

Cannabis spp. inflorescences have been known in medicine for thousands of years, but their mechanism of pharmacological action is not fully understood. Phytocannabinoids (PCs) are main components of medicinal cannabis responsible for its pharmacological activity. In plants, PCs are naturally synthesized in a less pharmacologically active acid form, e.g. tetrahydrocannabinolic acid (THCA), CBDA cannabidiol acid, and after heating (e.g. in the process of drug preparation) are they converted into neutral forms, e.g. Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD), which better penetrate the blood-brain barrier. As was shown before, the rate of conversion is different for each PC, therefore applied conditions of decarboxylation procedure impacts the pharmacological activity of final cannabis based medicine. In animals and humans, PCs are ligands of endogenous cannabinoid receptors (components of the endocannabinoid system) and are responsible for primary pharmacological effects of medicinal cannabis and cannabis-based medicines. The endocannabinoid system consists of endogenous cannabinoid receptors (CB1 and CB2), endogenous ligands (endocannabinoids), enzymes that synthesize and are responsible for the breakdown of endocannabinoids. More than 140 PCs have been identified in *Cannabis* spp. to date, each of them interacts differently with the endocannabinoid system. For example, Δ^9 -THC is a CB1 receptor agonist and a partial CB2 receptor agonist, while CBD is simplistically a CB1 and CB2 receptor antagonist. In our study, we plan to examine the influence of decarboxylation procedures developed in Hospital Pharmacy of St. Anna Hospital in Brno, Czech Republic and other recommended by Italian Society of Compounding Pharmacists (SIFAP) on so-called major PCs (e.g., Δ^9 -THC and CBD) and minor PCs (i.e. tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabichromene (CBC)). In addition, it is planned to monitor the content of the terpenes, which are volatile compounds also present in medicinal cannabis, and they exert synergistic effects with PCs and influence the final therapeutic effects of specific varieties. The levels of terpenes will be analyzed before and after decarboxylation. Overall, 13 PCs and 5 terpenes will be analyzed in this study, which is in accordance with newest recommendations of the US Pharmacopoeia draft. For the extraction of PCs, solvent-free technique named solid-phase microextraction (SPME) will be used at three stages: in growing plants prior to harvest, in post-harvest processing of plant material (before decarboxylation) and in dried plant material after decarboxylation. The SPME is an innovative technique involving the extraction of small molecules into a probe coated with a sorbent possessing high affinity to selected compounds. As demonstrated in our preliminary study, the SPME technique can be applied for the efficient extraction of PCs in growing cannabis plant (*in vivo* conditions), in dried plants and in decarboxylated plant material. This allows us to monitor the transformation of PCs of exactly the same inflorescence over a period of time. Extracts obtained by the SPME technique will be analyzed with the use of advanced analytical instruments based on mass spectrometry (MS). Moreover, analysis of terpenes will be performed during post-harvesting handling of medicinal cannabis plant material and after decarboxylation process. For extraction of terpenes, a dynamic head space (DHS) technique along with gas chromatography coupled to MS-based method will be employed. Since concentration of PCs and terpenes may vary between flowers, and it is highly influenced by the cultivation conditions, the whole process of plant growth and production of medicinal cannabis material will be closely monitored. Due to well established collaboration with the Cannabis Facility, ICRC-FNUSA, Brno, Czech Republic, which is highly specialized and certified facility that produces medicinal cannabis under strict and controlled cultivation conditions, it will be possible to precisely monitor the concentrations of multiple PCs in a single inflorescence, from the time of its harvest (*in vivo* SPME extraction) until analysis of decarboxylated plant material. Moreover, since there are premises that terpenes can have influence on therapeutic effect of medicinal cannabis-based medicines their concentration will be monitored before and after decarboxylation procedure.