Development of Rapid Detection and Estimation of Unexploited Cannabinoids from source Cannabis sativa and Cannabis indica by RP-HPLC

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Abstract: Siwan (Bihar) being a better surface to give loamy soil advances the development of a huge number of plants. Some basic hallucinogenic plants viz. Cannabis sativa L., and Cannabis indica (Lam.), were picked for the investigation of their dynamic constituents Tetrahydrocannabinvarin, Cannabinol and Cannabichromene. Cannabis has recaptured a lot of consideration because of refreshed enactment approving a wide range of employments and can be characterized based on the substance of Tetrahydrocannabinvarin (THCV), a psychotropic substance for which there are lawful impediments in numerous nations. For this reason, precise subjective and quantitative assurance is fundamental. The connection among Tetrahydrocannabinol (THC) and cannabidiol (CBD) is likewise critical as the last substance is invested with numerous particular and non-psychoactive legitimacies. Hence, it turns out to be progressively significant and earnest to use quick, simple, approved and blended systems for assurance of cannabinoids. This research work targeted to develop quick standard operating protocol for characterizing cannabinoids from the hallucination causing plants of locale of Hathwa. The methodology portrayed in this permits fast assurance of 3-cannabinoids from the inflorescences of Cannabis sativa L. just as Cannabis indica (Lam.) by the extraction with certain solvents (Acetonitrile: Methanol ; 70:30). Detachment and ensuing recognition are by RP-HPLC. Evaluation is performed by an outside standard technique through the development of adjustment blends utilizing unadulterated standard chromatograph substance confirmation of Tetrahydrocannabinvarin, Cannabinol and Cannabichromene were performed using reference mixtures. THCV was found to be the least amount. The limit of detection was 0.62 µg/ml, 0.32 µg/ml and 0.34 µg/ml for THCV, CAN and CBN respectively.

Keywords: Tetrahydrocannabinvarin, Cannabinol, Cannabichromene, Limit of quantification.

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1. INTRODUCTION

The genus Cannabis (Family Cannabaceae) is probably indigenous to wet habitats of Asiatic continent. The long coexistence between mankind and Cannabis led to an early domestication of the plant, which soon showed an amazing spectrum of possible utilizations, as a source of textile fibers as well as narcotic and psychoactive compounds. Nowadays, the species belonging to the genus Cannabis are represented by myriads of cultivated varieties, often with unstable taxonomic foundations. The nomenclature of Cannabis has been the object of numerous nomenclatural treatments. On the general platform Cannabis indica (L.) and Cannabis sativa (Lam.) both possess cannabinoids which are responsible for brawny hallucinations. In this research Photochemistry of Cannabis indica (L.) and Cannabis sativa(Lam.) has been explored through optimization of extraction procedure and Quantitation through HPLC. Cannabis is an unpredictable plant with more than 400 synthetic substances of which more than 60 of them are cannabinoid intensifies, some of them with contradicting impacts. Cannabis is additionally the most generally utilized unlawful medication on the planet and its utilization has been related with different psychological well-being issues, especially in the youthful. Despite the fact that cannabis has been utilized and developed by humanity for at any rate 6000 years. Our present study and information on its pharmacological properties depends on thoughts about which have occurred uniquely since the finish of the nineteenth century. The absolute first compound separated in unadulterated structure from the plant was cannabidiol. It was at first wrongly thought to be the principal dynamic compound of the plant liable for its psychoactive impacts. The subsequent compound discovered was cannabidiol (CBD) by . Gaoni and Mechoulam, 1967 in the next year disengaged the principle dynamic compound, delta-9-tetrahydrocannabinol d-9-THC. The cannabis plant has two primary subspecies, Cannabis indica (Lam.) and Cannabis sativa and they can be separated by their distinctive physical attributes. Indica-predominant strains are short plants with wide, dim green leaves and have higher cannabidiol content than the sativa plants in which THC content is higher. Sativa-prevailing strains are generally taller and have dainty leaves with a light green shading. Because of its higher THC content, C. sativa (L.) is the favored decision by clients. It is a mind boggling plant with around 426 substance elements, of which more than 60 are cannabinoid mix. The four major compounds are d-9-THC, CBD, d-8-THC and cannabolin, which have been most researched. In the plant, cannabinoids are orchestrated and gathered as cannabinoid acids, however when the home grown item is dried, put away and warmed, the acids decarboxylate step by step into their appropriate structures, for example, CBD or d-9-THC. Initially it was imagined that CBD was the metabolic parent to d-9-THC, however it was later discovered that its biosynthesis happens as indicated by a hereditarily decided proportion. Despite the fact that the synthetic structures of each of the four mixes are comparative, their pharmacological impacts can be altogether different. The most inquired about mixes of the plant are d-9-THC and CBD and in this manner we will chiefly concentrate on these two mixes and their disparities. Recently, in Italy the interest in Cannabis sativa L. has increased mainly due to the latest legislation. As a consequence, there is a request to develop cost-effective and easy-to-use quantitative and qualitative methods for analysis of cannabinoids. The Italian regulatory framework has classified two types of Cannabis sativa L. depending on the content of Δ9-THC. In particular, fiber-type plants of Cannabis sativa L., also called “hemp”, are characterized by a low content of Δ9-THC (0.6% w/w, it is considered as drug-type, also called “therapeutic” or “marijuana”. In our study we made a point by point standard working methodology (SOP) practiced with certain changes by Srikantharam as a strengthening data document, is moreover accessible, so any administrator with fundamental information on HPLC can without much of a stretch apply and make all the elution and alignment control checks utilizing monetarily accessible blends of extricates, which are more moderate and reasonable than single cannabinoid analysis in accordance to expenses and solvents utilized for alignment

2. MATERIALS AND METHODS

2.1 Cannabinoid Extraction

To extract the Phytoconstituents of Cannabis, an aliquot of powder sample of about 25 mg, was weighed using an analytical balance; 10 ml of methanol-chloroform extraction solvent 9:1 (v/v) was added as reported by and was placed first for 10 min on an oscillating oscillator set at 350 oscillations per minute and then for 10 min in an ultrasonic bath. The sample was centrifuged for 10 min at around 1000 rpm, and the supernatant was removed. The extraction was performed twice. The two different fractions containing cannabinoids were collected in a 25 ml volumetric flask and were brought to volume with methanol/chloroform (9:1, v/v). The samples were filtered with a 45 µm nylon filter.2 ml of the filtered extract (through 0.45 micrometers) was transferred to a glass tube. The solvent was removed, leading to dryness with the help of a weak nitrogen flow, and recovered with 500 µl acetonitrile. The solutions were then injected into an HPLC as method mentioned by .

2.2 Preparation of Standard Solution

Appropriate aliquots of a standard mixture of cannabinoids were diluted with acetonitrile to obtain solutions of known concentration, in particular eight points in a concentration range between 0.05 and 100 µg/ml (0.05, 0.50, 4.17, 8.33, 16.70, 25.00, 50.00, 100.00 µg/mL). The standard solutions were prepared to construct calibration curves for the 2 cannabinoids considered: CBD, THCV and CBN. The standard solutions were stored away from light at a temperature of -20°C. The stability of standard solutions stored at 20°C was evaluated.

2.3 HPLC Conditions

For the RP-HPLC investigation, the segment was thermostated at 35°C, and the autosampler was thermostated to 4°C. Test focus was 4 mg/ml and infusion volume was 5.0 µl. UV- discovery was utilized at 220 nm, and inclination elution was utilized at stream pace of 1.6 ml/min as indicated by the accompanying system. Eluent blend: Water + 0.085% phosphoric corrosive (An), acetonitrile + 0.085% phosphoric corrosive (B). Slope elution: 70% of B up to 3 min, 85% of B to 7 min, 95% of B to 7.01 up to 8.00 min, and 70% of B up to 10 min. The eluent blend was recently separated with a Millipore framework outfitted with a 0.2 µm nylon channel.
2.4 Linearity

The linearity was assessed with the help of calibration plot by plotting the graphs between area under the curve and retention time in minutes the standard calibration Tetrahydrocannabivarin 9714.3x+ 23795 and R² value is 0.9956 for the standard of Cannabinol the straight line equation 17806x + 200566 and the R² value is 0.9966. For the standard Cannabichromene the straight line equation will be y=1235.5x+ 24354 R² = 0.9805.

2.5 Quantification Limit, LOQ

The instrumental furthest reaches of measurement was dictated by an adjustment bend, as per the equations communicated in Section 3.6, taking into account that the sign-to-clamor technique is especially helpful to evaluate the cannabinoids present at lower focuses, for example, THC. As announced for the LODs, the instrumental furthest reaches of measurement (LOQ) values got for CBDA and CBGA were likewise lower than those detailed in the writing, while those for CBG and CBD were tantamount with those of different techniques depicted for comparative systems. Furthermore, the different cannabinoids (THCV and CBC) indicated low LOQs. The instrumental clamor was enrolled in µV, by performing 3 clear infusions with the ASTM technique given by the instrument, and a most extreme CV% of 3.49% was determined for every individual compound to decide the single LOD and LOQ, which was viewed as satisfactory.

3. RESULTS AND DISCUSSION

The aim of this work was to develop a new analytical method for determination of the left out cannabinoids in hemp samples which possess hallucinating properties not very catastrophic but are toxic as discussed by 17. They in any source could be harmful and even fatal a rapid development method through HPLC with the help of suitable extraction procedure of cannabinoids from regular obtaining sources. In fact, the method described below can be used as a routine quality. Different mobile phases were tested, and trials were performed with different compositions and gradient elution to optimize the separation of all 3 target compounds considered (which are not much exploited). The greatest difficulty was that of separating CBD and THC, which in many cases co-eluted also found in the work of Patel and Wene. It was also difficult to separate the isomer alkaloids. The best resolution of cannabinoids was obtained using a chromatographic column and, as an eluent mixture, water with 0.085% phosphoric acid and acetonitrile with 0.085% phosphoric acid. The quantification of cannabinoids was made at 220 nm after testing different wavelengths (220nm 228nm 230nm). This wavelength represents the best compromise for all the cannabinoids considered and was selected to detect and integrate all compounds of interest within the dedicated concentration range 18. As far as chromatographic analysis is concerned, before using the instrument, the system was conditioned for 20 min by fluxing the eluent mixture in the instrument under the same conditions as the method, and then a chromatographic run was performed by injecting 5 µl of acetonitrile to verify that the chromatographic system was adequately cleaned19. Simultaneously with the analysis of the sample, standard solutions were injected at different concentrations for the construction of calibration curves and to evaluate the separation and identification of each compound. The identification of cannabinoids was performed by comparing their retention times with those obtained by the injection of pure standards and by an enhancing procedure. Figure 1 shows a chromatogram of a standard mixture of cannabinoids viz. Tetrahydrocannabivarin, Cannabinol, Cannabichromene and Figure 2 shows a chromatogram of samples (Cannabis sativa. And Cannabis indica Lam.).
Fig 2(a) HPLC chromatogram of Cannabis sativa L. leaf extract having multiple peaks the peak of 5.283 minute demonstrates the standard of Tetrahydrocannabivarin, 2.425 minute Cannabinol, 3.383 minute Cannabichromene. Here an enormous amount of Cannabinol has been extricated out while a very slight amount was obtained of Cannabichromene.

Figure 2(b) HPLC chromatogram of Cannabis sativa L. leaf extract having multiple peaks the peak of 5.283 minute demonstrates the standard of Tetrahydrocannabivarin; 2.425 minutes Cannabinol and 3.383 minutes for Cannabichromene the related peaks in the above plot is 2.083 closely showing association to Cannabinol while there is a sharp peak at 3.825 minutes showing large detection of Cannabichromene.

Figure 2 (c) The above figure represents the extraction of Cannabinoids from seeds and leaves of Cannabis indica (Lam.) the peak in standards, the peak of 5.283 minutes demonstrates Tetrahydrocannabivarin, 2.425 minutes Cannabinol and 3.383 minutes for Cannabichromene.

Fig 2(d) Figure 2 (d) the figure above denotes Chromatogram of Cannabis indica seeds, the peak at around 2.633 minutes corresponds to standard Cannabinol while the peak at around 4.358 minutes corresponds to Cannabichromene, and the peak of Tetrahydrocannabivarin was not obtained. This depicts that Tetrahydrocannabivarin is present in a very limited amount.
In the four purified extracts of Cannabis sativa (L.) and Cannabis indica (Lam.) the contents of Tetrahydrocannabinol and Tetrahydrocannabinol were detected. The limit of detection on average and with standard deviation from the mean value appeared to be 0.34 µg/ml, Cannabinol 0.32 µg/ml and Cannabichromene 0.62 µg/ml. The limit of detection (LOD) is usually defined as the lowest quantity or concentration of a component that can be reliably detected with a given analytical method. While the concentration of Limit of Quantification which is actually the lowest analytes concentration that can be quantitatively detected with a stated accuracy and precision.

4. DISCUSSION

One of the most significant issues in scientific judgments for quality control, particularly when there are legitimate issues related with quantitation, for example, for cannabis, identifies with the capability of research centers. Along these lines, point by point and approved methods that are unreservedly accessible are fundamental for the full comprehension of any diagnostic advance and its cautious application. This is likewise valid for "day by day" techniques that can be effectively applied for quality control, completed utilizing conventional RP-HPLC and UV-Vis finders, with less productive execution than diode-cluster locators yet with lower costs, rendering them moderate in any event, for little research facilities. The approved technique portrayed in this permits the quantitative assurance of the 10 most pertinent cannabinoids utilizing a solitary wavelength (220 nm) in 8 min. A full division is acquired, even in the elution arrangement of troublesome goals, of the gathering of pinnacles identified with Tetrahydrocannabinol, Cannabinol And Cannabichromene (from 2.425 minutes to 3.383 minutes).

5. CONCLUSION

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6. AUTHORS CONTRIBUTION STATEMENT:

The above work has been written and performed by Priyanka Kumari under the supervision on Dr. Md. Sarfaraz Ahmad. The instrument authentication and data computation was well discussed and then mentioned in the paper.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

Table 1: Validation Framework of RP-HPLC method For Cannabinoids

<table>
<thead>
<tr>
<th>Compound</th>
<th>R²</th>
<th>LOD µg/ml</th>
<th>LOQ µg/ml</th>
<th>Intraday (Repeatability)</th>
<th>Inter day (Repeatability)</th>
<th>Reproducibility (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrahydrocannabinol</td>
<td>0.9956</td>
<td>0.34</td>
<td>1.05</td>
<td>6.65</td>
<td>5.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Cannabinol</td>
<td>0.9966</td>
<td>0.32</td>
<td>0.98</td>
<td>8.97</td>
<td>5.09</td>
<td>2.13</td>
</tr>
<tr>
<td>Cannabichromene</td>
<td>0.9805</td>
<td>0.62</td>
<td>1.87</td>
<td>5.65</td>
<td>6.09</td>
<td>2.17</td>
</tr>
</tbody>
</table>

Technological limitations in numerous nations which have Hallucinogens ban, with an absolute Cannabinol content above 0.4%, and also export import of hemp as well as hemp constituting products are banned as also referred by an early report. Because of its straight forwardness and rate, it can be utilized to check crude material or yields during the gathering time frame. In our study, we made a point by point standard working methodology (SOP) and practiced with certain changes by Srikantaramas, as a strengthening data document, it is moreover accessible, so any administrator with fundamental information on HPLC can without much of a stretch apply it and make all the elution and alignment control checks utilizing monetarily accessible blends of extricates, which are more moderate and reasonable than single cannabinoid analysis in accordance to expenses and solvents utilized for alignment.
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