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Gas Chromatography: Method Development and Validation for Identification and Quantification of delta-9-Tetrahydrocannabinol by Calibration Approach

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Cannabinoids like cannabidiol, cannabinol, delta-9-tetrahydrocannabinol (Δ9-THC) and several others are present in Cannabis drug. Under the NDPS Act of 1985, the cultivation, possession, use, transportation and trade of cannabis is crime. It is crucial forensic laboratory practice to identify and quantify Δ9-THC for prosecuting and reward purposes. In current study, a routine method for detecting Δ9-THC in cannabis based on fast gas chromatography coupled to flame ionization detector (fast GC-FID) was developed and validated. The method has analysis time of 7.0 min and outstanding linearity ($r^2 > 0.999$), with good repeatability (intraday < 2.55%; interday < 4.5%) and sensitivity (LOD = 1.86 ppm LOQ = 6.214). The fast GC-FID method suitability for detection and quantitation of Δ9-THC in cannabis, was tested successfully. Therefore, the developed Fast GC-FID method could be a valid alternative for a fast, robust and sensitive determination of Δ9-THC present in seized cannabis.

Keywords: Cannabis, delta-9-Tetrahydrocannabinol, Fast GC-FID, Linearity, Repeatability, Sensitivity.

INTRODUCTION

Cannabis is a tall, strong, dioecious annual herb that can reach heights of 3 to 15 feet. According to the World Drug Report 2021, the cultivation of cannabis plant was reported to UNODC by 151 countries, which was 97% of the world population [1]. Hashish, bhang and ganja are the different preparations obtained from various parts of cannabis plant. Hashish or charas is claimed to be pure resin in India [2]. delta-9-Tetrahydrocannabinol (Δ9-THC) is the principle active component present in the cannabis plant [3]. Other than Δ9-THC, cannabidiol (CBD), cannabinol (CBN) and other cannabinoids can be isolated from cannabis [4,5]. High Δ9-THC and low CBD and CBN concentrations are the desirable characteristics of cannabis abused for recreational purposes. Synthetic cannabinoids are a major class of new psychoactive substances (NPS) which are functionally similar to the Δ9-THC with structural modifications [6] such as JWH-018, JWH-073, JWH-398, *etc.* are becoming popular among drug addicts [7]. Unequivocal identification and quantification of Δ9-THC are the primary objectives of Cannabis analysis.

Analysis of Δ9-THC is carried out by various techniques such as thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), infrared spectroscopy (IR), near infrared spectroscopy (NIR), voltammetry, mass spectrometry (MS), *etc.* [8-11]. According to the scientific working group for the analysis of seized drugs, IR and MS are classified as A techniques while GC, HPLC and TLC as B techniques based on their discriminating power [12]. The TLC, IR, MS are useful only for the qualitative analysis but when both qualitative and quantitative analysis are required GC and LC are most useful.

In case of cannabis, it has been observed that the relative amounts of cannabinoids in a sample change with the geographical location [13]. Thus, GC and HPLC are the most helpful chromatographic techniques for comparisons in this kind of study [13]. A simple and precise HPLC approach using a diode array detector (DAD) was developed and validated by De Backer *et al.* [14] in 2019 for the quantification of the principal neutral and acidic cannabinoids (THC), THC acid (THCA), cannabidiol (CBD), CBD acid (CBDA), cannabigerol (CBG), CBG acid (CBGA) and cannabinol (CBN) in plant material [14]. It tooks more than 20 min for the separation of THC with 0.025% LOD and 0.05 LOQ for the same. Many other methods are also developed for Δ9-THC analysis using HPLC [15-17].

Casiraghi *et al.* [18] in 2018 developed and validate method for detection and quantification of cannabinoids by GC using flame ionization detector (FID) and MS detector, respectively. It was possible to detect CBD, Δ9-THC, CBN, CBD-A, THC-A within 21 min with GC-MS whereas with GC-FID method all four components were identified and quantified within 12 min using 1% BSTFA as derivatizing agent. Chan [19] demonstrated a cost effective validation procedure for profiling of cannabis by avoiding expensive chemical standards, whereas Cardenia *et al*. [20] proposed a fast GC-MS method for the detection and determination of tetrahydrocannabivarin (THCV), cannabidiol (CBD), cannabichromene (CBC), CBG acid (CBGA), THC acid (THCA), delta-9-tetrahydrocannabinol (Δ9-THC), cannabigerol (CBG) and CBG acid (CBGA) by silylation derivatization approach.

The GC techniques are rapid and cost effective compared to HPLC techniques for which reason GC techniques can preferably be used in forensic laboratories for analysis of delta-9-tetrahydrocannabinol (Δ9-THC). To identify and quantify cannabinoids, GC can be used with MS or FID. Therefore, the objective of the current study is to develop an alternate simple GC-FID method for detection and quantitation of THC, which is quick, easy to use and sensitive.

EXPERIMENTAL

delta-9-Tetrahydrocannabinol (Δ9-THC) was purchased from Cerilliant (Sigma-Aldrich, India), prepared in methanol having concentration of 1000 ppm. *n*-Hexane (99%) (HPLC grade) was obtained from SRL chemicals and acetone from Merck.

Trace 1310 gas chromatography (Thermo-Scientific, US) preinstalled with low bleed Trace GOLD TG-5MS 0.25 µm film thickness (30 m × 0.25 mm, internal diameter) capillary column was used for qualitative and quantitative determination of Δ9-THC. The gas chromatography was equipped with Triplus RSH auto sampler, flame ionization detector and combo gas generator (Claini Brezza), which was used for production of carrier gas *i.e.* nitrogen as well as hydrogen and air for makeup gas preparation.

Preparation of standards: A 1000 ppm of Δ9-THC certified reference standard solution was used for preparation of the working standard solutions. Working standards were prepared in acetone: *n*-hexane (9:1) used as a diluent. The calibration graph was plotted in the range of 1, 10, 20, 50, 75 and 100 ppm.

Sample preparation for case samples: Total 53 seized samples suspected of cannabis were ground to prepare a uniform sample. Each sample was diluted in acetone:*n*-hexane (9:1) and ultrasonicated for 5 min. The plant materials were mixed properly and then settle down after some time. After carefully filtering it with a syringe filter, an aliquot (1 mL) was transferred into a gas chromatography vial. Each aliquot was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Method development and optimization: The method development for Δ9-THC was based on its chemical and physical properties. Since Δ9-THC, a non-polar molecule, acetone: hexane (9:1) was utilized as a diluent, which is polar in nature. The low polar Trace GOLD TG-5MS capillary column was used as a stationary phase. The parameters of developed method were based on its boiling point, which is 157 ºC. The temperature programme was optimized. The injection port, oven and detector were all programmed to operate at isothermal mode with temperature of 275 ºC with a run time of 7 min. The split mode was chosen with split ratio of 95:5 having 5 mL/min split flow. Nitrogen is used as a carrier gas with a flow rate at 1 mL/min rate. The air, hydrogen and makeup flow of FID was 350, 35 and 40 mL/min, respectively. Δ9-THC had a retention time of about 5.597 min and a well-defined peak. The 1 µL injection volume was also reproducible and the peak response was considerable at the selected analytical concentration, according to preliminary precision and linearity measurements carried out during the method's development. Fig. 1 shows chromatogram of standard Δ9-THC and seized sample suspected as cannabis with presence of Δ9-THC.

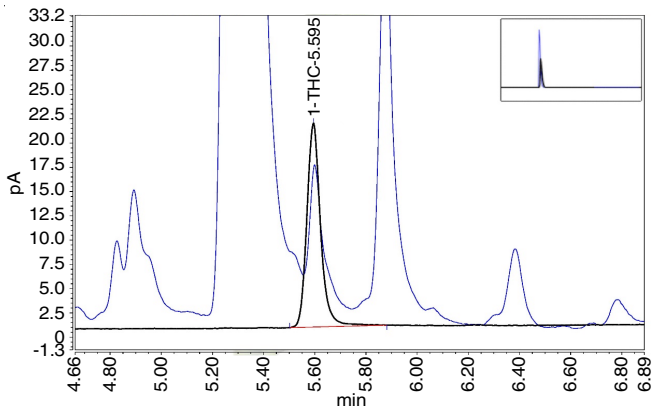

Fig. 1. GC-FID chromatogram of THC

Method validation

Linearity: The linearity was plotted in the range of 1-100 ppm. The peak area of Δ9-THC was proportionally changing with respect to concentration. The calibration curve offered excellent linearity *i.e.* $R^2 = > 0.999$ with less than 15% residuals (Fig. 2).

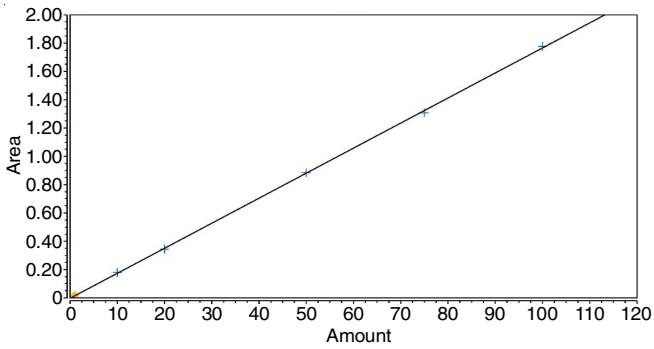

Fig. 2. Linearity plot of delta-9-tetrahydrocannabinol (Δ9-THC) detection

Precision: Two modes, intraday and interday precision, were used to evaluate the analytic method's precision. Three separate standard sample concentrations were examined in a

single day for intra-day precision, which was observed to be 2.55%. By analyzing three distinct concentrations once a day for 2 days, the interday precision was estimated and determined to be 4.5%.

Standard deviation (SD) and relative standard deviation (RSD): The SD and RSD were studied at concentrations similar for recovery study to know the deviation of individual concentration from its mean value. The SD and RSD of the method were calculated and found to be 0.315 and 0.9291, respectively.

Recovery: The recovery was examined at six different concentration levels, 5, 10, 12.5, 25, 50 and 100 ppm, to assess the accuracy by the proposed method. The ratio of observed to actual concentration was multiplied by 100 to determine the recovery. The total recovery of the present method was calculated to be 100.50%.

Limit of detection (LOD) and limit of quantitation (LOQ): Serial dilutions of Δ9-THC stock solutions were used to evaluate the LOD and LOQ in order to achieve signal-to-noise ratios of 3:1 for LOD and 10:1 for LOQ. It was observed that the analyte's LOD and LOQ values were 1.86 and 6.214 ppm, respectively. The results of this method were compared with others reported in the literature for the analysis of Δ9-THC using different analytical techniques (Table-1).

Population study: Population study was carried out to ascertain the reliability of the developed method. The study included 53 samples of cannabis sativa to detect and quantify the Δ9-THC (Figs. 3 and 4). Each of the samples were extracted as per the proposed method and the extracts obtained were analyzed quantitatively for Δ9-THC content.

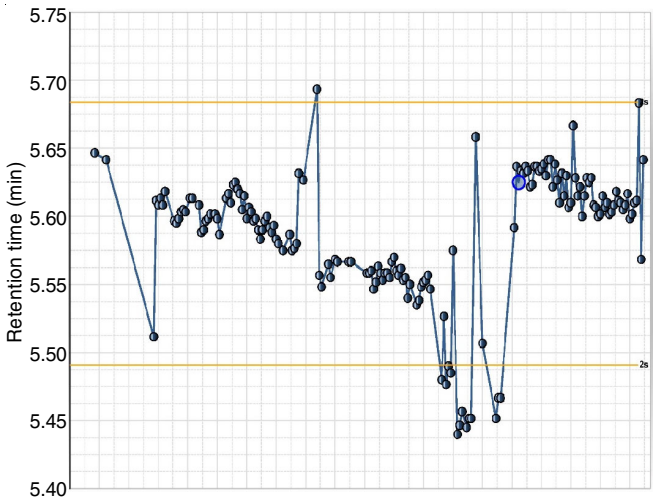

Fig. 3. Qualitative analysis of seized *Cannabis sativa* samples

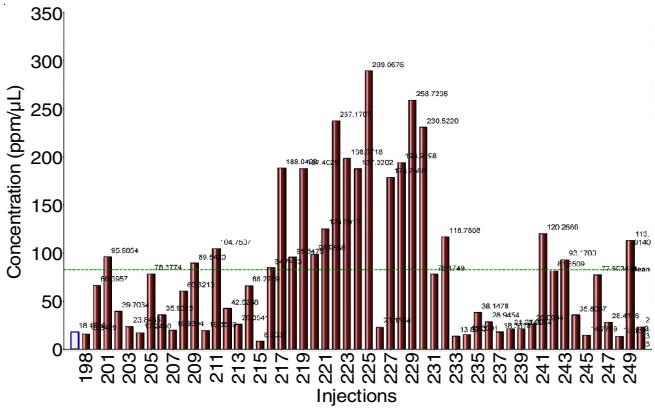

Fig. 4. Quantitative analysis of seized *Cannabis sativa* samples

Conclusion

In this work, delta-9-tetrahydrocannabinol (Δ9-THC) in the seized materials was determined using a new, simple, fast, sensitive, reliable, specific and accurate GC-FID method. Without any influence from the seized materials, the method described in the present paper has been employed effectively and efficiently to analyze Δ9-THC. Hence, the routine seized testing analysis of cannabis sativa in analytical and forensic preparations can be perform using the cost effective and rapid GC-FID method.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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TABLE-1
COMPARISON OF THE PROPOSED METHOD WITH OTHER METHODS OF THE ANALYSIS OF THC

Technique	Analyte	Linearity for THC	LOD	LOQ	Run time	Ref.
GC-MS	CBD, THC, CBG and CBN	0.976-1.000	–	–	5 min	[5]
HPLC-DAD	THC, THCA, CBD, CBDA, CBG, CBGA, CBN	0.9940	0.05%	0.025%	25 min	[14]
GC-FID	THC, CBD, CBN, THCA, CBDA	0.9989	0.03%	0.1%	12 min	[18]
GC-MS	THCV, CBD, CBC, CBDA, THCA, D9- THC, D8-THC, CBG, CBN and CBGA	0.9994	10.7 µg/mL	32.40 µg/mL	< 7 min	[20]
HPLC-DAD	CBD, CBDA, THC, CBN, THCA	0.9920	0.01-0.05 mg kg^{-1}	0.08-0.30 $mgkg^{-1}$	50 min	[21]
GC-FID	THC	0.9996	1.86 ppm	6.214ppm	7 min	This study

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