**Introduction**

At Next Frontier Biosciences, we use advanced pharmaceutical methods to develop best-in-class purified cannabis products for nasal and sublingual administration. Our development program is science-based and evidence-driven to achieve traditional pharmaceutical levels of quality or better. Our products are formulated using highly purified cannabinoids and GRAS excipients, and we use a high-resolution mass chromatography with HPLC-C to separate product potency, stability, and chemical identity of degradants and contaminants. Furthermore, we employ statistical tools such as design of experiment (DOE) to conduct systematic, high-quality studies, which allow us to efficiently screen and optimize many formulation ingredients for improvements in bioavailability and other pharmaceutical properties. For example, we conducted a factorial fractional DOE screening study of eight formulation factors (water/solvent ratio, buffer type and concentration, salinity, surfactant effect, cannabinoid loading, solubility enhancer, and a permeation enhancer), which would have required 256 experiments if conducted as a full factorial study. However, by utilizing our DOE methodology, we determined the main effects with just 16 experiments, and conducted two confirmatory replicates. From this study, we identified a hydroxyquinone degradant of cannabinoid (CBD) under certain pH conditions and obtained a clear understanding of cannabinoid solubility in the formulation space examined. In a three-component mixture DOE, we examined the solubility of CBD at varying ratios of a solvent, co-solvent, and water from 12 runs and 4 replicates. The surface response ternary plot obtained showed the optimal ratios that maximize CBD solubility. Our analytical tools and formulation approach enable us to develop unique and unparalleled high quality cannabinoid formulations for nasal and sublingual delivery.

**Experiment 1: Use of DOE in Determining Solubility Limits of Terpenoid Formulations**

- **Experimental goal** – to determine the solubility design space of CBD.
- **Focused on GRAS excipients for these solubility tests.**
- **Created restricted design space based on prior general knowledge about CBD solubility.**
- **Target loading concentration of CBD was 120 mg/mL.**
- **General DOE principles and JMP (statistical analysis program) used to generate mixture design to obtain a contour plot and prediction model for CBD solubility.**
- **Three solvents included in the surface response contour plot:**
  - Propylene Glycol (PG)
  - Ethanol (EtOH)
  - Deionized (DI) Water
- **Possible to formulate at 100 mg/mL, CBD or above in mixtures with large PG fractions.**
- **Addition of ethanol increased solubility significantly.**
- **Addition of DI water to a mixture of ethanol and propylene glycol rapidly decreased CBD solubility.**
- **To achieve a 100+ mg/mL, CBD concentration, mixture must be formulated within the forest green contour in Figure 2.**

**In Vitro Analysis**

- **Fraction of DI water in solution (Aq/PG) had strongest effect on CBD solubility (Figure 3).**
- **Cyclodextrin increased solubility by 2 to 3 fold in high aqueous solutions; however, overall solubility was still very low.**
- **Increased the viscosity of formulations.**
- **Maximize the information gained from a study while minimizing the amount of data.**
- **Example: 3 factors tested at two levels (low and high) (Figure 1)**
  - Full factorial study (one variable at a time): 2^n experiments
  - Fractional factorial (Resolution IV, main effects): 2^(n-1) experiments

**Experiment 2: Use of DOE in Enhancing Solubility Limits of Terpenoid Formulations**

- **Experimental goal** – to determine excipients and combinations of excipients useful for increasing solubility in propylene glycol, ethanol and DI water mixtures.
- **Additional experimental goal** – to screen for any excipients incompatible with CBD.
- **Eight factors varied at two levels:**
  1. DI Water to PG ratio
  2. Citrate Buffer
  3. Phosphate Buffer
  4. NaCl
  5. CBD
  6. Cyclodextrin
  7. EDTA
  8. THC
- **Changing only one variable at a time would require 256 or 2^n experiments (Figure 4).**
- **Full factorial study:**
  - 2^8 experiments
- **Fraction of DI water in solution (Aq/PG) had strongest effect on CBD solubility (Figure 3).**
- **Colors varied from orange to clear to purple (Figure 4).**
- **Phosphate buffer concentration (pH 7.4) had a high correlation with color change.**
- **Purple color development appears to be correlated with CBD hydroxyquinone formation.**
- **Cyclodextrin increased solubility by 2 to 3 fold in high aqueous solutions; however, overall solubility was still very low.**
- **EDTA increased the viscosity of formulations.**

**Results**

- **Possible to formulate at 100 mg/mL, CBD or above in mixtures with large PG fractions.**
- **Addition of ethanol increased solubility significantly.**
- **Addition of DI water to a mixture of ethanol and propylene glycol rapidly decreased CBD solubility.**
- **To achieve a 100+ mg/mL, CBD concentration, mixture must be formulated within the forest green contour in Figure 2.**

**Conclusions**

- **Solubility enhancers can increase the solubility of CBD or THC 2- to 3-fold.**
- **Many common solubilizers and co-solvents affect solution pH and have potential to degrade cannabinoids.**
- **Purple color development is associated with the degradation of CBD to CBDHQ.**
- **pH can be a critical parameter in medical cannabis products.**
- **More research should be done on shelf stability of cannabinoids in solutions to ensure patients and consumers are correctly receiving the labeled doses of cannabinoids.**

**Relevant Literature**


**Acronyms**

- CBD = Cannabidiol
- CBDHQ = Cannabidiol hydroxyquinone
- DOE = Design of Experiment
- EtOH = Ethanol
- GRAS = Generally Regarded As Safe
- HPLC = High Performance Liquid Chromatography
- pH = Potential of Hydrogen
- THC = Δ9-Tetrahydrocannabinol
- XIC = Extracted Ion Chromatogram

**Acknowledgments**

Adapted from Telford 2007

**Figure 1:** Three factor, two level DOE visualization Adapted from Telford 2007

**Figure 2:** Ternary contour plot of CBD solubility in restricted design space of EtOH, PG, and H2O

**Figure 3:** Scatterplot matrix of DOE responses and factors

**Figure 4:** Selected samples from Experiment 2 demonstrating color variation

**Figure 5:** XIC for the CBDHQ exact molecular weight (329.211 ± 0.006 Da)