Confidential

Sunny BioDiscovery™

Final Report

March 28, 2019

Intestinal Absorption Potential of CiBiDinol and CBD neat Test Materials using the Millipore PAMPA Membrane Model

(Method Development)

Report 1021PAMPA-HPLC

Prepared for

Xceptor

972 EAST MAIN STREET SANTA PAULA, CA 93060 PHONE (805) 229 7580

E-MAIL: INFO@SUNNYBIODISCOVERY.COM

1. Introduction & Objective

The objective of this project was to assess the intestinal absorption potential of the test materials listed in Table I, using the Millipore PAMPA membrane model designed to approximate passive transcellular absorption, such as at the level of the intestinal epithelium.

Table I Material tested.

Test Material	Lot #	SBD Identifier	Reception date	Storage
CBD (neat)	N/A	CBDneat	July 13, 2017	RT
CiBiDinol - CBD Complex 30%	1812106	CiBiDinol	Feb. 21, 2019	
Cyclodextrin	N/A	CCD	July 13, 2017	

2. Materials & Methods

Test Materials & Read-out system

Test materials (Table I) were dissolved in DMSO at 10mg/ml and further dissolved in 5%DMSO in PBS to 200µg/ml (CiBiDinol), 70µg/ml (CBDneat) or 140µg/ml (CCD), following the CBD solubility information provided by Cayman Chemicals (Ann Arbor, MI) and guidance from the maker of the PAMPA plates (Millipore Sigma, Burlington, MA).
All samples were analyzed using Agilent HPLC series 1100 (Agilent Technologies, Palo Alto, CA) station equipped with the Autosampler G1313A, Zorbax SB-C8 reverse phase column, diode array UV/Vis detector and Chemstation software, at 235nm (A_{235nm}) and 254nm (A_{254nm}). Ethanol:Methanol:Ethyl Acetate (2:2:1) was used as mobile phase.

Intestinal absorption model

Intestinal absorption is approximated here with the Parallel Artificial Membrane Permeation Assay (PAMPA) using MultiScreen Filter/Receiver Plates from Millipore (cat. MAIPNTR10 and MATRNPS50). This simple and robust assay is designed to measure the permeability of a substance from a donor compartment through a phospholipid-coated PVDF filter into an acceptor compartment. Because the membrane has no transporters or efflux systems, only passive permeability is observed.

The method followed in this project is based on the application note “Evaluation of the reproducibility of Parallel Artificial Membrane Permeation Assays (PAMPA)” by Millipore Corporation. Briefly, it consists in applying 150µl of test materials and controls on the filters immediately after preconditioning them with 5µl of 1% (w:v) L-α-phosphatidylcholine (a natural phospholipid, Sigma, cat.# 3644). The incubation with test materials is pursued at room temperature in a humidified chamber for 20h under constant slow movement on an orbital shaker. At the end of the experiment five µl from each receiver chamber (total volume 300µl) are injected to the HPLC system. The elution profile of the injected sample is then matched to the elution profile of 20µg/ml solution of the CBDneat injected in a 5µl volume.

3. Results and Discussion

A. Proof of concept: Establishment of the elution profile of CBDneat.

Figure 1 shows the elution profile of the diluent with no sample in it.

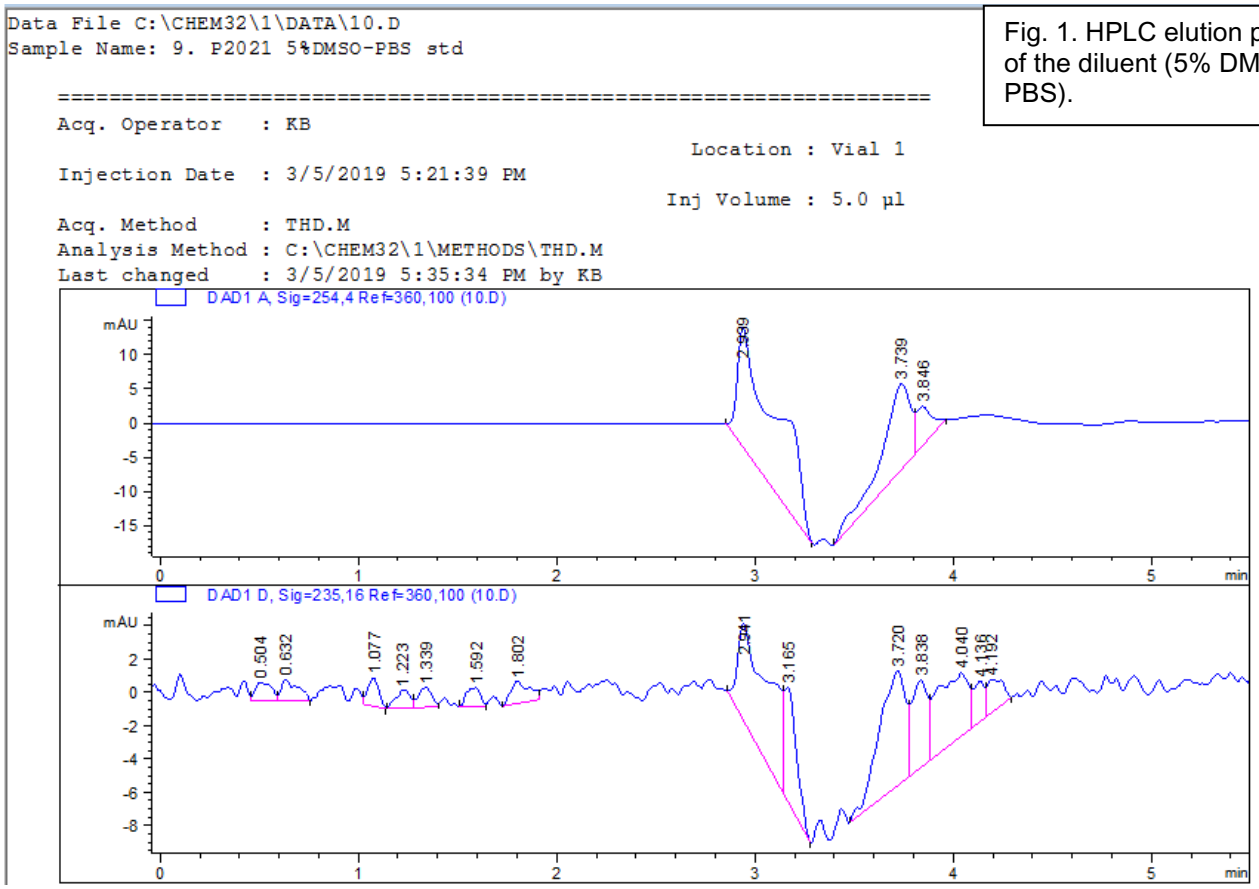

Fig. 1. HPLC elution profile of the diluent (5% DMSO in PBS).

Figure 2 shows the elution profile of CBDneat (20µg/ml) standard in the same diluent measured at the optimum wavelength (wv) for cannabidiol (235nm) and at a non-optimal wv (254nm). A peak of an area of 14.8 AU (arbitrary units) at elution time ~3.5min. represents the compound of interest.

B. PAMPA absorption of CBDneat permeate.

Figure 3 shows the elution profile of CBDneat permeate. The peak area is 0.5AU, which corresponds to ~ 0.7µg/ml – 1.3% of the applied donor solution.

```
Data File C:\CHEM32\1\DATA\1. P2021 CBD NEAT 20UG-ML STD.D
Sample Name: CBD neat 20ug-ml std

    ========================================================================
    Acq. Operator   : KB                          Location : Vial 1

    Injection Date  : 3/5/2019 12:25:03 PM         Inj Volume : 5.0 µl

    Acq. Method     : THD.M
    Analysis Method : C:\CHEM32\1\METHODS\THD.M
    Last changed    : 3/13/2019 11:49:46 AM by KB
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
```

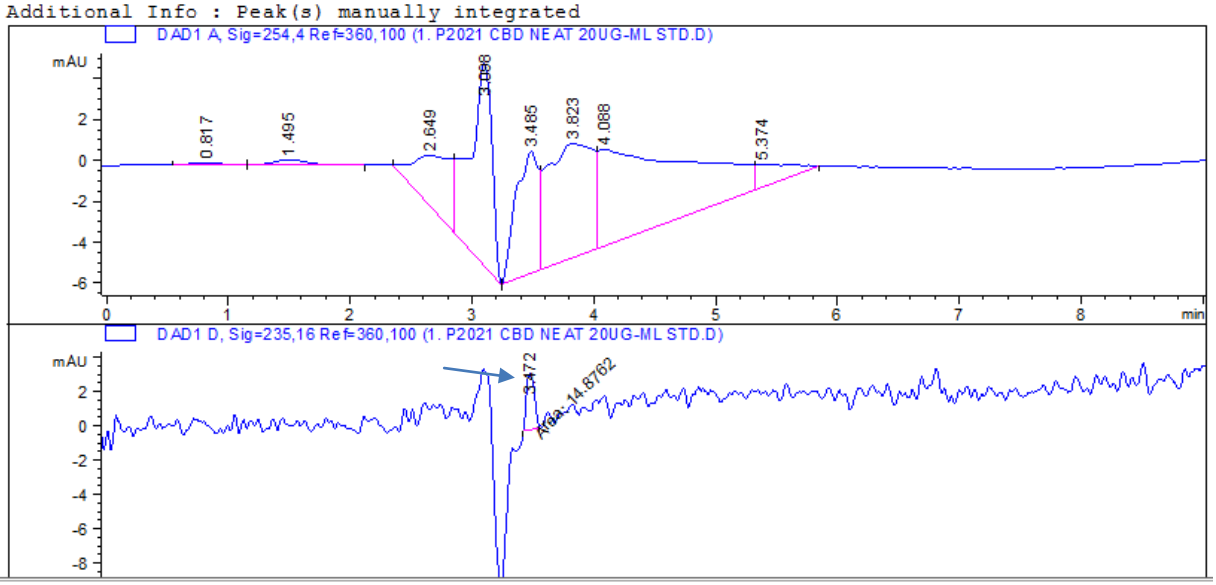

Fig. 2. HPLC elution profile of the 20ug/ml CBDneat standard solution in 5% DMSO in PBS. Arrow indicates CBDneat elution peak.

```
Data File C:\CHEM32\1\DATA\18.D
Sample Name: 18.CBD Neat Pass through 2

    ========================================================================
    Acq. Operator   : KB                          Location : Vial 1

    Injection Date  : 3/9/2019 12:49:28 PM         Inj Volume : 5.0 µl

    Acq. Method     : THD.M
    Analysis Method : C:\CHEM32\1\METHODS\THD.M
    Last changed    : 3/13/2019 11:30:25 AM by KB
                      (modified after loading)
    Additional Info : Peak(s) manually integrated
```

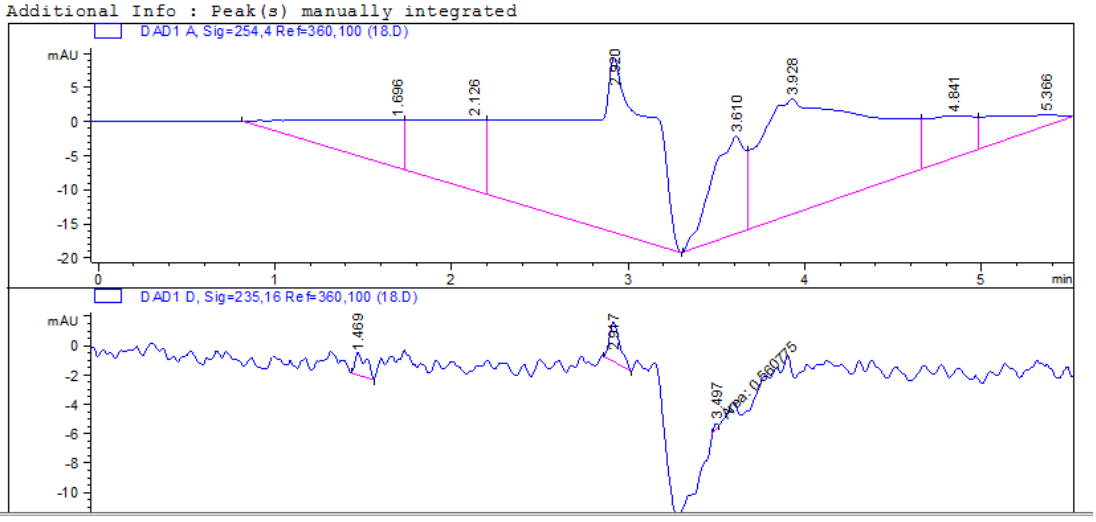

Fig. 3. HPLC elution profile of CBDneat permeated from the donor chamber through a phospholipid-coated filter after 20h.

C. PAMPA absorption of CiBiDinol permeate.

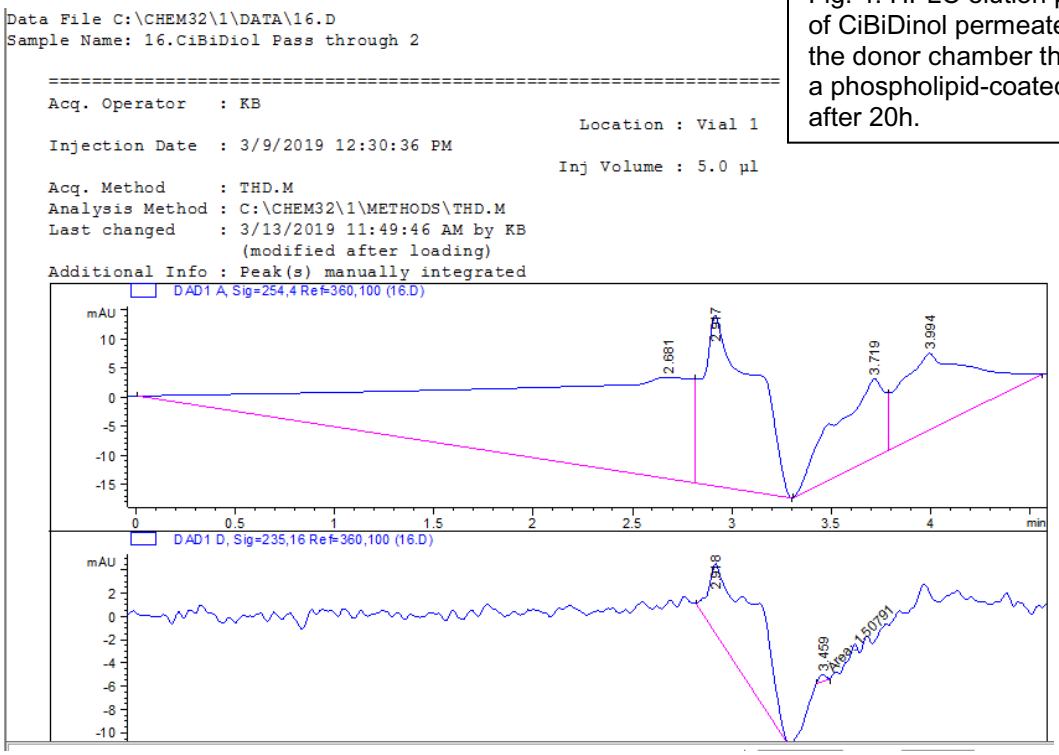

Fig. 4. HPLC elution profile of CiBiDinol permeated from the donor chamber through a phospholipid-coated filter after 20h.

Figure 4 shows the elution profile of CiBiDinol permeate. The peak area is 1.5AU, which corresponds to ~ 2µg/ml – 4% of the applied donor solution.

In conclusion, this series of experiments indicates that CiBiDinol may permeate up to three times better than CBDneat over the 20h incubation period in the passive intestinal absorption PAMPA model.

Experiments performed and Report prepared by:

Krys Bojanowski, *PhD*

Krys Bojanowski

Signature Valid

Digitally signed by Krys Bojanowski
DN: cn=Krys Bojanowski, o=Sunny BioDiscovery, Inc., c=US
Date: 2019.03.28 12:25:12 -08'00'