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BIOANALYTICAL APPLICATIONS OF CANNABINOIDS IN MEDICINE

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Comprehensive bioanalysis is key at every stage of product development and at ABS Laboratories, part of ACM Global Laboratories, bioanalysis is intertwined with our discovery, preclinical and clinical capabilities. From method development through clinical sample analysis, we are dedicated to providing accurate, reliable and streamlined bioanalytical services for the most challenging programs to help you make confident research decisions.

In this white paper we will explore the medicinal use of cannabinoids, and showcase how ABS has been directly involved with one of the foremost cannabinoid medications, Sativex®.

CANNABINOIDS DEFINED

Cannabinoids, which are classically 21-carbon terpenophenolics of which cannabis contains 108, have distinct pharmacologic properties. These properties include analgesic, antiemetic, antispasmodic, antioxidative, neuroprotective, antidepressant, anxiolytic, and anti-inflammatory properties, as well as the capacity for glial cell modulation and tumor growth regulation. Particularly promising is the application of cannabinoids in pain management as they inhibit pain in supraspinal, spinal, and peripheral regions and do not have a risk of accidental lethal overdose (Aggarwal *et al.*, 2009).

As a class of compounds, the cannabinoids have vast therapeutic potential, which has led to extensive scientific investigation. They fall into three main categories: single molecule pharmaceuticals, cannabis-based liquid extracts, and phytocannabinoid-dense botanicals (Aggarwal *et al.*, 2009).

In recent years the knowledge of cannabinoid biology has progressed rapidly. This is paving the way for new, promising avenues for potential drug development. Research has revealed a variety of cellular pathways through which potentially therapeutic drugs could act on cannabinoid receptor systems. These potential drugs might include chemical derivatives of plant-derived cannabinoids, or those compounds that occur naturally in the body, or even of other drugs that act on the endocannabinoid system or cannabinoid receptors (National Academies of Sciences, Engineering, and Medicine, 1999).

BRIEF HISTORY OF THE MEDICINAL USE OF CANNABIS

The medicinal use of cannabinoids has a long and varied history. For thousands of years cannabis was used in Asian medicine, particularly in India, and it was first introduced to the western world by a British doctor, WB O'Shaughnessy, returning to England from service abroad in the mid-nineteenth century (*The Medicalization of Cannabis,* 2009).

During the mid-1960s and early-1970's, research in the pharmacology of cannabinoids saw a rapid increase. This was mainly due to the widespread use

of cannabis as a recreational drug in the US and UK (Pertwee, 2006). However, as a result there was less interest in the therapeutic potential of cannabinoids, and cannabinoid experiments were primarily focused on the psychoactive properties of cannabis.

In 1973, Raphael Mechoulam, an Israeli organic chemist, discovered the structure of THC as the principle active ingredient in cannabis. This led to various pharmaceutical companies actively researching programs aimed at creating THC-like synthetic compounds that would retain the benefits of THC without having unwanted psychoactive effects (Pertwee, 2006).

As discussed in the British Journal of Pharmacology, an important prerequisite for seeking out the modes of action of any drug is the availability of quantitative bioassays. For the cannabinoids, two bioassays that proved to be successful measured 'static ataxia' in dogs and changes such as sedation, ptosis and body sag in monkeys. These bioassays yielded data that supported the hypothesis that Δ^9-THC is the main psychotropic constituent of cannabis. Eventually, in vitro assays for cannabinoids were also developed and it was two of these in particular, a bioassay that measures adenylate cyclase activity and a radioligand binding assay, that provided conclusive evidence for the existence of the cannabinoid CB_1 receptor (Pertwee, 2006).

In the 1990's, therapeutic interest was renewed with the description of cannabinoid receptors and the identification of an endogenous cannabinoid system in the brain. A new and more consistent cycle of the use of cannabis derivatives as medication began, since treatment effectiveness and safety started to be scientifically proven.

Accumulating evidence suggests that the endocannabinoid system is a promising target for the treatment of a variety of health conditions. Two paths of cannabinoid drug development have emerged. One approach is focused on developing medications that are directly derived from the cannabis plant. The other utilizes a single molecule approach whereby individual phytocannabinoids or novel cannabinoids with therapeutic potential are identified and synthesized for pharmaceutical development (Bonn-Miller *et al.*, 2018).

APPLICATIONS OF CANNABINOIDS IN MEDICINE

Beginning in 1985, a synthetic form of THC, Δ^9-THC (trans-9-tetrahydrocannabinol), called Nabilone® was used to treat the side-effects of chemotherapy, namely nausea and vomiting, in cancer patients who had otherwise failed to respond to conventional treatments (Aggarwal *et al.*, 2009).

The 1980s and 1990s also saw the use of cannabinoids for Multiple Sclerosis (MS) treatment. Organizations such as the MS Society conducted anonymous surveys that revealed a large number of MS patients were willing to use Marijuana, which was still illegal during that time, because of the medical benefits they claimed to be receiving from it (*The Medicalization of Cannabis,* 2009). In Britain, the Medical Research Council eventually sponsored the first large-scale clinical trial of cannabis in MS.

This study recruited over 600 patients and lasted for up to 12 months. Though results were mixed, there was genuine positive evidence of the benefit on symptoms of pain and spasticity. The changing attitudes toward cannabinoids prompted Geoffrey Guy to form the company GW Pharmaceuticals to undertake the systematic development of a standardized herbal cannabis extract and a novel sublingual spray delivery system for their product Sativex® (*The Medicalization of Cannabis,* 2009).

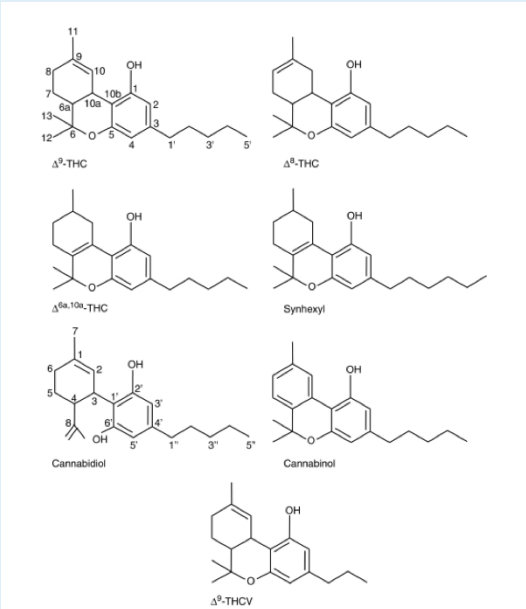
BIOANALYSIS AND CANNABIS TODAY

Sativex®

Sativex® (Nabiximols in the US) is an oromucosal spray of a formulated cannabis extract that contains a controlled ratio of the principal cannabinoids, delta-9-tetrahydrocannibinol (THC) and cannabidiol (CBD), as well as specific minor cannabinoids and other non-cannabinoid components.

Regulatory approval has been obtained in numerous countries outside the United States for the use of Sativex® in the treatment of spasticity (muscle stiffness/spasm) due to multiple sclerosis (MS), as well as for the treatment of severe neuropathic-related cancer pain (GW Pharmaceuticals, *Sativex®*).

ABS Laboratories supported the development of Sativex through multiple preclinical, phase 1, phase 2 and phase 3 studies. Our expert scientists and support teams collected samples and provided assays for the analysis of CBD, THC and 11-OH-THC in plasma and a variety of tissues, and looked at the efficacy of this compound on a variety of therapeutic areas including multiple sclerosis, cancer pain and addiction.



Diagrams of the structures of some major plant cannabinoids and of certain structurally related synthetic cannabinoids (The Medicalization of Cannabis, 2009*)*

CBD FULL CASE STUDY

Support Up to Regulatory Submission to MHRA & IND Submissions

Compound	Studies	Specific Support Provided by ABS	Additional Information
Sativex® Company X	Multiple Preclinical	Assays for the analysis of CBD, THC, and its' active metabolite, 11-hydroxy delta-9-tetrahydrocannibinol (11-OH-THC) in plasma and a variety of tissues.	Supported a variety of preclinical studies in rat, dog, and rabbit.
	Phase 1 Study	Plasma samples were collected at the designated time-points for analysis of CBD, THC, and its' active metabolite, 11-hydroxy delta-9-tetrahydrocannibinol (11-OH-THC).	Study to Assess Food Effect on Sativex Bioavailability https://clinicaltrials.gov/ct2/show/NCT01322464?term+Sativex%C2%AE&lead=GW&rank=12 Eur J Clin Pharmacol (2013) 69:825-834 C. G. Stott et al A phase 1 study to assess the effect of food on the single dose bioavailability of the THC/CBD oromucosal spray https://www.ncbi.nlm.nih.gov/pubmed/23052407.
	Multiple Phase 1, 2, and 3 studies	Analysis of CBD, THC, and its' active metabolite, 11-hydroxy delta-9-tetrahydrocannibinol (11-OH-THC).	Multiple Phase 1 studies looking at the bioavailability of various formulations of CBD and THC. Multiple Phase 2 and 3 studies looking at the efficacy of variety of formulations of CBD and THC for multiple sclerosis, cancer pain and addiction .

ABS CANNABINOID CAPABILITIES

At ABS, we specialize in complex assay method development and validation for the quantification of drugs, metabolites, and biomarkers in biological samples for preclinical and clinical trials from research to final regulatory submission.

For over a decade, our lab has used validated LC-MS/MS and GC-MS assay techniques to determine the bioavailability of various cannabinoids, and their metabolites, from a variety of different formulations and routes of administration. And, we've facilitated studies designed to investigate potential clinical applications of cannabinoids for the treatment of various diseases and health conditions such as oncology, metabolic diseases, pain, addiction, epilepsy, multiple sclerosis, and other therapeutic areas.

Our company has significant experience in supporting pharmaceutical companies, academic institutions, and government agencies in the US, UK, and other countries around the world. ABS offers fully validated GLP assays in a range of matrices and models for CBD, THC and it's metabolite, 11-hydroxy THC. We also develop bespoke assays for other cannabinoids as needed and use validated assays to profile the pharmacokinetics (PK) of cannabinoids whether the administered drug is synthetic or a medicinal extract.

ABS CANNABINOID EXPERTISE

Assays Available

- CBD, THC & 11-OH THC in plasma to support regulatory preclinical and clinical studies since 2000
  - Initial assay was using GC-MS
  - Current assay LC-MS/MS using AB Sciex 6500
    - » Method validated over 100 ng/mL
    - » Stability in human plasma (Li Hep) is:
      - ○ 4 freeze thaw cycles
      - ○ 24 hours at ambient and 4°C
      - ○ Frozen at -20°C approx. 12 weeks

- Total 11-COOH THC in urine (1 to 500 ng/mL)
- Endo cannabinoids in plasma
  - Anandamide (AEA)
    - » 0.1 to 5 ng/mL
  - 2-arachidonoylglycerol (2-AG)
    - » Stabilisation required as 2-AG is prone to spontaneous isomerisation to 1-AG
    - » 1 to 50 ng/mL

ABS has experience assaying a variety of canabinoids and their metabolites such as CBDV and THCV.

Note: Sponsor provided the reference materials when not commercially available.

To learn more about how you can put our experience to work for your next cannabinoid or endocannabinoid research study, contact us today at **acmgloballab.com/bioanalytical-services**

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