
**VALIDATION OF PRODUCTION AND PACKAGING PROCESS OF CANNABIS OIL
25:25 THC:CBD (25MG/ML THC: 25MG/ML CBD)**

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Abstract: Validation of manufacturing processes is a critical element within Good Manufacturing Practice (GMP), particularly in the production of products containing pharmacologically active substances such as the oil derived from *Cannabis sativa L.*. The chemical complexity of the plant, together with regulatory restrictions, necessitates the systematic establishment and demonstration of reproducible and controlled manufacturing conditions.

This study aims to present a framework for the validation of critical steps in the production and packaging of cannabis oil 25:25 THC:CBD (25 mg/mL THC : 25 mg/mL CBD), thereby defining and establishing standards and parameters which can subsequently be considered reproducible and applicable for every subsequent batch of production and packaging of the final product.

A GMP-oriented methodology was applied, including: identification of critical control points, establishment of acceptable limits for heating, scaling, mixing with heating, filling, labeling, and packaging. To conduct this study, three validation series were produced.

The results of the validated process testing confirmed that the production and packaging process complies with the quality specification. Furthermore, compliance was ensured with pharmacopeial requirements regarding residual solvents, pesticides, heavy metals, and microbiological purity.

Validation of the process for cannabis oil production represents an essential prerequisite for ensuring the quality, safety, and efficacy of the final product. Standardization of manufacturing steps and strict control of critical parameters provide the foundation for regulatory compliance and support the clinical application of cannabis-based medicinal products.

The production and packaging process of cannabis oil 25:25 THC:CBD (25 mg/mL THC : 25 mg/mL CBD) can be further developed with additional approaches and long-term modifications to the already established framework, with the aim of improving and simplifying the process, while maintaining the primary goal of achieving a high-quality, effective, and safe product.

The manufacturing and packaging process of 25:25 THC:CBD cannabis oil (25 mg/mL THC : 25 mg/mL CBD) may be further optimized through additional and innovative approaches in the long term, with the overall goal of improving and simplifying the process to ensure a high-quality, effective, and safe product. Furthermore, continuous monitoring and iterative improvement of the process will allow adaptation to evolving regulatory standards and emerging scientific knowledge. Such efforts will ultimately contribute to broader clinical availability and patient access to standardized, reliable cannabis-based therapies.

Keywords: Cannabis sativa L., Good Manufacturing Practice, validation, specification.

1. INTRODUCTION

Cannabis sativa L. is a plant (plant species) with a long history of use for medicinal, industrial and recreational purposes and with high economic value and a global market worth billions of dollars. However, the long history of prohibitions has resulted in limited scientific research on this plant, and biotechnological approaches are still in the process of development. The introduction and application of modern biotechnological techniques has the potential to overcome some of the specific challenges of this species, thereby increasing productivity and deepening our understanding of its biology (Andre et al., 2016). With the gradual relaxation of regulatory restrictions in many regions around the world, a significant increase in research aimed at cannabis biotechnology is noticeable.

The components in cannabis (cannabinoids), also known as phytocannabinoids, are a group of compounds naturally found in cannabis that act by interacting with the endocannabinoid system in the human body (Russo, 2011). These interactions can result in a wide range of physiological and psychological effects – from stimulating and energizing to relaxing and calming. The most notable of these are tetrahydrocannabinol (THC) and cannabidiol (CBD), but

over 110 different cannabinoids have been isolated to date, so the potential for future discoveries remains significant (Andre et al., 2016).

Cannabis extracts can be classified into three basic types according to their composition and pharmacological profile: full-spectrum extracts, broad-spectrum extracts, and isolates. The starting material used for the preparation of this paper is an extract (FSO).

Validation of the manufacturing process is an essential part of the pharmaceutical industry and aims to ensure that the product is continuously manufactured in accordance with previously defined specifications and quality standards. Process validation provides scientifically based confirmation that each step in the manufacturing process – from the extraction of active ingredients to the packaging of the finished product – is stable, controlled and reproducible.

Process validation is traditionally carried out in several basic forms:

- Prospective Validation – carried out before the start of routine production;
- Concurrent Validation – performed in parallel with current production;
- Retrospective Validation – based on historical data from already produced batches;
- Revalidation – re-validation of the process after significant changes;
- Continued Process Verification (CPV), as a modern and advanced concept that provides continuous assurance in the stability and control of validated processes throughout their entire life cycle.

Prospective validation lays the foundation for all subsequent validation phases and for the continuous monitoring of the process, as it provides baseline data and evidence that the process is well understood, critical parameters are controlled, and the results are reproducible.

This study was performed in accordance with prospective validation principles

2. MATERIALS AND METHODS

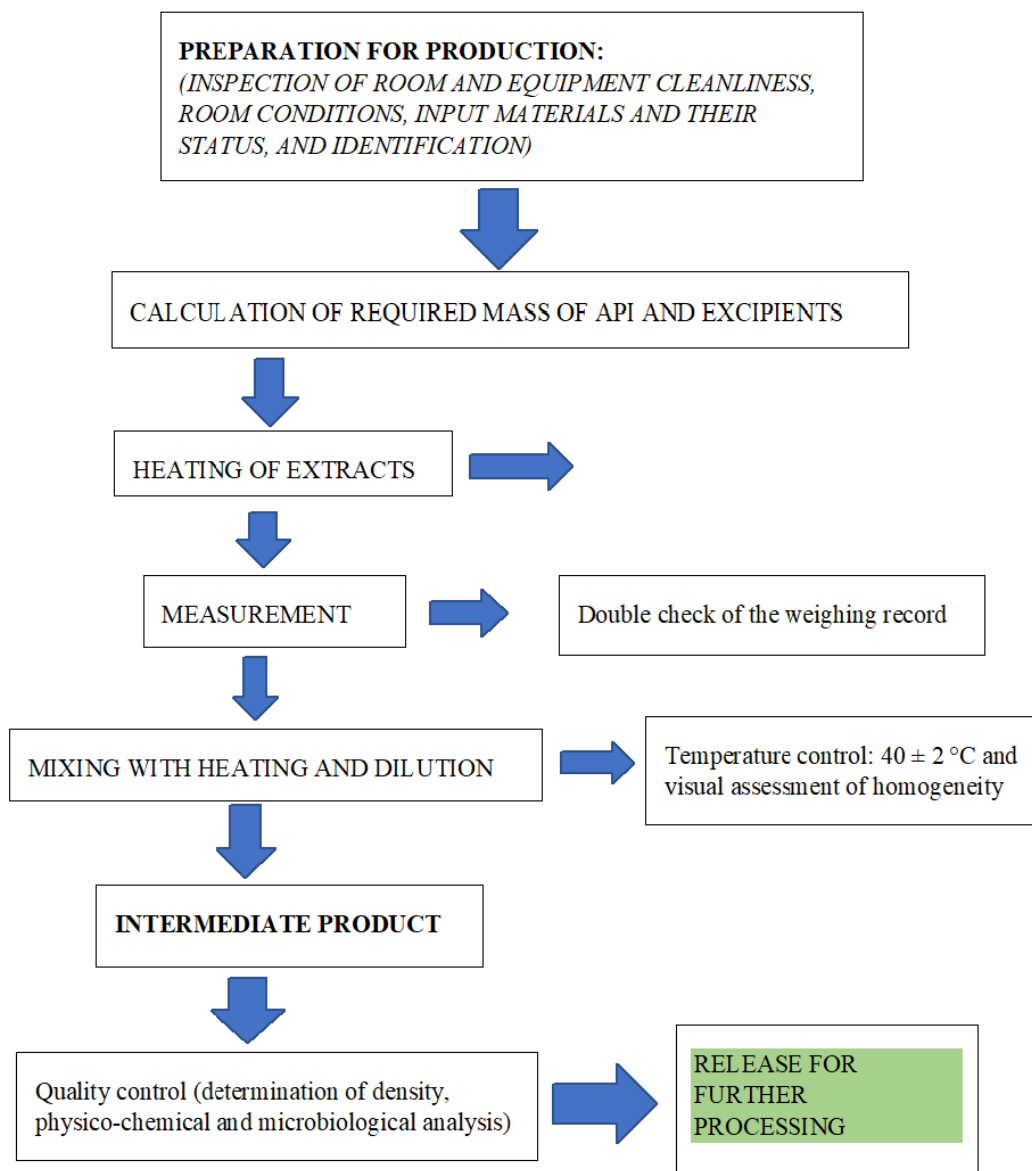
The study was conducted according to predefined procedures to ensure accuracy and reproducibility. All methods were performed in compliance with relevant guidelines and standard operating procedures. The applied methods are based on the principles of Good Manufacturing Practice (GMP) and on the regulatory guidelines of the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) for process and prospective validation. The study encompasses all key parameters of the manufacturing process — from the receipt of raw materials and equipment preparation to the packaging and final control of the finished product.

The validation is performed on three batches (FP0199, FP0201 and FP0203) each with a nominal volume of 6,000 mL, in order to assess the reproducibility of the process, the repeatability of key process parameters, and the consistency of the final product in terms of potency, purity, and safety.

2.1. Production of Cannabis Oil 25:25 THC:CBD (25 mg/mL THC : 25 mg/mL CBD)

The following diagram (Figure 1) illustrates the main phases and subsequent activities in the production process of cannabis oil 25:25 (THC:CBD). The diagram provides a visual representation of the workflow. The purpose of the diagram is to ensure a clear and systematic overview of the sequence of operations, as well as to facilitate understanding and monitoring of the GMP-required steps within the manufacturing process. This schematic representation ensures transparency and consistency in process execution, which is particularly important for guaranteeing the quality, safety, and regulatory compliance of the product.

Figure 1. Schematic representation of the production process of cannabis oil 25:25 (THC:CBD) up to the intermediate product

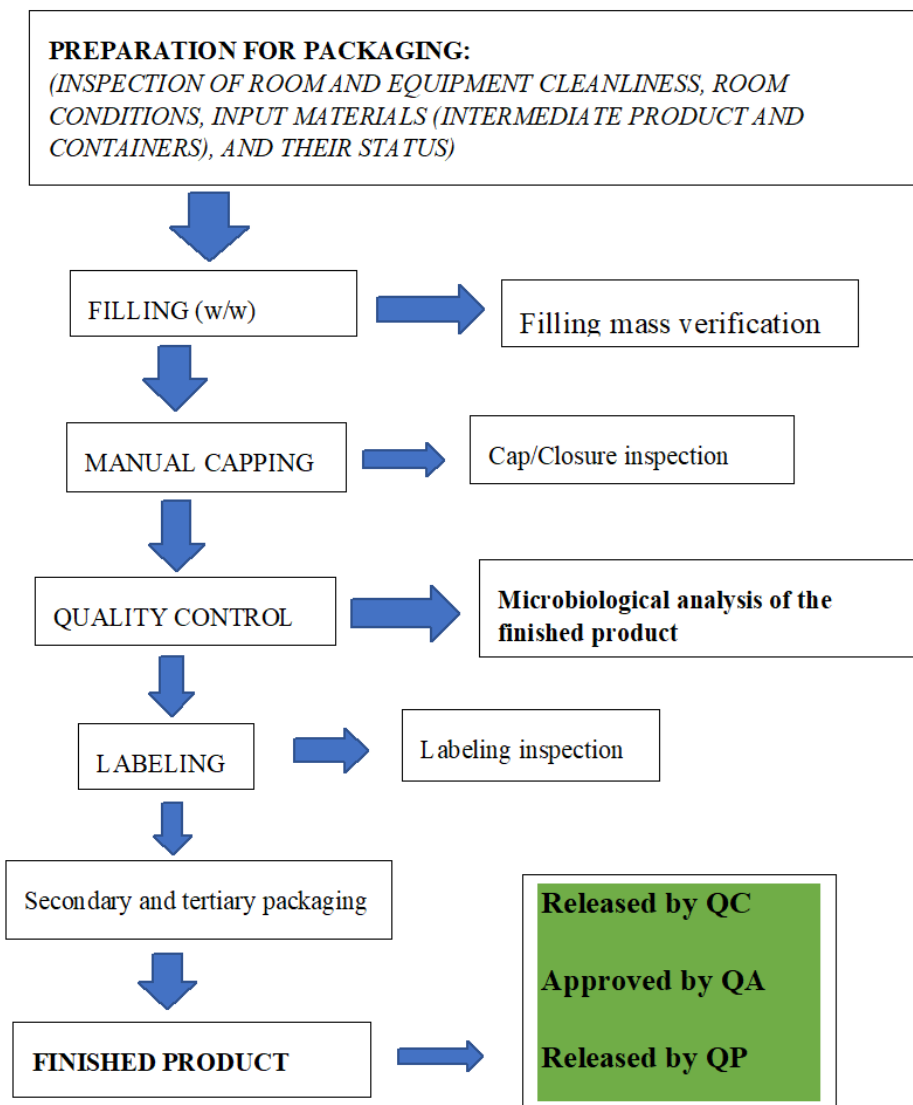


Source: Authors' elaboration

2.2. Packaging of Cannabis Oil 25:25 THC:CBD (25 mg/mL THC : 25 mg/mL CBD in 30 mL amber glass bottle)

The packaging of cannabis oil 25:25 (THC:CBD) represents a critical phase in the manufacturing process, which goes beyond merely physically filling the product into containers. It also involves ensuring complete protection of the active substances from external factors such as light, oxygen, and moisture, which can cause their degradation and reduce pharmacological activity. Considering that the active components THC and CBD are sensitive to light, temperature, and oxygen, the choice of appropriate packaging — in this case, a 30 mL amber glass bottle — is crucial for maintaining their stability and quality throughout the entire shelf life. The following flow diagram (Figure 2) illustrates all the steps in the packaging process of cannabis oil 25:25 (THC:CBD).

Figure 2. Schematic representation of packaging process of cannabis oil 25:25 (THC:CBD) in a 30 ml amber glass bottle



Source: Authors' elaboration

3. RESULTS AND DISCUSSION

The results of the conducted controls and analytical tests on the finished product — cannabis oil 25:25 (THC:CBD) — indicate full compliance with the defined specifications and regulatory requirements, including the Batch Manufacturing Record (BMR), the finished product specification, and relevant pharmacopeial standards. The results are presented in the following table, arranged according to the intended specification.

Table 1. Tabular presentation of results from three validated batches

Parameter	FP0199	FP0201	FP0203	Requirements / Limits
Appearance	Complies	Complies	Complies	Homogeneous liquid, with greenish/yellowish to brown color
Identification TLC (Ph.Eur.2.2.27) DABcurrent edition	Complies	Complies	Complies	Characteristic zones of CBD and Δ^9 -THC
Identification	Complies	Complies	Complies	The retention time of the peaks of CBD, Δ^9 -THC, Δ^8 -THC and CBN that elute in the

HPLC (Ph. Eur. 2.2.29)				chromatogram of the Test solution during the analysis of the parameter Assay correspond to the retention times of CBD, Δ^9 -THC, Δ^8 -THC and CBN in the chromatogram of the Diluted Standard solution.
Identification UV-Vis Spectrophotometry (Ph. Eur. 2.2.25)	Complies	Complies	Complies	Spectral characteristics of the peaks of CBD, Δ^9 -THC, Δ^8 -THC and CBN that elute in the chromatogram of the Test solution during the analysis of the parameter Assay, correspond to the spectral characteristics of the peaks of CBD, Δ^9 -THC, Δ^8 -THC and CBN in the chromatogram of Diluted Standard solution (spectral evaluation during elution time with Diode Array detector)
Water content Method A- Direct titration (Ph.Eur. 2.5.12)	0,007 %	0,065 %	0,051 %	Max. 0,5 %
Content (Assay) HPLC (DAB 2021)	27,35 mg/ml	27,50 mg/ml	27,50 mg/ml	22,5mg/ml -27,5mg/ml (90-110% of the declared content)
Δ^9-THC				
CBD	22,52mg/ml	22,88 mg/ml	22,63 mg/ml	
Impurity HPLC (DAB 2021) Cannabinol (CBN)	0,09%	0,09%	0,09%	≤ 2.5 %
Average fill (USP/NF <698>)	Complies	Complies	Complies	Minimum of 30 mL of oil per bottle
Fill variation (USP/NF <698>)	Complies	Complies	Complies	At least 95% of the declared fill volume
Residual solvents: -Ethanol HS-GC (Ph.Eur. 2.2.28)	< LoD (LoD = 162,17ppm)	< LoD (LoD =162,17ppm)	< LoD (LoD =162,17ppm)	≤ 5000 ppm
Pesticides (GC/MS and LC MS/MS Ph. Eur. 2.8.13)	≤ LoQ (LoQ = 0,01 ppm)	0,16 ppm	0,08 ppm	According to the limits of Ph. Eur. current edition
Heavy metals Ph. Eur. 2.4.27 (AAS, Ph. Eur. 2.2.23; ICP-AES, Ph.Eur.2.2.57; ICP-MS, Ph. Eur. 2.2.58)	< LoQ	< LoQ	< LoQ	As ≤ 1.0 ppm
	0,07045 ppm	0,12480 ppm	0,09932 ppm	Cd ≤ 1.0 ppm
	0,01408 ppm	0,02268 ppm	0,04306 ppm	Pb ≤ 5.0 ppm
	0,00071 ppm	0,000116 ppm	0,00031 ppm	Hg ≤ 0.1 ppm
	0,33137 ppm	0,58674 ppm	0,91219 ppm	Ni -Indicative
	< LoQ	< LoQ	< LoQ	Zn - Indicative
Aflatoxins Ph. Eur. 2.8.18 (Ph. Eur. 2.2.29, HPLC- UV method)	ND	ND	ND	Aflatoxin B1 < 2μg/kg
	ND	ND	ND	sum of aflatoxin B1, B2, G1, G2 < 4μg/kg
Ochratoxin A Ph. Eur. 2.8.18 (Ph. Eur. 2.2.29, HPLC- UV method)	ND	ND	ND	< 20μg/kg
Microbiological quality Ph. Eur. 5.1.4 (Table 5.1.4.-1)	≤ 10 ⁴ CFU/g	≤ 10 ⁴ CFU/g	≤ 10 ⁴ CFU/g	TAMC (2.6.12) ≤ 10 ⁴ CFU/mL
	≤ 10 ² CFU/g	≤ 10 ² CFU/g	≤ 10 ² CFU/g	TYMC (2.6.12) ≤ 10 ² CFU/mL
	≤ 10 ² CFU/g	≤ 10 ² CFU/g	≤ 10 ² CFU/g	Bile-tolerant gram-negative bacteria (2.6.31) ≤ 10 ² CFU/mL
	Absent / 10 g	Absent / 10 g	Absent / 10 g	Salmonella (2.6.31) Absent / 10 mL
	Absent / 1 g	Absent / 1 g	Absent / 1 g	Escherichia coli (2.6.31) Absent / 1 mL
	Absent / 1 g	Absent / 1 g	Absent / 1 g	Staphylococcus aureus Absent / 1 mL
Relative density	0,95 g/ml	0,95 g/ml	0,95 g/ml	N/A
Packaging	Complies	Complies	Complies	30 mL oil solution in amber glass pipette vial

Source: Authors' elaboration

Notes:

- **LoD** = Limit of Detection
- **LoQ** = Limit of Quantification
- **ND** = Not Detected

3.1. Active Substance Content (THC and CBD)

Analytical measurements showed CBD at 22.5 mg/mL and THC at 27.5 mg/mL, within the allowed deviation. These results confirm correct dosing, homogenization, and control of critical process parameters (temperature, time, mixing speed). Homogeneous distribution ensures dose consistency and efficacy.

3.2. Physicochemical Properties

Product density (0.95 g/mL) is within the expected range for MCT oils (0.92–0.95 g/mL), indicating correct carrier selection and stable composition. Appearance and odor are as expected – clear, golden-yellow liquid with mild vegetal scent, free from foreign matter, reflecting proper production conditions and storage.

3.3. Microbiological Purity

Microbiological testing (Ph. Eur. 2.6.12) confirmed total aerobic microbial count, molds, and yeasts within limits. No pathogenic microorganisms (*E. coli*, *S. aureus*, *P. aeruginosa*) were detected, demonstrating proper controlled production, validated cleaning, and quality control of packaging materials.

3.4. Stability and Storage Control

Three-month stability studies showed no significant changes in appearance, density, or active substance content. The product remains homogeneous, with MCT oil ensuring solubility and protection against oxidation. Dark glass bottles protect light-sensitive cannabinoids, particularly THC.

3.5. Packaging and Labeling Control

Packaging complies with label accuracy, visibility, regulatory, and aesthetic requirements. No deviations were observed, confirming effective visual inspection and batch tracking procedures.

3.6. Overall Assessment and Compliance

The finished product meets all quality, safety, and stability criteria. Manufacturing follows GMP principles with validated procedures and full documentation. Integrated quality systems ensure consistency and traceability from raw material receipt to final release. The product is fully compliant with specifications and regulatory requirements, safe, stable, and suitable for its intended shelf life.

The production of 25:25 (THC:CBD) cannabis oil is a complex process requiring precise control of raw materials, extraction, stability, and packaging to ensure a consistent and safe final product. Analytical results demonstrate that all validated batches meet the specified criteria, with THC and CBD concentrations within acceptable limits, confirming process reproducibility and effective extraction and dosing methods.

Active cannabinoids remained stable throughout production and storage, highlighting the importance of controlled temperature, light protection, and proper carrier oil to prevent degradation. Physicochemical parameters, including color, viscosity, density, and pH, were consistent across batches, indicating controlled manufacturing and appropriate storage.

Microbiological testing confirmed the product's safety, with no pathogenic microorganisms detected, reflecting adherence to GMP, hygienic practices, and sterile packaging conditions. The 25:25 THC:CBD ratio may provide synergistic therapeutic effects, with CBD modulating THC's psychoactive effects, emphasizing the importance of precise concentration control for predictable clinical outcomes.

Stability studies showed minimal variations over time and under validated storage conditions, confirming the product's robustness. Continuous monitoring and extended stability studies are recommended, alongside further pharmacological and clinical evaluations to support long-term therapeutic use.

Overall, the results confirm the reproducibility, safety, and quality of the 25:25 cannabis oil production process, providing a reliable foundation for future research and development of standardized therapeutic cannabis oils.

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