass Spectra of 371 Target Ion
Image represents the standard sample. X-axis: Retention time; Y-axis: the intensity of the signal. Each peak shows the presence of active compounds in which target ion 371 is present (circled)

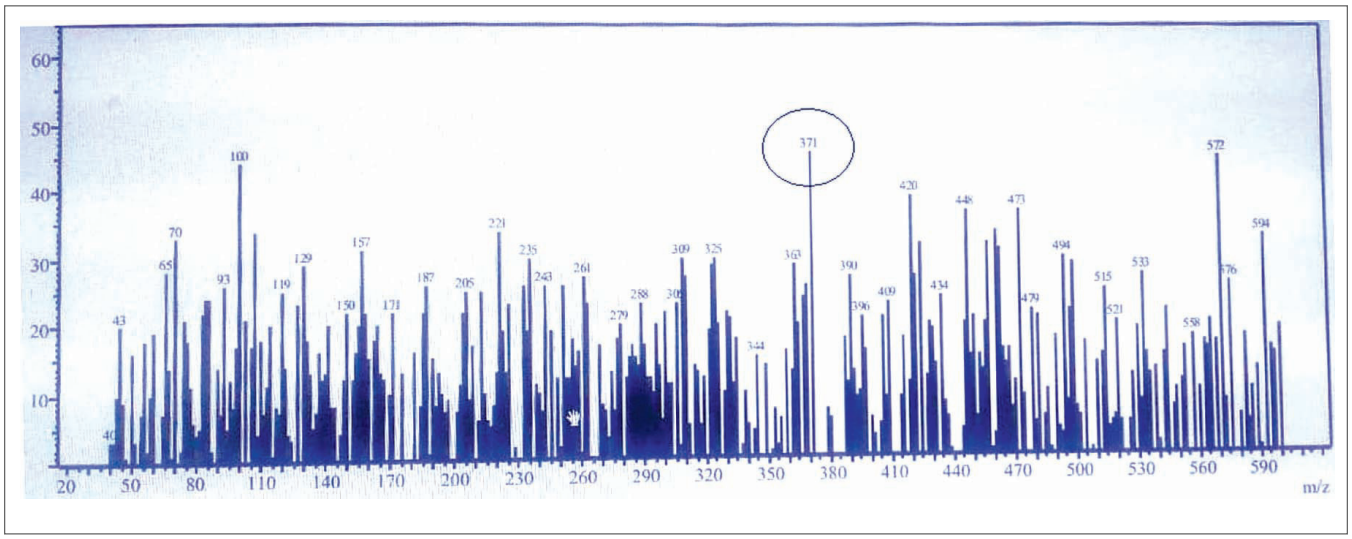

Figure 4: Pubic Hair Sample Mass Spectra of 371 Target Ion
The image represents the pubic hair sample. X-axis: Retention time; Y-axis: the intensity of the signal. Each peak shows the presence of active compounds in which target ion 371 is present (circled)

Thin Layer Chromatography (TLC), and Elisa fluorescence polarization radioimmunoassay [13]. In the present study, GC-MS analysis has successfully identified and confirmed the presence of 11-Nor-9-carboxy-Δ9 -Tetrahydrocannabinol using 371 target ion identification in mass spectroscopy. Studies reported that the target ions used for THC-COOH metabolite could be confirmed in urine through the GC-MS method [4,14]. Therefore, in the present study, the GC-MS method was applied to the pubic hair of the cannabis abuser to identify THC-COOH cannabis metabolites using 371 target ions. The target ion mass spectra of the blank sample (Figure 2), standard (Figure 3), and sample (Figure 4) were represented in the figure. It detected the sample and standard 371 target

ion height variation; hence the selected target ion can be used for the quantitative purpose of cannabis metabolite.

CONCLUSION

The study demonstrated 371 target ion detection for the confirmation of cannabis metabolites in pubic hair. This method can be used in various drug-facilitated crimes for forensic toxicological analysis. The target ion selection method is easy to quantify and identify cannabis metabolites. In the future, for single drug exposure cases, the target ion method can be applicable for confirming the drug of abuse cases.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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