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**Artigo**

# CANNABIS THIN LAYER CHROMATOGRAPHY (TLC) CHEMO TYPING: HOW ACCURATE IS TLC COMPARED TO HIGH PRESSURE LIQUID CHROMATOGRAPHY?

## DIGITAÇÃO DE QUIMIOTERAPIA DE CANNABIS THIN LAYER CHROMATOGRAPHY (TLC): QUAL A PRECISÃO DO TLC EM COMPARAÇÃO COM A CROMATOGRAFIA LÍQUIDA DE ALTA PRESSÃO?

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**ABSTRACT:** Problem: Brazilian Cannabis Associations (BCA) search for methods of reducing their costs and increasing their quality control of Cannabis *spp. Oil* and extracts. The significant difficulties presented by ABRACAM (Associação Brasileira Cannabis Medicinal) to UFPI professors were to standardize cannabinoids profiles of its plants and guarantee the same levels of cannabinoids on its artisanal extracts. ABRACAM has difficulty standardizing plant cannabinoids profiles due to Cannabis *spp.* Phenotypic plasticity, or the ability of Cannabis spp. to change attributes like the size of leaves, levels of secondary metabolites, stems structures, flowering period, among others. The second problem is that artisanal extract combines different production batches of non-tested extractions. It is necessary to quantify and qualify each cannabinoid and how much is on artisanal Cannabis production. Aiming to solve the ABRACAM production problem, UFPI and CANNAPI suggested manual testing practices to test cannabis and its derivates. This article results from validation tests performed at ABRACAM to determine how precise manual methods are to quantify cannabinoids. Methods: This research compared the cannabinoids results of an off-the-shelf Thin Layer Chromatography (TLC) cannabinoids profile test kit with the results of a High-Pressure Liquid Chromatography (HPLC). UFPI perfect the method from the vendor manual after running sixty quantifications from one sample. Results: The TLC error compared to HPLC was between approximately 0,5% and 1%. ABRACAM differentiated the

Chemotype Profile of its High THC varieties and High CBD varieties. For High CBD variety, the outdoor cultivation proportionated greater cannabinoids levels. Conclusions: The comparison pointed that TLC may be used as testing equipment for this Brazilian Association due to its artisanal production license. This article concludes that precision is the key to medication production, and the TLC error may be accepted for artisanal production but not as a standard for precision. For scientific purposes, TLC is a strong hypothesis builder, and for agriculture, purposes may be a vital tool point the right time to harvest.

**KEYWORDS**: Thin Layer Chromatography (TLC), Cannabis Quality Control, Cannabis Social Clubs, Brazilian Medical Cannabis Associations.

**RESUMO:** Problema: Associações Brasileiras de Cannabis (BCA) buscam métodos para reduzir seus custos e aumentar seu controle de qualidade de Cannabis spp. Óleo e extratos. As dificuldades significativas apresentadas pela Associação Brasileira Cannabis Medicinal aos professores da UFPI foram padronizar os perfis canabinóides de suas plantas e garantir os mesmos níveis de canabinóides em seus extratos artesanais. ABRACAM tem dificuldade em padronizar perfis de canabinóides de plantas devido a Cannabis spp. Plasticidade fenotípica, ou a capacidade de Cannabis spp. para alterar atributos como o tamanho das folhas, níveis de metabólitos secundários, estruturas do caule, período de floração, entre outros. O segundo problema é que o extrato artesanal combina diferentes lotes de produção de extrações não testadas. É necessário quantificar e qualificar cada canabinóide e quanto está na produção artesanal de Cannabis. Com o objetivo de resolver o problema de produção da ABRACAM, a UFPI e a CANNAPI sugeriram práticas de teste manual para testar a cannabis e seus derivados. Este artigo resulta de testes de validação realizados na ABRACAM para determinar como os métodos manuais precisos são para quantificar canabinóides. Métodos: Esta pesquisa comparou os resultados de canabinóides de um kit de teste de perfil de canabinóides TLC (Cromatografia de Camada Fina) disponível no mercado com os resultados de uma cromatografia líquida de alta pressão (HPLC). A UFPI aperfeiçoou o método do manual do fornecedor depois de executar sessenta quantificações a partir de uma amostra. Resultados: O erro de TLC em comparação com HPLC situou-se entre aproximadamente 0,5 % e 1 %. A ABRACAM diferenciou o perfil quimiotipado de suas variedades de alto THC e variedades de alto CBD. Para a variedade High CBD, o cultivo ao ar livre proporcionou maiores níveis de canabinóides. Conclusões: A comparação apontou que o TLC pode ser utilizado como equipamento de teste para esta Associação Brasileira devido à sua licença de produção artesanal. Este artigo conclui que a precisão é a chave para a produção de medicamentos, e o erro de TLC pode ser aceito para a produção artesanal, mas não como um padrão de precisão. Para fins

científicos, o TLC é um forte construtor de hipóteses, e para a agricultura, os propósitos podem ser um ponto de ferramenta vital no momento certo para colher.

**PALAVRAS-CHAVE:** Thin Layer Chromatography (TLC), Controle de Qualidade da Cannabis, Clubes Sociais da Cannabis, Associações Médicas Brasileiras da Cannabis.



## 1. Introduction

This research article is the result of the combined effort of its authors to implement a quality control testing function at ABRACAM, a Brazilian Cannabis association. During six months, the organizations ABRACAM, Cannapi (Cânhamo Piauiense), and UFPI (Federal University of Piauí) researchers performed eight six thin-layer chromatography (TLC) identifying the best time to measure cannabinoid concentration.

Brazilian legislation did not recognize TLC as a valid method to measure cannabinoid concentration; however, Brazilian universities use TLC as a primary test regarding academic production as dissertation and thesis. Cannabis testing in Brazil is still incipient, and the Brazilian Cannabis spp. Subspecies are still unknown since classification needs chemo typing or the determination of concentrations of cannabinoids and terpenes [1].

Some authors [1] point out that HPLC (high-pressure liquid chromatography) must be the Cannabis testing standard for chemo typing - identifying and quantifying cannabinoids and terpenes of each variety. Authors agree about HPLC precision and difficulty coping with the financial obligations imposed by Brazilian legislation to purchase Cannabis testing equipment.

There are cannabis organizations known as Cannabis Social Clubs (CSC). These are Cannabis non-profit organizations that supply their members with Cannabis and its derivatives, allowing them to avoid contact with the black market [2]. For CSCs, quality products often maintain customers; however, CSC quality is not, in its majority, obtained by government labels or processes nor through laboratory testing; quality is a subjective variable based on clients' feedback [2].

Cannabis quality control is not an isolated Brazilian problem; CSCs also have quality control problems in other countries. In Brazil, CSCs are known as Cannabis Associations like ABRACAM and usually are organizations that initially are not well-financed and lack quality control via testing.

Belgian, one of the first countries to allow CSCs to operate, needed help maintaining quality control. A 2018 research pointed out that most Belgian CSCs used non-professional practices for quality control [3]. Client feedback is the leading non-professional practice mentioned by the authors, and TLC testing is considered a professional method that allows qualitative and semi-quantitative results [3].

CSC quality control is a significant factor in protecting public health since these non-profit organizations aim to proportionate users with potentially better cannabis products than unregulated production [4].

Medical cannabis patients joined CSC in Europe and South America to obtain Cannabis either to artisanal produce cannabis products or purchase medication [5]. Medical patients often receive more significant amounts of Cannabis than adult users; there is also a practice of discounting for medical purposes [5]. To the authors in Europe, limited CSCs work exclusively on medical users, as adult users are the prominent influencers of CSC financiers [5]. ABRACAM is an example of a Medical CSC that works exclusively with medical patients.

Authors [2,3,4,5] agree that European, American, and South American legislation for adult use and medical purposes has advanced since 2014. They

also agree that quality control by testing, mandatory in current legislation, is still challenging. It is difficult to enforce and control medical CSCs to cope with quality control standards [2,3,4,5].

The primary issue with TLC testing is knowing how accurate it can be. UFPI researchers used ABRACAM cannabis extract results of a high-performance liquid chromatography (HPLC) test as standard and later matched results with TLC quantification.

UFRN (Universidade Federal do Rio Grande do Norte) Neurochemistry Laboratory at Instituto Cérebro analyzed ABRACAM extracts to identify the concentration of significant Cannabinoids using HPLC-UV. UFPI (Federal University of Piauí) professors visited ABRACAM facilities to quantify and document the TLC readings.

According to the ANVISA (Brazilian Health Agency) technical note RDC N 166/2017, HPLC quantification is the only valid method in Brazil for measuring cannabinoids [6]. HPLC methods standardization for cannabinoid quantifying in Brazil is very expensive due to the necessity of importing the reference patterns for each tested cannabinoid [6].

UFRN professors agree with how expensive testing can be; the Instituto Cérebro at the time only had purchased *tetra-hydrocanbinol* (THC), *cannabidiol* (CBD), and *cannabinol* CBN references. The TLC test purchased measures, besides THC, CBD, and CBN, *tetrahydrocannabivarin* (TCHV), *cannabigerol* (CBG), and *cannabichromene* (CBC).

TLC is a method used for decades to measure cannabinoid concentrations in cannabis-based products [7]. United Nations (UN) recommends using TLC as an accurate and low-cost technique [8]. TLC is a source of reliable data at a low cost [9]. One of the problems with TLC is that human error may lead to unreliable readings, such as hand-spotting, temperature/humidity samples control, and sample weighting, among other procedures that could be performed better [10].

TLC can precisely detect cannabinoids in cannabis products as extracts or flowers, but only well-trained laboratory technicians may obtain accurate results [11]. Some authors compared TLC quantification with Gas Chromatography (GC), having errors above 20%. According to one of the authors, in a personal interview, the reported errors are due to untrained laboratory technicians using the TLC methodology [11].

There is a high-performance thin-layer chromatography (HPTLC) that automates the mobile and reading phases, and this technique results in no more than 0.5% (+/-) error as compared to an HPLC [12].

TLC passes through similar procedures as HPLC since both are liquid chromatography (LC) that dilutes cannabis samples in solvents before moving them to the stationary and mobile phases. The TLC stationary phase first marks the dropping spots target at silica gel plates, injecting diluted solution at silica gel plates. In contrast, the stationary phase involves positioning the silica gel plate on a running jar with a solvent.

HPLC stationary phase consists of preparing a coated column and injecting the sample from the column top. In contrast, the mobile phase uses a powered pump to mix the sample with solvent and move this mixture into the column at a constant rate.

The significant difference between methods is that the HPLC molecule detector is used to quantify cannabinoids more precisely. TLC is a non-automated method in which human and environmental conditions may affect results. Quantification is the linear result of measuring the bands of each separated component with a ruler.

There is one more aspect of Cannabis production, not yet treated the phenotypic plasticity. Cannabis *spp.* can change its characteristics according to environmental conditions by changing its chemotype profile [13].

Artisanal cannabis oil production relies on Cannabis *spp.* Subspecies chemotype profiles and Cannabis *spp* grown under the tropics tend to produce

more THC than CBD [14], a problem for Brazilian Associations needing products with low THC.

Phenotypic plasticity is a phenomenon that ABRACAM aims to control since its production is based on Cannabis *spp—subspecies* with high THC and subspecies with high CBD. Changes in plants' chemotypes interfere with the concentration levels of Cannabis extract medication and further patients' treatment success.

In this introductory chapter, we presented the quality control problem of ABRACAM on quantifying Cannabis and extracts with HPLC due to its high cost, positioned the quality control problem as a current and not resolved issue at CSCs worldwide, presented HPLC as a reference method used to set parameters in Cannabis testing and discussed TLC as a possible low-cost method to quantify cannabinoids.

The next chapter presents the method and the experiment conducted at ABRACAM to measure several cannabis and extract samples. The sample sent to UFRN was an extract from Brazilian Creoles Cannabis, a blend of Manga Rosa, Alecrim, and Rabo de Raposa Subspecies.

## 2. Material and Methods

This research aims to verify how precise thin-layer chromatography (TLC) solutions can be for cannabis products. This research design uses three steps (setting parameters, identifying TLC kits with low reading errors, and testing at the ABRACAM site). Setting the quantification parameters was the first step. ABRACAM sent a sample of Cannabis extract to the Neurochemistry Laboratory in Instituto Cérebro of UFRN (Federal University of Rio Grande do Norte). The extract results using HPLC-UV were 603,9 mg/g of tetrahydrocannabinol (THC), 0,0 mg/g of cannabidiol (CBD), and 0,0 mg/g of cannabinol (CBN).

The second step was identifying TLC kits and choosing a solution with the lowest error margin for readings. Cannapi chose the Alpha-CAT Cannabinoids test kit due to the lowest error margin published by vendors, which was 0.5% (-/+). Cannapi, the research sponsor, imported a test kit and donated it to ABRACAM. The Alpha-CAT kit quantifies six major cannabinoids: THC, CBD, CBN, TCHV, CBG, and CBC, as seen in Figure 1. The Alpha-CAT kit also qualifies organic composts as olivetol (cannabinoids precursor) and acids cannabinoids, as seen in Figure 2.

The kit has 1 CBD and 1 THC calibration chart, standards that allow accurate % quantification, 2 Test plates, 2 Dye powder (Fast BB Salt) microtubes (0,06 gr), 1 Flask of alpha-CAT test fluid (10 ml), eight Eppendorf tubes (1,5 ml), one Developing jar, one Dipping tray, one Pipette (3 ml), one Syringe (1 ml), one Vial with ten capillary tubes (1 ul), four Nitrile gloves, one Becher (25 ml), one capillary pipette bulb.

The third step was to transfer laboratory knowledge on conducting TLC. The Federal University of Piauí (UFPI) provided online courses to ABRACAM, and one professor traveled to ABRACAM to run the testing, photograph the procedure, and compare the results.

## 2.1 Samples Preparation (Weighting and Extracting Cannabinoids)

TLC Testing procedures start with sample weighting. In this step, the necessary materials are a scale measuring 0,01 grams, an Eppendorf, and a small spatula. Researchers used the spatula to insert and weigh 40 mg of cannabis extract in a previously weighted Eppendorf (Table 1).

Table 1 – Sample's dilution and Multiplication factor Millimeters [13,14]

| **Samples** | **Sample weight** | **Solvent** | **Plate Drop** | **Multiplication Factor (MF)** | **Reading** | **Reading Scale** |
|---|---|---|---|---|---|---|
| Low Concentration | 200 mg | 1ml | 8 ul | 0,125 | 0,2%-5% | 0,2% 1,25mm |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Medium Concentration | 100 mg | 1 ml | 2 ul | 1 | | 2%-20% | 2,0% 1,25mm |
| High Concentration | 40 mg | 1 ml | 1 ul | 5 | | 20%-100% | 10,0% 1,25mm |

Source: Authors adapted from [13,14] by adding: Reading – the minimum and maximum recommended measurable percentage, Reading Scale: millimetric scale to improve precision

After weighting the samples, researchers advanced to sample dilution. The provided Kit diluting materials are a 2 ml graduated pipet and 30 ml solution. The laboratory analyst pipetted 1 ml of solution, added it to the Eppendorf, and shook it for 2 minutes until the extract homogeneously dissolved.

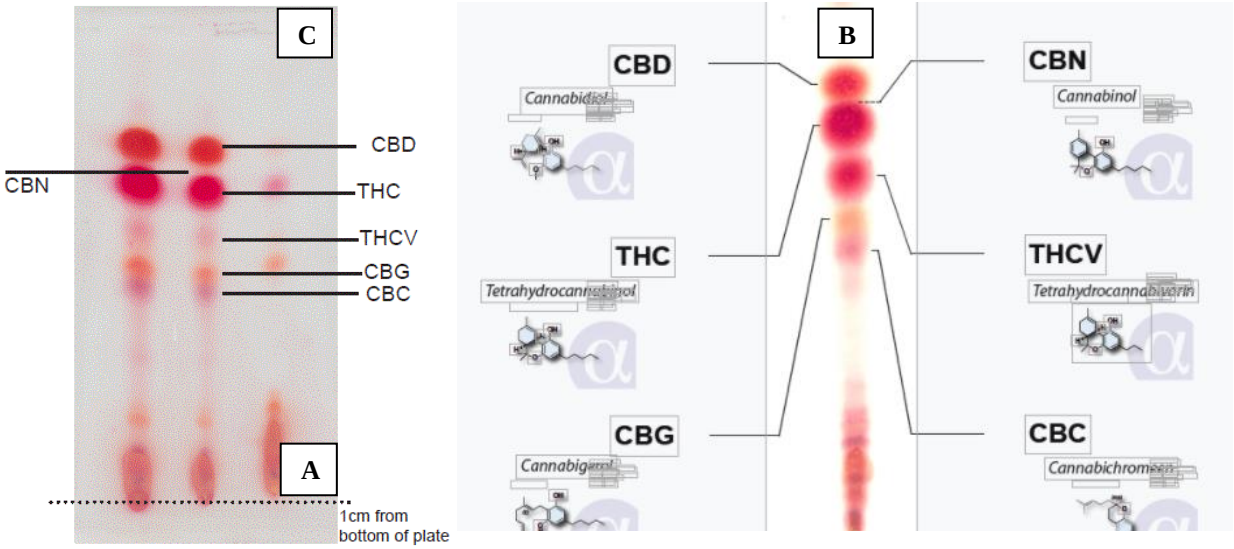
## 2.2 Stationary Phase

The stationary phase steps are the plate preparation to receive diluted cannabinoids and inserting cannabinoid extraction at the plate. These phase materials are a 5x10 cm silica-coated plate, a pencil, a ruler, a timer, an electric oven, a thermometer, and microcapillary tubes.

ABRACAM laboratory technician used the ruler and the pencil to mark down four dots, each 1,25 cm from each and 1 cm from the plate bottom (fig. 1A). The capillarity tube volume is one microliter (ul). After filling the capillarity tube, the technician dropped the Eppendorf solution at the pencil mark origin point.

The kit manual suggests a specific dilution and specific amounts of solutions to measure Cannabis concentrates as extracts or rosin (high concentrates), flowers (medium concentration), or diluted materials as edibles or medicinal oils (low concentration) (Table 1). ABRACAM extract is high concentration, so one capillarity tube 1 ul measured the solution added to the plate. The plate was dried in a preheated oven at 150 Celsius for five minutes.

Fig. 1 – Cannabinoid's qualification chemo typing [13].




Source: Adapted [14], A: dots marked with a pencil at 1,25 cm, B: Cannabinoids Chemotype color identification, C: Silica Plate.

## 2.3 Mobile Phase

The Kit provides only one solvent to dilute the samples, insert them in silica-coated plates (stationary phase), and move cannabinoids in silica-coated plates (mobile phase). In the mobile phase, cannabinoids move at different points, named Rf, concentrating spots in specific places of the silica-coated plate (Figure 1 C, the dots at the silica plate). This phase requires a glass jar, a plate prepared at the stationary phase, water, fast bb salt, a revealing tray, 2 ml of kit solution, and a pipette.

UFPI professor at ABRACAM added 2 ml of the kit solution to the jar and inserted the plate vertically with the drop-downside at the bottom. He closed the jar and allowed 25 minutes for the solution to run until it reached close to the plate top. After 25 minutes, the plate needs to dry vertically for 5 minutes.

The technician mixed 2 mg of fast bb salt with 25 ml of water and placed it on the revealing tray. He emerged the plate with the silica-coated

face down for one second, removed and let it dry vertically. At this moment, colored spots appeared on the plate, as in Figure 1 C.

## 3. Results

UFPI professors performed 60 cannabinoid tests before setting the best time frame and imaging technique for quantifying cannabinoids using the calibration chart. This research's main result is that the THC readings in Table 2 are different from the HPLC reading by 1,01% (TLC 1s reading) and -0,645% (TLC 2nd reading), close to the +/- 0,5 error informed by the Kit's manual [13].

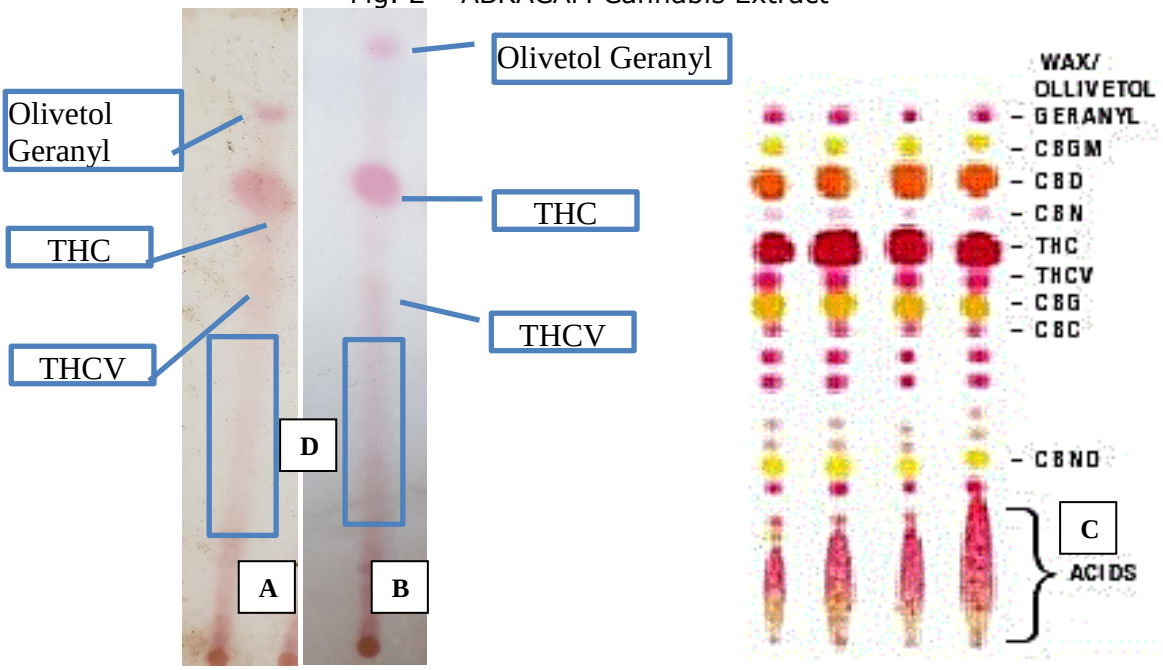
Table 2 – HPLC and TLC comparison between cannabis extract quantification.

| | **HPLC** | **TLC 1ˢ reading** | **TLC 2nd reading** |
|---|---|---|---|
| **Cannabis extract** | 60,39% | 61,00% | 60,00% |
| **Readings Difference** | Standard | 0,61% | -0,39% |
| **Readings Error** | | 1,01% | -0,645% |

Source: Authors – Results from UFRN and the best reading at 2 minutes and 50 seconds.

The UFRN HPLC reading searched only for THC, CBD, and CBN because those were the only references available. At the same time, the TLC could detect THC, THCV, Olivetol Geranyl, and other cannabinoid traces. TLC test also confirmed the absence of CBD and CBN (Figure 2 A and B).

Fig. 2 – ABRACAM Cannabis Extract




Source: Authors – Legend - Fig. A and Fig. B are TLC 1st and 2nd run with the same ABRACAM cannabis extract; Figure C - Chemotype profile from Alpha-CAT/Cannalytics Supply [15], Figure D – cannabinoids traces.

TLC precision does not substitute an HPLC reading. However, TLC may be a reasonable hypothesis builder for researchers and a way of chemo-typing plants and extracts for Cannabis Associations in Brazil with a small error.

## 3.1 Chemotype

The test on HPLC run by Instituto Cérebro at UFRN only used THC, CBD, and CBN references. As noted in the introductory chapter, HPLC testing in Brazil is expensive, and universities opt to purchase fewer references, such as THCV, CBG, or CBC.

The TLC test in Figures 2A and 2B are the first and second runs that use the same extract quantified by UFRN. There are two cannabinoids identified (THC, THCV) and Olivetol Geranyl. There are other cannabinoids trace but not at a measurable definition.

3.2 Cannabinoids Quantification

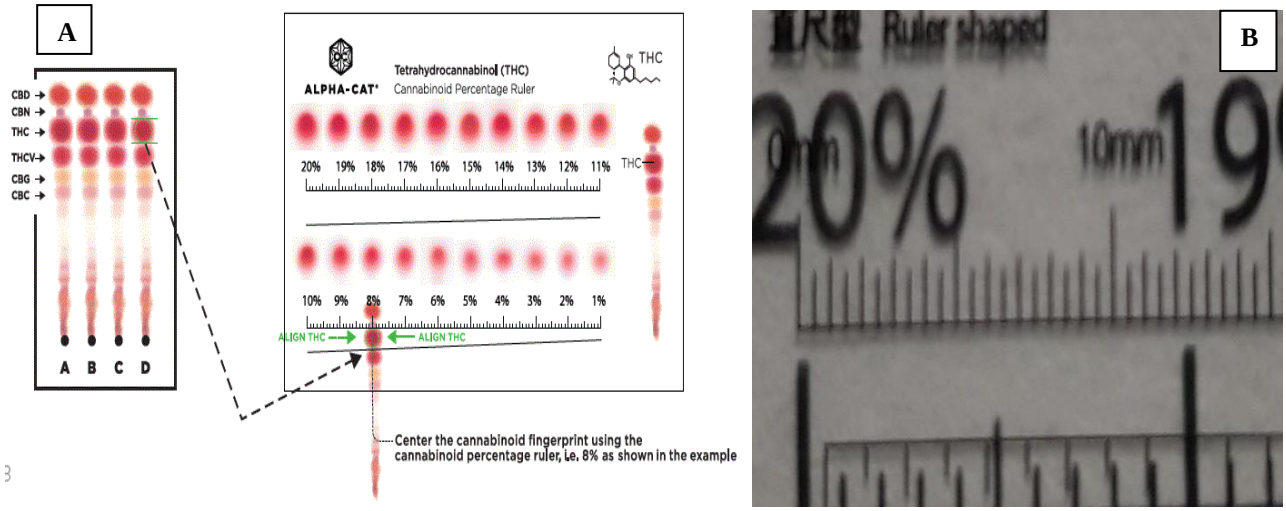
Quantification uses the dilution and amount inserted at the plate (Table 1) for medium concentration as a reference. The multiplication factor (MF) suggested in Kit's manual is MF = (100 mg X 2 µl) / (samples weight (mg) X extraction fluid (µl)).

The calibration chart works with 100 mg/ml dilution and a 2 µl plate drop. The chart measures from 2% to 20% with this specific setting. Each interval at the calibration chart measures 2% (Figure 2), and each interval corresponds to 0,125 cm (Table 1).

For concentrated samples, as the extract tested previously by HPLC, the calculated MF is 5 using the formula (100 mg X 2 µl) / (40 (mg) X 1 (µl)). That means the results readings must be multiplied by 5, or each calibration chart interval measures 10% (Table 1).

The chart also has two other functions: the qualitative pattern test (Figure 3A) and a percentage ruler (Figure 3B). The qualitative pattern suggests the color intensity of THC for different concentrations. As shown in image Y, the percentage ruler is 12.5 mm long, where each percentage space corresponds to 0,125 cm.

Fig. 3 – Fig. A Calibration Chart for Tetrahydrocannabinol (THC) and Fig. B its metrics correspondence [16].




Source: Authors adapted from [16] and laboratory images.

The color spots on the calibration chart are the alpha-CAT suggestion to help users better hypothesize about the concentration. After selecting a spot on the graph with color intensity like the one revealed at the plate, place the calibration chart mark measuring the bands of cannabinoids separated at the silica-coated plate (Figure 4A, 4B, 4C).

TLC showed some advances in chemo-typing cannabis samples. The method is low-cost (approximately 7,95 U$ per test). This test could help CSCs decide the best time to harvest plants with the desired number of cannabinoids and check the profile of Cannabis, extracts, and cannabis medication (cannabis extract diluted in oil/water/gel).

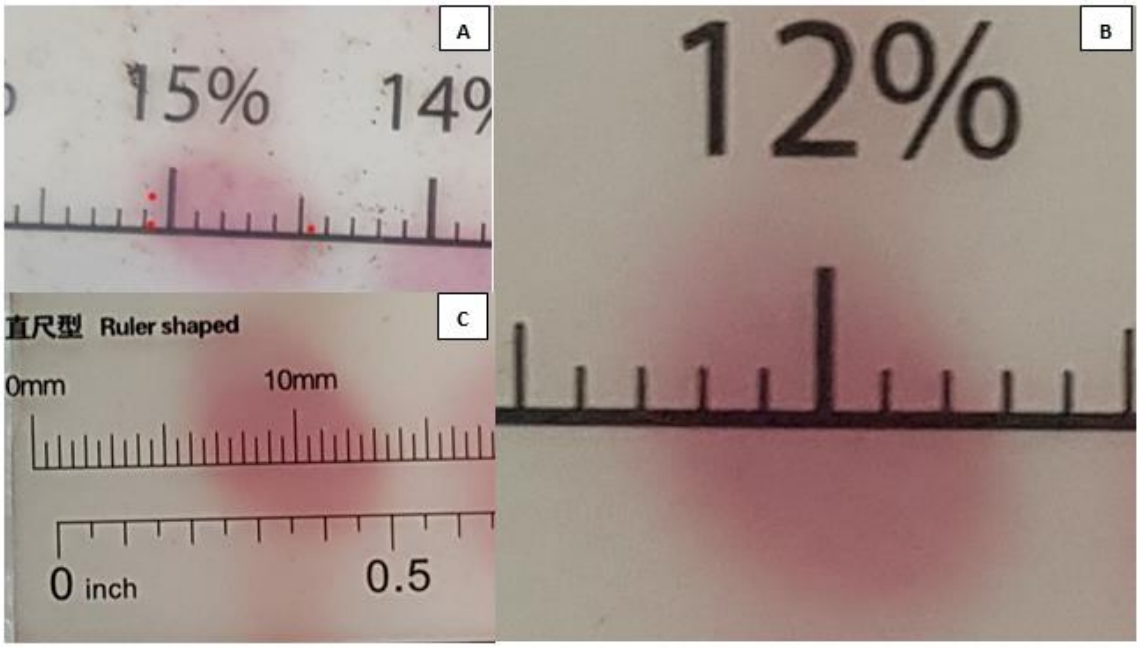
One disadvantage of TLC quantitation is that eye vision is not enough to record cannabinoids' presence with precision. Readings with the lowest error (Figures 3B, 4A, 4B, and 4C) were recorded by a camera and later, with amplified imaging measured with precision. This method needs a microscope or a camera with image zooming to record measuring.

The second disadvantage of this method is the presence of image halos around cannabinoids after 5 minutes of revealing the plates with fast bb salt (Figure 5A). The solution to reducing error was to photograph the calibration

chart above the cannabinoid's spots after one and half minutes past the revealing phase (dropping the plate at the revealing tank with fast bb salt) (Figure 5B). The two-minute and fifty-second -time frame eliminated the halo effect and allowed cleaner readings.

We consider the new time frame to contribute to the Alpha-CAT method since we could reach errors close to/under 1% (Table 2 and Figures 4A and 4B).

Figure 4 – Cannabinoids measurements images.



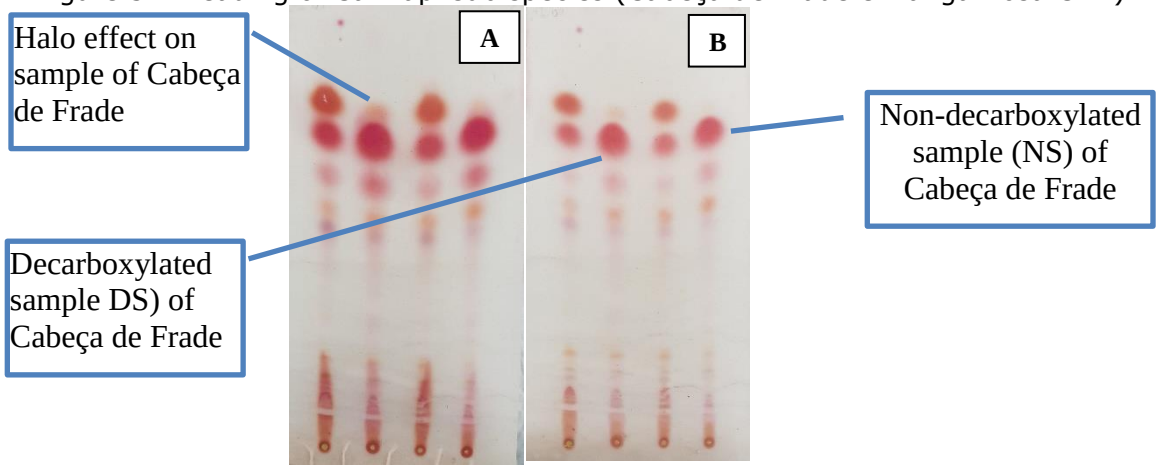

Source: Authors. Legend - THC levels: Figure A – ABRACAM extract first run, Figure B – ABRACAM extract second run, Figure C – Abracam extract on metrics scale.

Another contribution to the Alpha-CAT method, as seen in Figure 4C, was converting measuring to metric scale. Measuring using the kit calibration chart is less precise due to more significant gaps between each calibration chart landmark.

The calibration chart may not be as precise because of its more significant gaps, although it sets the limits of the parameters translated into

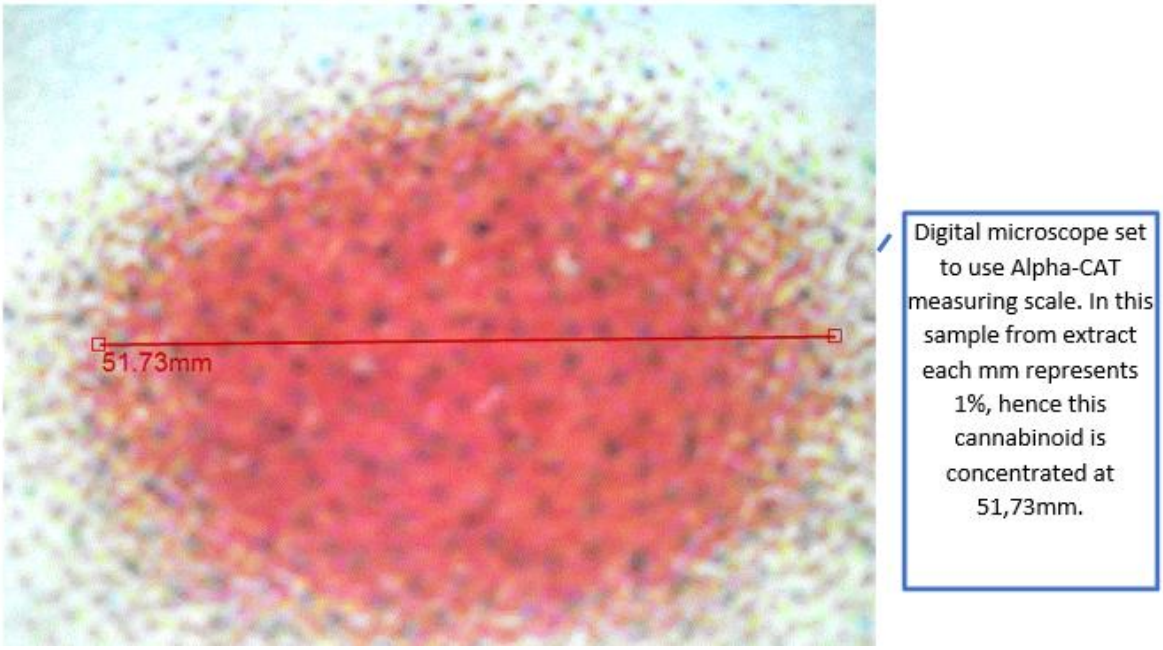
metric scales for imaging. This reference aided UFPI professors in calibrating digital microscopes to read the spot's diameter (Figure 6).

Figure 5 – Reading of Cannapi sub species (Cabeça de Frade e Manga Rosa CBD)




Source: Authors – Legend - Difference from Cabeça de Frade and Manga Rosa CBD of decarboxylated and non-decarboxylated samples. Figure A – Silica coated plate with 5 minutes after revelation, Figure B – Silica-coated plate after one-and-a-half-minute revelation.

Figure 6 – Microscope Calibration with Alpha-CAT calibration chart limits.




Source: Authors

Table 3 readings can add more information about the cannabis extract tested by HPLC. ABRACAM made the referred extract combining flowers from Brazilian creole varieties (Manga-Rosa, Alecrim, and Rabo de Raposa).

Manga-Rosa shows a broad spectrum of cannabinoids, with THC at almost 15% (Table 3). When using the quantification from UFRN, it is impossible to account for the richness of this variety of chemotype profiles (other cannabinoids).

The TLC quantification also has the advantage of quantifying acids and cannabinoids. The quantification is possible by testing the same sample in two different columns, being that part of this sample is decarboxylated prior at 150 degrees Celsius for 5 minutes and the other not (Figure 5).

Different sample treatments allow differentiating cannabinoid levels at two columns. Quantifying acids cannabinoids results from subtracting decarboxylated sample (DS) levels from the non-decarboxylated sample (NS) levels. The formula DS-NS=AC allows for identifying the amounts of acids and cannabinoids (AC).

Table 3 - Cannabinoids Profiles of ABRACAM strains using TLC (using digital microscope and setting parameter reading with Alpha-CAT Calibration Chart)

| **Cannabinoids** | **Manga Rosa** | **Alecrim** | **Rabo de Raposa** | **Candida CD-1 (Indoor)** | **Candida Cd1 (outdoor)** |
|---|---|---|---|---|---|
| | **Brazilian Landraces** | | | **High CBD Genetics** | |
| **CBD** | | | | 19,58% | 20,89% |
| **CBDA** | | | | | |
| **THC** | 14,92% | 11,27% | 7,09% | 5,84% | 6,23% |
| **THCA** | 1,072% | 2,53% | 2,55% | | |
| **THCV** | 1,60% | | | 0,95 % | 1,24% |
| **THCVA** | 1,21% | | | | |
| **CBG** | 1,95% | | | 0,5% | 0,3% |
| **CBGA** | 1,39% | | | | |
| **CBC** | 0,69% | | | | |
| **CBCA** | 0,73% | | | | |

Source: Authors

Another significant result obtained from cannabinoid quantification refers to best cultivation practices for obtaining higher levels of CBD. ABRACAM cultivated the same variety, Candida CD1, in two different

environments (indoor and outdoor). Results in Table 3 point to more cannabinoid production outdoors compared to the ABRACAM indoor system.

Higher cannabinoid profiles indicate conscientious use of energetic force to produce Cannabis. The results do not suggest that outdoor is the best cultivation system in general, but it is undoubtedly more cost-efficient for the ABRACAM case.

## 4. Conclusions

CSC quality control of Cannabis and its derivates is a known worldwide issue. In Brazil, no legislation guarantees laboratory testing on cannabis products produced nationally by CSCs. TLC techniques show disadvantages as not being recognized as a valid form of quantifying cannabinoids in Brazil and because it requires extensive human training to guarantee reliable quantification.

However, TLC showed several advantages when using the additional methods elaborated by UFPI professors, such as the best time for filming readings, imaging zooming, and scale conversion from volume to metric.

We conclude that this TLC kit may be a low-cost and reliable hypothesis builder able to measure with 99% precision major cannabinoids from extract samples. However, profound laboratory experience and rigorous methods must obtain such results.

This research admits the error of approximately 1% (+1,01 and -0,645) in the highly concentrated extract for other concentrations as medium and low. However, researchers found difficulties quantifying cannabis oil with less than 1% cannabinoids (10 mg/ml) in lower concentrations.

Like cannabis oil, edibles, and pastes, lower concentration samples are the essential final Medical CSCs product, like ABRACAM. We strongly suggest testing samples during all phases (cultivation, harvesting, processing

extracts, and diluting extracts in medication) to ensure higher quality control for artisanal production of Cannabis extracts.

TLC quantification does not replace the necessity for HPLC quantification. Instead, it should be an additional method for extensive testing to lower total test expenses. CSCs acquiring this knowledge may advance faster in controlling the quality of their products.

In the present case, a gap between universities and CSCs can be straightened by transferring laboratory practices and methods. ABRACAM became aware that quantification precision requires training and should be a central part of Cannabis artisanal extract production.

We suggest that future researchers use ultraviolet (UV) lights to replace the raveling with fast bb salt, as the second increases possible health risks. This suggestion may come to practice integrating UV revealing with smartphone imaging. Cabinets are low-cost equipment, and smartphone cameras showed very effective and good quality images, in most cases, better than microscope imaging. However, digital microscopes have a rescale function. CSCs may initially use a photo cabinet before purchasing a microscope with excellent digital imaging.

The photo cabinet suggestion follows the two-minute-and-fifty-second time frame to avoid the halo effect. It is difficult for inexperienced laboratory technicians to adjust the silica-coated plate under the microscope, adjust focus, and record the image in approximately 3 minutes. Another suggestion is the investigation by ANVISA to verify the possibility of inserting TLC as one of the valid methods for quantifying cannabinoids.

TLC may be a valuable tool for building initial abilities to guarantee minimum quality control for CSCs. Cannabinoid's laboratory analysis is essential for selecting cannabis varieties, the best time to harvest, the best production system yields, and reducing costs at massive testing environments.

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