

# Development and validation of analytical method for microbiological quality control of Cannabis oil solution

Marjan Velkovski\*, Damjan Shushleski, Irena Slaveska Spirevska, Elena Metcheva, Silvana Ristevska, Ivana Karchicka

Replek Farm, st. Kozle 188, 1000 Skopje, N. Macedonia

## Introduction

Medicinal cannabis (*Cannabis sativa*) has become a topic of great debate within the recent oncology literature. The history of cannabis within the medical community dates back to the 19th century when Dr. William Brooke O'Shaughnessy first published his data on the pharmacology and toxicology properties of cannabis, where he states that it is a powerful analgesic, anti-convulsant, and muscle relaxant through his experimental treatment of patients suffering from rheumatism, cholera, tetanus, and seizures (MacGillivray, 2015).

As of January 22, 2021, the US FDA has approved Cannabidiol (CBD) and Tetrahydrocannabinol (THC) containing medications for therapeutic use. Exogenous cannabinoids, such as CBD and THC, and their effect on the endocannabinoid system are frequently discussed regarding their role in oncology related diseases (Bodine and Kemp, 2022).

Considering the facts of the popularity and the necessity of this type of natural treatment approach, many authorized pharmaceutical companies are developing cannabinoid-containing products. These types of products are generally made by extracting CBD and THC from the cannabis plant, and then diluting the extracts with carrier oil. European Pharmacopeia (Ph. Eur.) specifies reference criteria for microbiological quality control of such products as "Special Ph. Eur. provisions for oral dosage forms containing raw materials of natural origin". According to this reference, the specified microbiological quality control parameters are: Total Aerobic Microbial Count (TAMC), Total Combined Yeasts and Molds Count

(TYMC), Total Bile-tolerant gram-negative bacteria (TBTGN), as well as Absence of *Escherichia coli*; Absence of *Salmonella* and Absence of *Staphylococcus aureus* (Ph.Eur. 10.0, 2019).

## Materials and methods

### Materials

During the method validation, standard microbiological laboratory instrumentation and equipment was used: Biosafety Cabinets Grade A, microbiological incubators within incubation intervals: 20-25°C, 30-35°C and 42-44°C, laboratory balances, Bunsen burners, Vortex and orbital shakers, as well as standard sterile glassware for microbiological use. Ready to use sterile solutions and culturing media from certified media suppliers: BioMérieux (France), Oxoid (Germany) and Merck (Germany) were used during this validation. Quantitative certified reference materials from authorized culture collections were used as challenge microorganisms for the specified testing parameters as required by European Pharmacopeia for the method suitability test: *Staphylococcus aureus* ATCC 6538; *Pseudomonas aeruginosa* ATCC 9027; *Bacillus subtilis* ATCC 6633; *Candida albicans* ATCC 10231; *Aspergillus brasiliensis* ATCC 16404; *Escherichia coli* ATCC 8739 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 (Ph.Eur. 10.0, 2019).

\*marjan.velkovski@replek.mk

## Methods

For determination of microbial properties and method suitability selection, individual challenge tests for all specified parameters were performed. All testing sample dilutions were made in Tryptic Soy Broth (TSB) as chosen solution for dilution and enrichment.

Surface-spread method using primary suspension of the product to be examined (1:10 dilution in TSB) is determined to be validated as suitable for the conduction of the quantitative tests for determination of TAMC - CFU/mL and TYMC - CFU/mL, as defined in Ph. Eur. 2.6.12. Test for TBTGN - CFU/mL using primary suspension of the product to be examined (1:10 dilution in TSB) is determined to be validated as suitable for the conduction of the quantitative test for determination of Bile-tolerant gram-negative bacteria, as defined in Ph. Eur. 2.6.13. Test for Absence of specified microorganisms using primary suspension of the product to be examined (1:10 dilution in TSB) is determined to be validated as suitable for the conduction of the qualitative tests for determination of *Staphylococcus aureus*/mL, *Escherichia coli*/mL and *Salmonella*/10mL, as defined in Ph. Eur. 2.6.13. Stabilization of the primary suspension of the fatty product in the water-based diluent is achieved by preheating the TSB diluent to 40 °C and maintaining the temperature for 15min while mixing the product until complete dispersion is achieved.

The challenge tests are conducted by individual inoculation of the sample with reference microorganisms in range of 10-100 cfu/0.1mL. Each test is conducted in duplicate and the validation process is performed on three batches of the testing product. The temperature and the incubation are depended of the type of the medium. Tryptic Soy Agar (TSA) were incubated for 3 days at 30-35 °C, Sabouraud Dextrose Agar (SDA) were incubated for 5 days at 20-25 °C, TSB was incubated for 24h at 30-35 °C, MacConkey Broth (MCB) was incubated for 24h at 42-44 °C, Mossel Broth (MSB) was incubated for 3.5h at 20-25 °C, Rappaport Vassiliadis Broth (RVS) was incubated for 24h at 30-35 °C, and Mannitol Salt Agar (MSA), MacConkey Agar (MCA), Violet Red Bile Glucose Agar (VRBGA) and Xylose Lysine Deoxycholate Agar (XLDA) agar were incubated for 24h at 30-35 °C.

The Recovery percent for quantitative and qualitative parameters is calculated by comparing the Test inocula to the Viability Control inocula of the same microorganisms in absence of product. The Recovery percent is limited by the values of 50-200% on all tested microorganisms, followed with Recovery Factor for method suitability compliance by value not greater than 2 (Ph.Eur. 10.0, 2019).

## Results and discussion

The Recovery Percent and Recovery Factor results obtained from all qualitative and quantitative challenge tests show that the tested Cannabis oil solution product, doesn't demonstrate significant product matrix interference or antimicrobial properties against any reference microorganisms while using primary suspension of the product to be examined (1:10 dilution in TSB).

During the plate reading some formations of oil drops on the plate surface can be observed. These observations can be easily distinguished from colony forming units, and do not interfere with the quantitative determination for TAMC/mL, TYMC/mL and TBTGN/mL, thus resulting readable plate count and Recovery factor values  $\leq 2$  for all testing microorganisms.

The challenge for qualitative tests for Absence of *Staphylococcus aureus*/mL, *Escherichia coli*/mL and *Salmonella*/10mL, demonstrated positive recovery on all three testing iterations, with relative Recovery rate of 100% for all specified microorganisms while using primary suspension of the product to be examined (1:10 dilution in TSB).

## Conclusion

The Cannabis oil solution: THC 40 mg + CBD 40 mg/1 mL doesn't demonstrate product matrix or antimicrobial interference by 1:10 dilution in Tryptic Soy Broth, without the necessity for addition of any surface active agent, such as isopropyl myristate or polysorbate 80. The stabilization of the primary suspension of the product is achieved by temperature controlled dispersion.

## References

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