Supplementary Material

A Phase 2 Randomized Trial to Explore the Potential for Pharmacokinetic Drug-Drug Interactions with Stiripentol or Valproate when Combined with Cannabidiol (CBD) in Patients with Epilepsy

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Cannabidiol (CBD) and 7-carboxy-cannabidiol (7-COOH-CBD): Plasma Protein Binding Displacement (PPB) with Valproate (VPA)

To assess for the potential of a PPB interaction between CBD and VPA, an *in vitro* study was carried out using plasma pooled from three healthy donors. CBD, 7-COOH-CBD, and ^{14}C-VPA incubations were prepared to reflect 0.1× maximum observed plasma concentration (C_{max}), C_{max} and 5× C_{max} values. The concentrations assayed within each combination, along with the PPB percentage values, are detailed in Supplementary Table 1.

Ultracentrifugation was used to assess the PPB in each of the conditions outlined in Supplementary Table 1. This method was chosen due to the physical chemical properties of the compounds being tested and the results of a preliminary suitability study, which assessed the recovery of test compound from the assay matrix. Following ultracentrifugation, supernatant samples were removed, protein precipitated and then vortex mixed prior to liquid chromatography with tandem mass spectrometry analysis. For samples measuring ^{14}C-VPA, duplicate aliquots were taken for liquid scintillation counting analysis.

The PPB of CBD and 7-COOH-CBD was not concentration-dependent. The PPB of VPA was concentration-dependent, with a marked increase in the VPA fraction unbound at 625 µg/mL. VPA did not alter the PPB of CBD or 7-COOH-CBD when incubated at therapeutically relevant concentrations. Neither CBD nor 7-COOH-CBD altered the PPB of VPA when incubated at therapeutically relevant concentrations.

Supplementary Table 1 Mean PPB of CBD, 7-COOH-CBD and VPA (*N* = 2)

Mean percentage CBD PPB in the presence of ^{14}C-VPA			
	CBD (ng/mL)		
^{14}C-VPA (µg/mL)	33	330	1650
0	90.1	86.2	88.4
12.5	87.4	82.9	90.5
125	86.7	85.2	90.4
625	84.5	80.2	83.9
Mean percentage 7-COOH-CBD PPB in the presence of ^{14}C-VPA			
	7-COOH-CBD (ng/mL)		
^{14}C-VPA (µg/mL)	982.4	9824	49120
0	98.5	98.8	98.0
12.5	98.2	98.2	98.9
125	97.2	97.6	97.7
625	92.8	96.8	96.3
Mean percentage ^{14}C-VPA PPB in the presence of CBD			
CBD (ng/mL)	^{14}C-VPA (µg/mL)		

	12.5	125	625
0	93.2	84.7	50.2
33	94.5	86.8	60.4
330	94.4	86.5	58.4
1650	95.6	90.1	64.1

Mean percentage ^{14}C-VPA PPB in the presence of 7-COOH-CBD

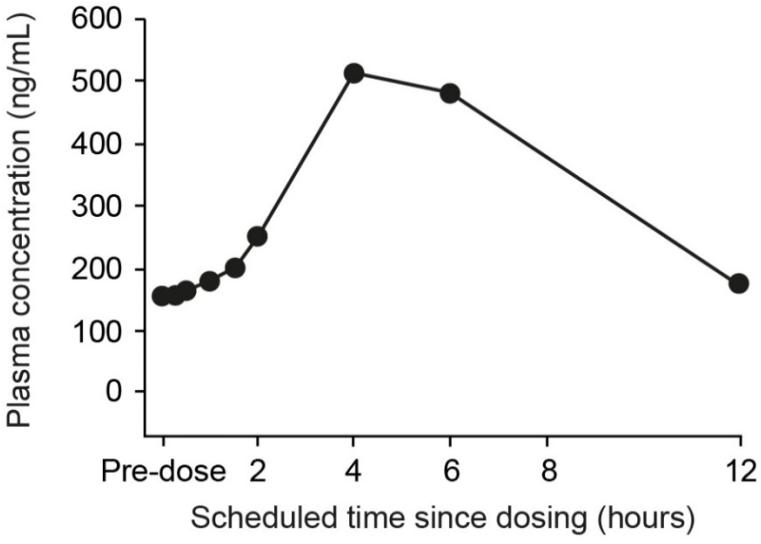
	^{14}C-VPA (µg/mL)		
7-COOH-CBD (ng/mL)	12.5	125	625
0	93.3	80.4	52.0

982.4	92.2	85.6	45.7
9824	90.0	85.6	47.7
49120	92.1	82.6	49.3

7-COOH-CBD 7-carboxy-cannabidiol, *CBD* cannabidiol, *PPB* plasma protein binding, *VPA* valproate

Geometric mean plasma CBD concentration versus time at steady state in the STP and VPA arms

Supplementary Figure 1 Geometric mean plasma CBD concentration versus time at steady state in the STP arm (Day 26, PK population, n = 10)



Supplementary Figure 2 Geometric mean plasma CBD concentration versus time at steady state in the VPA arm (Day 26, PK population, n = 10)