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STUDY REPORT

V21238

Bioaccessibility of cannabidiol (CBD) from two forms of CBD in the dynamic model of the upper gastrointestinal tract (TIM-1) simulating fasted state conditions.

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Study director

Katja Lange

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Date

Contents

Abbreviations

GI tract	Gastro-intestinal tract
HKW	Housekeeper wave
TIM-1	TNO gastro-Intestinal Model of the stomach and three-compartmental small intestine

1 General

1.1 Study Sponsor

Sponsor:	OLEO
Monitor:	Derick Anderson
Phone:	+1-206-290-5125
E-mail:	Derick@oleolife.com

1.2 Test facility

Triskelion B.V.	www.triskelion.nl
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1.3 Responsible Personnel

Project manager:	Ronald Schilderink, PhD ronald.schilderink@triskelion.nl Phone: +31 6 1125 6065
Study Director	Katja Lange, PhD katja.lange@triskelion.nl Phone: +31 6 5000 7604
Scientific contributor Bio-analysis	Brigitte Buscher, PhD

1.4 Time schedule

Delivery test products and analytical reference API:	June 6, 2018
Performance of phase 1 pilot TIM run:	June 21, 2018
Performance of phase 2 duplicate TIM runs:	July 10-11, 2018
Summary report of phase 2 results:	July 27, 2018

2 Introduction

1.1 Objective

The aim of this study was to measure the bioaccessibility of 2 forms of cannabidiol (CBD), crystalline Isolate (99%) and a micro-encapsulated CBD isolate. This was tested under fasted state conditions. Both formulations were subjected to the *in vitro* dynamic, computer-controlled model of the stomach and small intestine (TIM-1).

1.2 Background information

A key element of developing oral dosage forms is having predictive in-vitro tests that accurately predict its in-vivo behaviour. OLEO is developing products containing CBD and would like to determine the CBD bioaccessibility from two forms of CBD using the TIM-1 system under fasted state conditions.

3 Study plan and deviations

3.1 Study plan

The study was conducted according to study plan P21238 entitled 'Bioaccessibility of cannabidiol (CBD) from two forms of CBD in the dynamic model of the upper gastrointestinal tract (TIM-1) simulating fasted state conditions.'. The study plan was approved by the project manager on 20-06-2018.

3.2 Deviations

No deviations from the study plan occurred.

4 Materials and methods

1.3 Test products

The test products were supplied by the sponsor together with information about the physical chemical properties and safety measures.

API

Name:	Cannabidiol (CBD)
Batch	VS051718-01
Purity	99.81%
Log P	6.6
pKa	9.64
BCS Class	N.A.
MW (g/mol)	314.2

Solubility (mg/mL)

Aqueous solubility	0.0126 mg/mL (computer calculated number)
Organic solvents:	
Ethanol & DMSO	23.59 mg/mL

Storage conditions	Ambient temperature
Light sensitivity	Yes
Stability info	In general stable

Test product #1

Name:	Cannabidiol Isolate
Description:	White Crystalline
Strength:	998.1 mg/g
Batch:	VS051718-01
Storage conditions:	Ambient temperature
Dose in TIM-1:	25 mg Cannabidiol = 25 mg test product

Test product #2

Name:	OLEO Micro-Encapsulated Cannabidiol
Description:	White Powder
Strength:	166.43 mg/g
Batch:	SD051718-01
Storage conditions:	Ambient temperature
Dose in TIM-1:	25 mg Cannabidiol = 153 mg test product

1.4 Test system: TIM-1

The study was performed in the TNO dynamic, multi-compartmental *in vitro* system of the stomach and small intestine (TIM-1). The system has been described in detail in several publications: Minekus et al. 1995, Blanquet et al. 2004, Brouwers et al. 2011, Barker et al. 2014

and Verwei et al. 2016. The TIM-1 system consists of a stomach compartment and three small intestinal compartments: the duodenum, jejunum and ileum (Figure 1).
Each compartment is composed of two glass units with a flexible silicone inner wall enclosing the luminal material. The space between the inner and outer walls is filled with water. Peristaltic mixing of the chyme is the result of alternate compression and relaxation of the flexible inner wall. The compartments are connected by peristaltic valve pumps that successively open and close, allowing the chyme to transit over time through the compartments. In this way, oral dosage forms/API are exposed to locally changing and physiological relevant conditions in the stomach and the three parts of the small intestine.

Prior to the performance of each experiment the secretion fluids (e.g. gastric juice with enzymes, electrolytes, bile, and pancreatic juice) were freshly prepared, the pH electrodes calibrated, and semipermeable membrane (polysulfone plasma filter with a cut-off of 50 nm and a surface area of 0.3 m3, Plasma Flux P1 dry) units installed.

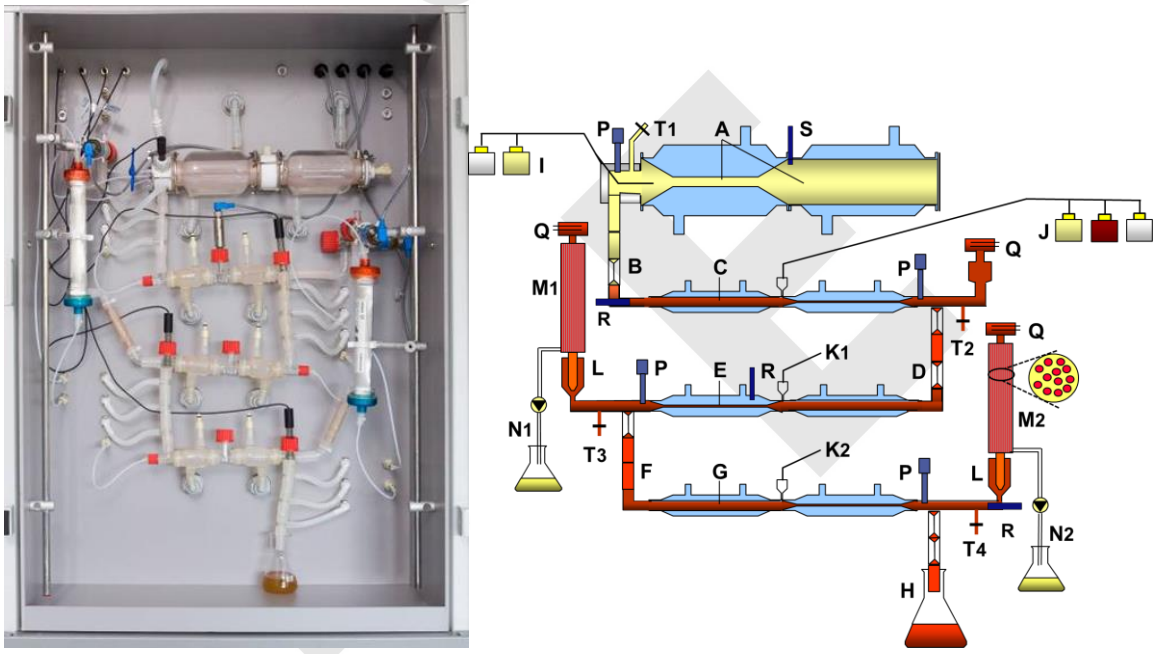

Figure 1. TNO dynamic model of the upper GI tract, simulating the stomach and the compartments of the small intestine (TIM-1 system): A. gastric compartment; B. pyloric sphincter; C. duodenum compartment; D. peristaltic valve; E. jejunum compartment; F. peristaltic valve; G. ileum compartment; H. ileo-cecal sphincter; I. gastric secretion; J. duodenum secretion; K. jejunum/ileum secretion; L. pre-filter; M. semi-permeable membrane; N. collection of filtrate; P. pH electrodes; Q. pressure sensor, R. temperature sensor, S. level sensors, T: pressure sensor, food intake.

1.5 Test conditions

Average test conditions of the fasted state were simulated which includes parameters such as intake of the dosage form with a glass of water; gastric emptying (incl. housekeeper wave); the concentration of secreted bile, pancreatic juice, and enzymes; pH profile in the stomach and pH set points in small intestinal compartments, and small intestinal transit (Table 1).

Table 1. Gastrointestinal (GI) parameters mimicked in the TIM-1 system under simulation of average GI physiological conditions of healthy young adults.

TIM-1	Fasted state
Stomach	
Intake (total)	270 g
Glass of water / high fat meal	240 g
Gastric starting fluid	30 g
Gastric emptying T ½	20 min
House keeper wave	60 min
Gastric pH	3.0 to 1.8 in 30 min
Small Intestine	
Concentration bile	20 %
pH duodenum	6.3
pH jejunum	6.5
pH ileum	7.4

1.6 Gastric intake

The bioaccessibility of Cannabidiol (CBD) was measured while simulating the fasted state conditions and the intake of a glass of water in phases 1 and 2.

1.7 Transit of formulations

The housekeeper wave (HKW) was simulated after 60 minutes for fasted state conditions by transferring residual material from the gastric compartment to the duodenal compartment.

1.8 Sampling

During the 5-hour (fasted state) experiments in the TIM-1 system the following samples were collected:

Filtrate (bioaccessible fraction)
The removal of released and dissolved/solubilized drug molecules from the intestinal lumen by a semipermeable membrane unit allows the assessment of the so-called bio-accessible fraction, i.e. the fraction of the drug which is potentially available for small intestinal absorption. Filtrate fractions from the jejunum and the ileum were collected each 60 minutes till completion of the experiment. The collected volume per 60-min periods were measured, and sub-samples were taken and stored until analysis.

Ileal effluent
The ileum effluent, fluid content that is emptied from the small intestine to the large intestine was collected each 60 minutes till completion of the experiment.

Gastric- and small intestinal residues
Upon completion of the TIM-1 experiment the residues of the stomach, the duodenum, the jejunum and the ileum was collected separately. Any remains of the formulation were collected separately. Each compartment was rinsed twice; with appropriate solvent. This rinse was pooled with the residue samples and stored until analysis. These samples were analyzed for total API content.

Storage of spare samples
The spare TIM samples (back-up samples) were stored at -20°C for 1 month for optional later analysis. Thereafter the samples will be destroyed.

Remaining test products will be returned to the sponsor.

1.9 Sample analysis

TIM study samples was analysed using an UPLC/UV method. The sponsor shared the analytical method with Triskelion, as described in Table 2 and Table 3:

Table 2 UPLC-UV equipment and conditions

UPLC-UV system:	**Acquity (Waters)**
UPLC column:	Acquity BEH C18; 2.1 x 100 mm; 1.8 µm (Waters)
UPLC column temperature:	40 °C
Autosampler temperature:	15 °C
Flow rate:	0.5 ml/min
Injection volume:	2 µl
Mobile phase A:	Milli-Q water
Mobile phase B:	Acetonitrile
UV detection:	210 nm
Retention time:	4.4 min
Run time:	5.0 min

Table 3 UPLC gradient

Time (min)	**Flow rate (ml/min)**	**Mobile phase A (%)**	**Mobile phase B (%)**
0.0	0.5	80	20
0.5	0.5	80	20
4.0	0.5	0	100
5.0	0.5	0	100
5.1	0.5	80	20

Step I. Feasibility study

Based on the information received from the sponsor, an analytical method (UPLC-UV) was set-up for the analysis of the API in a standard solution. The parameters of investigation were the sensitivity of the method and the linear range. The sensitivity of the method was investigated by the analysis of blank solvent. The linear range was investigated by the analysis of blank solvent spiked with the API in a range from approximately 0.050-50 µg/ml.

Step II. Method qualification in TIM matrices

The developed method was applied to the analysis of the two TIM matrices spiked with the API at three concentration levels (QC low, QC medium and QC high) in one qualification run.
Quality controls were prepared in two representative fasted TIM matrices: filtrate matrix and ileal effluent (used for the residue and ileal effluent samples) matrix. These matrices were collected pooled samples from blank TIM-1 runs. The parameters of investigation are the selectivity of the method, calibration, accuracy and repeatability.

Calibration

Calibration standards (6-8), prepared in blank solvent, were analyzed in duplicate. Linear regression analysis with weighting factor 1, 1/x of 1/x2 were performed. At least 75% of the calibration samples should not deviate more than 15% from the actual concentration (≤20% for the lowest calibration standard).

Selectivity

Blank TIM matrices were analyzed to determine the selectivity of the method. The signal obtained from blank TIM matrix should be lower than 50% of the signal in the lowest calibration sample.

Accuracy and repeatability

Quality Control samples were prepared by spiking the four TIM matrices with the API at three concentration levels (QC low, QC medium and QC high). QC levels were selected based on expected concentrations in the actual TIM study samples. The three QC samples were 10-fold diluted, and analyzed in triplicate. After dilution, the QC samples were stored at 2-10°C until analysis (same storage time as the TIM study samples). The precision, expressed as the coefficient of variation (CV, %), should be within 20% at each QC level in each TIM matrix. The mean back-calculated analyte concentrations in the QC samples should not deviate more than 20% from the actual concentrations.

Step III. Analysis of TIM samples (pilot study and main study)

The qualified method were applied to the analysis of the API in TIM samples. The TIM samples were analysed in single-fold, together with calibration standards (analysis in duplicate), QC samples (3 levels per matrix, analysis in duplicate) and blank TIM samples. The samples were collected, diluted (e.g. 10-fold) and analysed within 48 hours from collection (stored at 2- 10°C).

1.10 Calculation of results

The absolute amount of the API recovered in a sample was calculated by multiplying the analysed concentration in the sample with the collected volume (equation 1).

$$A(mg) = C_{sample}(\mu g / ml) \cdot 10^{-3} \cdot V_{sample}(ml) \quad (1)$$

The recovery of the API was determined by the sum of all amounts measured in the jejunum and ileum filtrate fractions, in the ileum effluent and in the residues and rinse fractions of the gastric- , duodenum-, jejunum- and ileum compartment.

The total recovery is expressed as % of amount added (equation 2).

$$\textit{Recovery (\%)} = \frac{\sum A_{filtrate}(mg) + \sum A_{effluent}(mg) + \sum A_{residues}(mg)}{A_{added}(mg)} \cdot 100\% \quad (2)$$

In general, a recovery of 80% or higher is considered as adequate. Recoveries observed between 70-80% would be discussed with the sponsor whether optimization of the protocol is needed in subsequent TIM runs. A recovery below 70% of the API is an indication that either the protocol should be evaluated and if possible optimized for the specific API or the API is unstable under the simulated GI conditions and possible metabolites should also be measured.

The bioaccessibility (% of intake) is calculated by expressing the amount of API in the filtrate samples as a percentage of the intake (equation 3).

$$\textit{Bioaccessibility (\% of intake)} = \frac{\sum A_{filtrate}(mg)}{A_{added}(mg)} \cdot 100\% \quad (3)$$

The results of the duplicate TIM-1 runs were presented as mean ± range.

5 Results

5.1 Phase 1

Phase 1 describes the setup of the analytical UPLC/UV method (see appendices) and performance of 1 fasted state TIM-1 run with TP#1 at a 25 mg dose to validate the analytical method for analyzing API in the TIM-1 samples.

The analytical method to analyze Cannabidiol in the TIM matrices was set up successfully. Analysis of TIM samples collected from the pilot TIM-1 run indicated a high Cannabidiol recovery (91.1 % of intake). A low Cannabidiol bioaccessibility was observed (1.2 % of recovery) and high amounts of API were measured as residue after completion of the 5 hours fasted state TIM-1 experiment (Table 4 and Figures 2-3).

Table 4 Cannabidiol under fasted state conditions in TIM-1 (0-5h), n=1

Sample fraction	% of recovery (TP #1 VS051718-01)
Jejunal bioaccessibility	1.0
Ileal bioaccessibility	0.2
Total bioaccessibility (jejunum+ileum)	**1.2**
Ileal effluent	11.1
Gastric residue	42.4
Duodenum residue	3.2
Jejunum residue	23.4
Ileum residue	12.8
Total residue	**87.7**

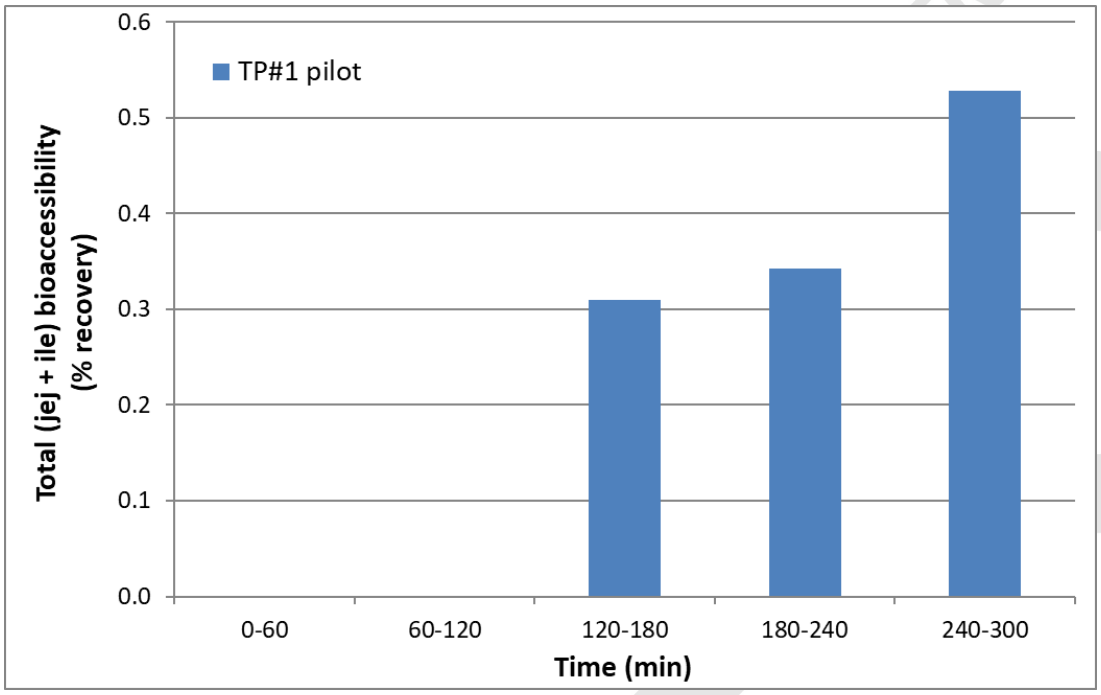

Figure 2 Bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 (n=1) at 25 mg dose expressed as percentage of recovery.

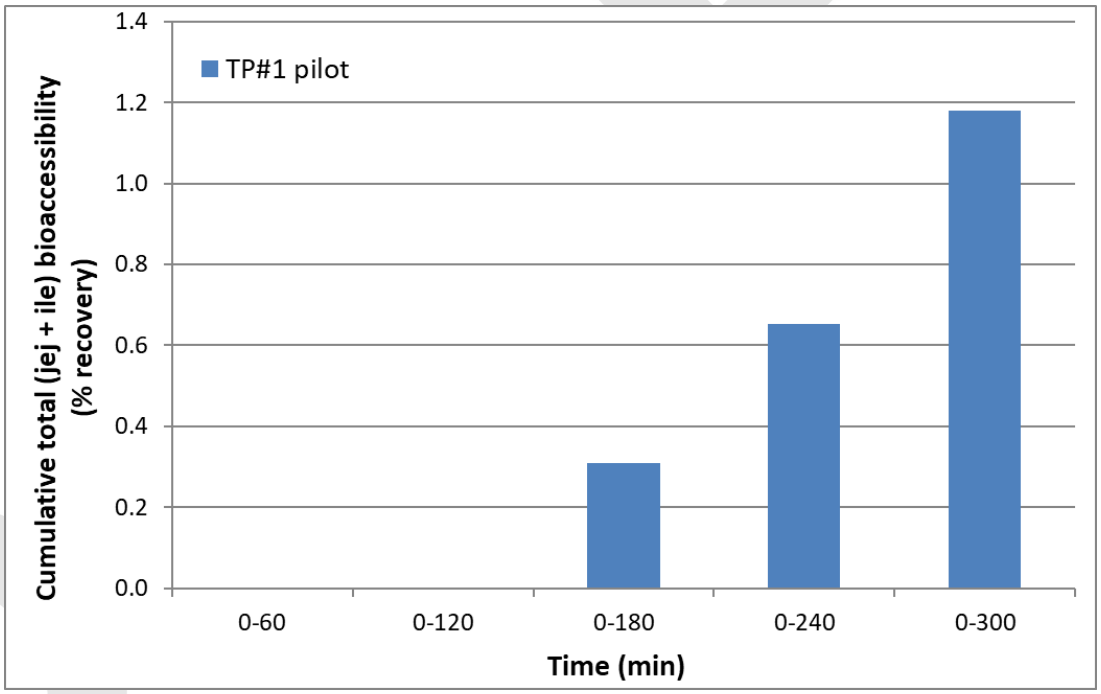

Figure 3 Cumulative bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 (n=1) at 25 mg dose expressed as percentage of recovery.

5.2 Phase 2

Since the phase 1 results indicated a successful analysis of Cannabidiol in the TIM samples and a good API recovery, the study continued with phase 2 using the same experimental set-up.

This second phase included the performance of duplicate fasted state TIM-1 experiments with TP#1 and TP#2 at a dose level of 25 mg. For both products, the total Cannabidiol recovery was adequate (97.6±6.0% and 82.0±1.7% of intake for test products #1 and #2, respectively). Results are shown in Table 5 and Figure 4-5.

Cannabidiol bioaccessibility from test product #1 was 6.1%±1.2 of recovery became bioaccessible, which corresponds with 1.5mg±0.2. Cannabidiol bioaccessibility from test product #2 was approximately two fold higher (13.7%±1.0 of recovery), corresponding with 2.8mg±0.3.

Despite the high variability between duplicate experiments in the first hour of testing, a similar Cannabidiol bioaccessibility was observed for both test products until 60 minutes from start of the experiment. In the samples collected from 60 minutes onwards, higher Cannabidiol bioaccessibility was observed from test product #2 compared to test product #1.

Table 5 Cannabidiol under fasted state conditions in TIM-1 (0-5h) for TP #1 and TP #2 , n=2, mean ±range

Sample fraction	% of recovery (TP #1) VS051718-01	% of recovery (TP #2) SD051718-01
Jejunal bioaccessibility	4.0±0.7	11.9±1.0
Ileal bioaccessibility	2.1±0.5	1.9±0.1
Total bioaccessibility (jejunum+ileum)	**6.1±1.2**	**13.7±1.0**
Ileal effluent	25.5±5.5	5.1±0.5
Gastric residue	21.7±.9	15.3±3.3
Duodenum residue	3.1±0.9	4.6±0.4
Jejunum residue	25.1±0.2	49.2±3.0
Ileum residue	18.5±2.6	12.1±1.2
Total residue	**68.3±4.2**	**81.2±0.5**

The higher Cannabidiol bioaccessibility from test product #2 was caused by the higher amounts of Cannabidiol measured in the jejunum filtrate, since Cannabidiol amounts in the ileal filtrate were comparable for both test products (Table 5).

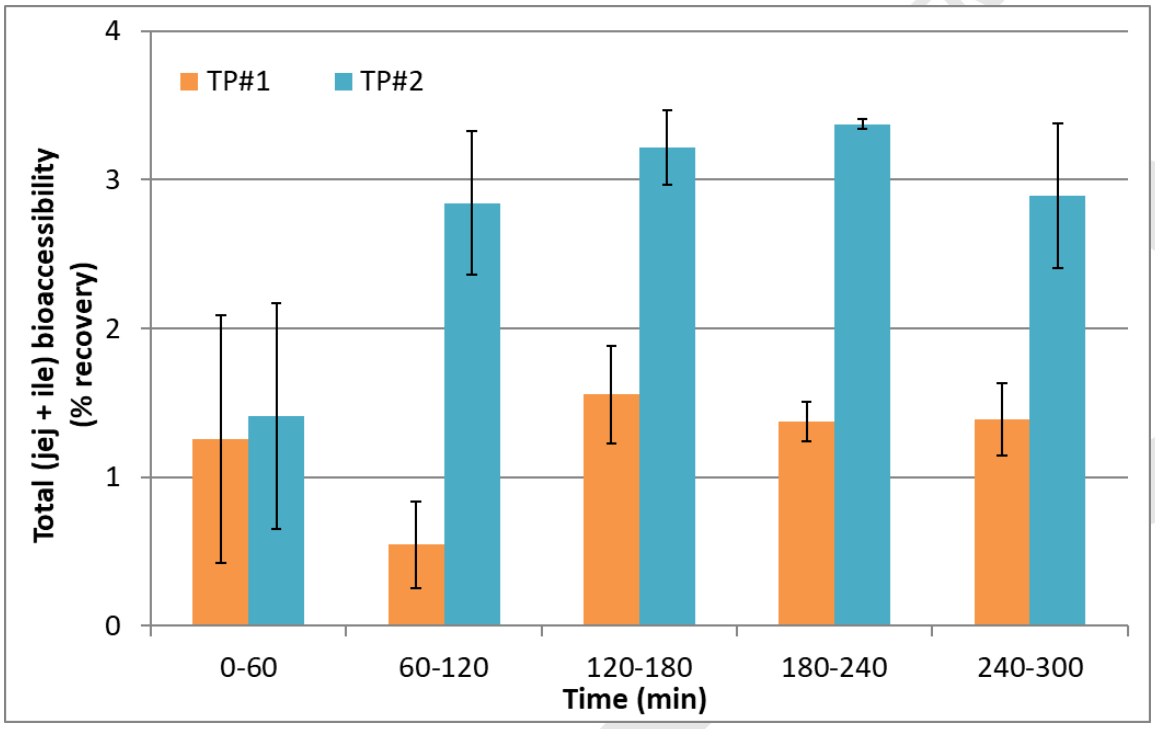

Figure 4 Bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 and TP#2 SD051718-01 at 25 mg Cannabidiol dose expressed as percentage of recovery (n=2), mean ±range.

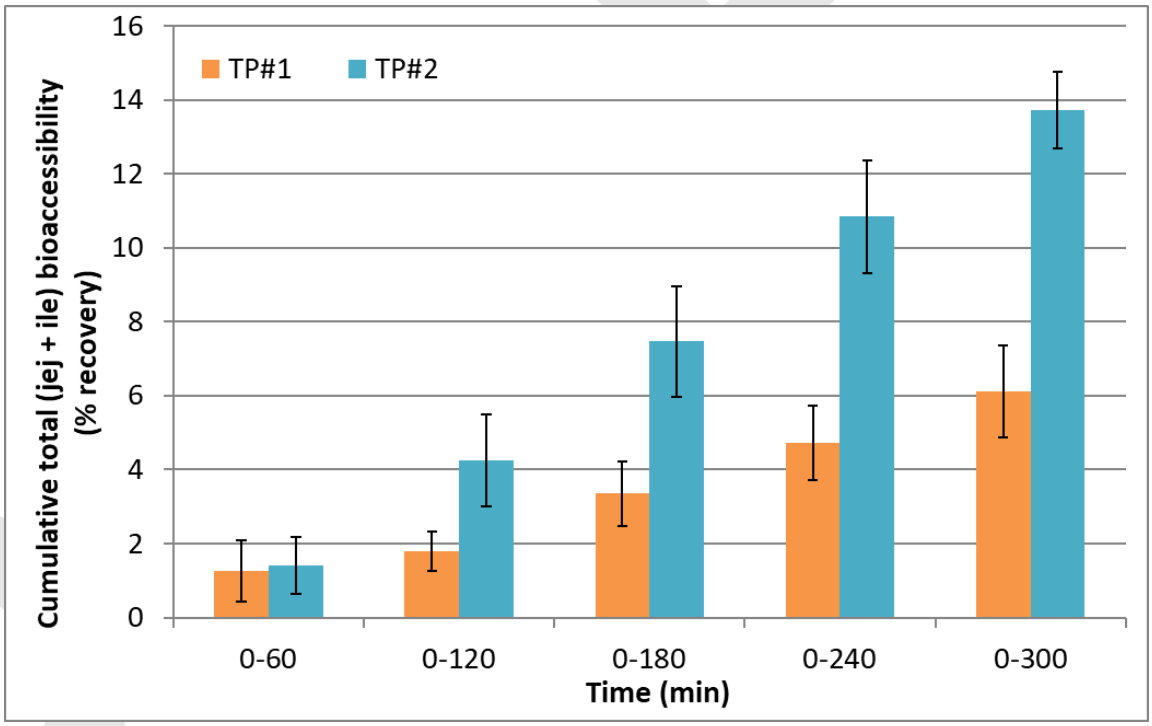

Figure 5 Cumulative bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 and TP#2 SD051718-01 at 25 mg Cannabidiol dose expressed as percentage of recovery (n=2), mean ±range.

6 Discussion and conclusions

The aim of this study was to measure the bioaccessibility of Cannabidiol from two different test products. One test product contained Cannabidiol isolate and the other micro-encapsulated Cannabidiol. The study was performed in the *in vitro* dynamic gastrointestinal model, TIM-1, which closely simulates the luminal conditions of the stomach and small intestine. Both test products were tested under simulated fasted state conditions.

Results indicated a twofold higher Cannabidiol bioaccessibility from test product #2 compared to test product #1 (13.7±1.0% versus 6.1±1.2%, respectively).

While the onset of Cannabidiol bioaccessibility was comparable for both test products in the first hour of testing, a higher Cannabidiol bioaccessibility was observed from test product #2 in the samples collected from 60 minutes until completion of the run at 300 minutes from start. The higher Cannabidiol bioaccessibility from test product#2 was predominantly measured in the jejunum filtrate. This is most likely the effect of micro encapsulation of the Cannabidiol resulting in an increased dissolution and subsequent higher jejunal API bioaccessibility.

In conclusion, the micro-encapsulated test product #2 resulted in a higher Cannabidiol bioaccessibility compared to Cannabidiol isolate under simulated fasted state conditions, tested in the *in vitro* dynamic model of the stomach and small intestine, TIM-1.

7 Documentation and retention of records and samples

The following study specific materials be archived for 5 years:
- Raw data (or true copies if unstable)
- Correspondence

The following study specific materials will be archived for 15 years
- Original study plan and final report, and any amendments thereof

General raw data will be retained for at least 25 years, after which they may be destroyed without further notice. These may include, but are not necessarily limited to:
- Facility-based documents
- Calibration and quality control data
- General registrations potentially used for more than one study

At the end of the archiving period, the sponsor will be asked whether the study plan, final report, amendments, raw data and correspondence should be discarded, retained for an additional period, or transferred to the archives of the sponsor.

All materials will be retained in the archives of TNO, Utrechtseweg 48, 3704 HE Zeist, The Netherlands. The archiving period for starts on the cover date of the final report.

8 References

Minekus, M., Marteau, P., Havenaar, R., Huis in 't Veld, J.H.J. (1995). A multi compartmental dynamic computer-controlled model simulating the stomach and small intestine. Alternatives to Lab. Animals (ATLA) 23: 197-209.

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Barker, R., Abrahamsson, B., Kruusmägi, M. (2014). Application and validation of an advanced gastro-intestinal in vitro model for evaluation of drug product performance in pharmaceutical development. J. Pharm. Sci. 103 (11): 3704-3712. doi: 10.1002/jps.24177.

Verwei, M., Minekus, M., Zeijdner, E., Schilderink, R., Havenaar, R. (2016). Evaluation of two dynamic *in vitro* models simulating fasted and fed state conditions in the upper gastrointestinal tract (TIM-1 and tiny-TIM) for investigating the bioaccessibility of pharmaceutical compounds from oral dosage forms. Int. J. Pharm. 498: 178-186.

Appendices

Analytical method qualification

Reference substance

Name:	Cannibidiol (CBD)
Supplier:	Oleolife
CAS:	13956-29-1
Batch:	VS051718-01
Purity:	99.81%
Storage:	ambient (15 – 25 °C)
Expiry date:	05/22/20

The reference substance was used for the preparation of the calibration standards and quality control (QC) samples.

Preparation of diluent

Ethanol was used as Diluent.

Calibration (CAL) stock solution

The CAL stock solution was prepared by dissolving approx. 10 mg of the reference substance in 10 ml diluent. After preparation, the stock solution was stored at 2-10 °C for a maximum of 7 days.

Calibration standards

Calibration standards were freshly prepared prior to the analytical run by diluting the CAL stock solution with diluent. For method qualification, eight calibration levels were prepared of which the concentrations ranged from approximately 0.05 µg/ml to 50 µg/ml. For the analysis of the pilot samples and Main study samples the calibration range was changed to 0.01-20 µg/ml because of expected low concentrations in the TIM study samples.

Quality control (QC) stock solution

The QC stock solution was prepared by dissolving approx. 10 mg of the reference substance in 10 ml diluent. After preparation, the stock solution was stored at 2-10 °C for a maximum of 7 days.

Quality control samples

For method qualification, QC samples were prepared at three levels (approximately 2, 20 and 200 µg/ml) in blank lipid fasted TIM matrices (coded Ma and Ie). After preparation, the QC samples were diluted 10-fold with diluent. The diluted QC samples were stored at 2-10 °C for a maximum period of 1 week. For the analysis of the pilot samples and Main study samples the QC samples were prepared at approximately 0.5-5-50 µg/ml because of expected low concentrations in the TIM study samples.

Analysis of blank TIM matrices

To monitor the selectivity of the method blank matrix samples, 10-fold diluted with diluent, were analysed in triplicate for the method qualification, and in duplicate for other runs.

UPLC-UV equipment and conditions

UPLC-UV system:	Acquity (Waters)
UPLC column:	Acquity BEH C18; 2.1 x 100 mm; 1.8 µm (Waters)
UPLC column temperature:	40 °C
Autosampler temperature:	15 °C
Flow rate:	0.5 ml/min
Injection volume:	6 µl
Mobile phase A:	Milli-Q water
Mobile phase B:	Acetonitrile
UV detection:	210 nm
Retention time:	4.4 min
Run time:	5.1 min

UPLC-gradient:

Time (min)	Flow rate (ml/min)	Mobile phase A (%)	Mobile phase B (%)
0.0	0.5	80	20
0.5	0.5	80	20
4.0	0.5	0	100
5.0	0.5	0	100
5.1	0.5	80	20

Calibration results

The calibration results are presented in Table 6. The 8 freshly prepared calibration standards were analyzed in duplicate. Linear regression with weighting factor 1/x was applied. The calibration curve was accepted because at least 75% of the calibration standards had a maximum deviation of ±15% (±20% at lowest calibration level).

Table 6 Calibration results of Cannabidiol in diluent. The calibration range was from approximately 0.05 to 50 µg/ml. Linear regression analysis with weighing factor 1/x was applied.

Sample code	Actual concentration (µg/ml)	Calculated concentration (µg/ml)	Dev (%)	Calculated concentration (µg/ml)	Dev (%)
CAL 1	0.0620	0.0659	6.4	0.0662	6.8
CAL 2	0.124	0.123	-0.7	0.127	2.7
CAL 3	0.248	0.252	1.5	0.245	-1.3
CAL 4	0.620	0.600	-3.2	0.603	-2.8
CAL 5	2.48	2.40	-3.2	2.41	-2.8
CAL 6	6.20	6.09	-1.7	6.08	-1.9
CAL 7	24.8	24.7	-0.4	24.7	-0.4
CAL 8	62.0	61.8	-0.2	62.7	1.2

Quality control results
Table 7 shows the quality control results. The QC samples were analyzed successfully in both TIM matrices at all levels (QC low, QC medium and QC high): the accuracy was within 20% for all QC samples.

Table 7 Quality control results

Matrix		QC 1		QC 2		QC 3	
	Actual conc. (ug/ml)	1.95	dev. (%)	19.5	dev. (%)	195	dev. (%)
FST Ma	Replicate 1	1.96	0.9	18.4	-5.5	192	-1.2
	Replicate 2	1.97	1.2	18.4	-5.6	192	-1.3
	Replicate 3	1.98	1.5	18.4	-5.6	190	-2.1
	mean	1.97	1.2	18.4	-5.6	192	-1.6
	s	0.006		0.014		0.987	
	CV (%)	0.3		0.1		0.5	
FST Ie	Replicate 1	1.99	2.2	18.3	-5.9	196	0.9
	Replicate 2	1.93	-1.1	18.3	-6.1	196	0.9
	Replicate 3	1.94	-0.4	18.3	-5.8	197	1.1
	mean	1.95	0.2	18.3	-5.9	197	1.0
	s	0.03		0.04		0.24	
	CV (%)	1.7		0.2		0.1	

All QC samples were diluted 10 fold in diluent (ethanol) and analyzed without freeze-thaw cycle. Between preparation and analysis the diluent QC samples were stored at 2-10°C.

Analysis of blank TIM matrices
To investigate the selectivity of the method both TIM matrices were analysed in triplicate. The selectivity of the method was accepted because no signal was observed at the retention time of the analyte.

Individual values (% of recovery) for each TIM-1 run and timepoint

Table 8. Individiual values expressed as percentage of recovery

Percentage of recovery %	Time (min)	Pilot run 1	TP#1 run 1	TP#1 run 2	TP#2 run 1	TP#2 run 2
Jejunal filtrate	**0-60**	0.0	1.7	0.3	1.8	0.5
	60-120	0.0	0.0	0.5	2.8	1.6
	120-180	0.3	1.2	0.8	3.2	2.6
	180-240	0.3	0.9	0.7	3.0	3.0
	240-300	0.3	1.0	1.0	2.1	3.2
total jejunal filtrate		**1.0**	**4.8**	**3.3**	**12.9**	**10.9**
Ileal filtrate	**0-60**	0.0	0.4	0.2	0.4	0.2
	60-120	0.0	0.3	0.3	0.5	0.7
	120-180	0.0	0.7	0.4	0.3	0.4
	180-240	0.0	0.6	0.5	0.5	0.3
	240-300	0.2	0.6	0.2	0.3	0.2
total ileal filtrate		**0.2**	**2.6**	**1.6**	**1.9**	**1.8**
jej+il filtrate	**0-60**	0.0	2.1	0.4	2.2	0.7
	60-120	0.0	0.3	0.8	3.3	2.4
	120-180	0.3	1.9	1.2	3.5	3.0
	180-240	0.3	1.5	1.2	3.4	3.3
	240-300	0.5	1.6	1.1	2.4	3.4
total jej+il filtrate		**1.2**	**7.4**	**4.9**	**14.8**	**12.7**
Ileum Effluent	**0-60**	3.0	2.2	2.1	2.6	3.3
	60-120	5.5	4.8	13.5	1.4	0.7
	120-180	1.0	7.7	11.4	0.3	1.2
	180-240	0.7	2.5	3.0	0.2	0.3
	240-300	0.8	2.8	1.0	0.1	0.1
total ileum effluent		**11.1**	**20.1**	**31.0**	**4.6**	**5.6**
Residues	**Stomach**	42.4	22.6	20.7	18.6	12.0
	Duodenum	3.2	4.0	2.2	5.0	4.2
	Jejunum	23.4	24.8	25.3	46.2	52.2
	Ileum	12.8	21.1	15.9	10.8	13.3
		87.7	**72.6**	**64.1**	**80.7**	**81.7**

Individual values (% of intake) for each TIM-1 run and timepoint

Table 9. Individiual values expressed as percentage of intake

Percentage of intake	Time (min)	Pilot run 1	TP#1 run 1	TP#1 run 2	TP#2 run 1	TP#2 run 2
Jejunal filtrate	**0-60**	0.0	1.5	0.3	1.5	0.4
	60-120	0.0	0.0	0.6	2.4	1.3
	120-180	0.3	1.1	0.9	2.7	2.1
	180-240	0.3	0.8	0.7	2.5	2.4
	240-300	0.3	0.9	1.0	1.8	2.6
Total jejunal filtrate		**0.9**	**4.4**	**3.4**	**10.8**	**8.7**
Ileal filtrate	**0-60**	0.0	0.4	0.2	0.3	0.1
	60-120	0.0	0.2	0.3	0.4	0.6
	120-180	0.0	0.6	0.4	0.2	0.3
	180-240	0.0	0.6	0.6	0.4	0.3
	240-300	0.2	0.6	0.2	0.2	0.1
Total ileal filtrate		**0.2**	**2.4**	**1.6**	**1.6**	**1.4**
Jej+ile filtrate	**0-60**	0.0	1.9	0.4	1.8	0.5
	60-120	0.0	0.2	0.9	2.8	1.9
	120-180	0.3	1.7	1.3	2.9	2.4
	180-240	0.3	1.4	1.3	2.8	2.7
	240-300	0.5	1.5	1.2	2.0	2.7
Total jej+ile filtrate		**1.1**	**6.7**	**5.0**	**12.4**	**10.2**
Ileum effluent	**0-60**	2.8	2.0	2.1	2.2	2.7
	60-120	5.0	4.4	14.0	1.1	0.5
	120-180	0.9	7.1	11.8	0.3	0.9
	180-240	0.7	2.3	3.1	0.1	0.2
	240-300	0.7	2.6	1.0	0.1	0.1
Total ileum effluent		**10.1**	**18.4**	**32.1**	**3.8**	**4.5**
Residues	**Stomach**	38.6	20.7	21.5	15.6	9.6
	Duodenum	2.9	3.7	2.3	4.2	3.4
	Jejunum	23.4	22.7	26.2	38.7	41.9
	Ileum	11.7	19.3	16.5	9.1	10.7
Total residues		**80.0**	**66.4**	66.4	**67.5**	**65.6**
Recovery		**91.1**	**91.6**	**103.6**	**83.7**	**80.2**

Table 10. Cannabidiol under fasted state conditions in TIM-1 (0-5h), n=1

Sample fraction	% of intake (TP #1 VS051718-01)
Jejunal bioaccessibility	0.9
Ileal bioaccessibility	0.2
Total bioaccessibility (jejunum+ileum)	**1.1**
Ileal effluent	10.1
Gastric residue	38.6
Duodenum residue	2.9
Jejunum residue	23.4
Ileum residue	11.7
Total residue	**80.0**
Recovery	91.1

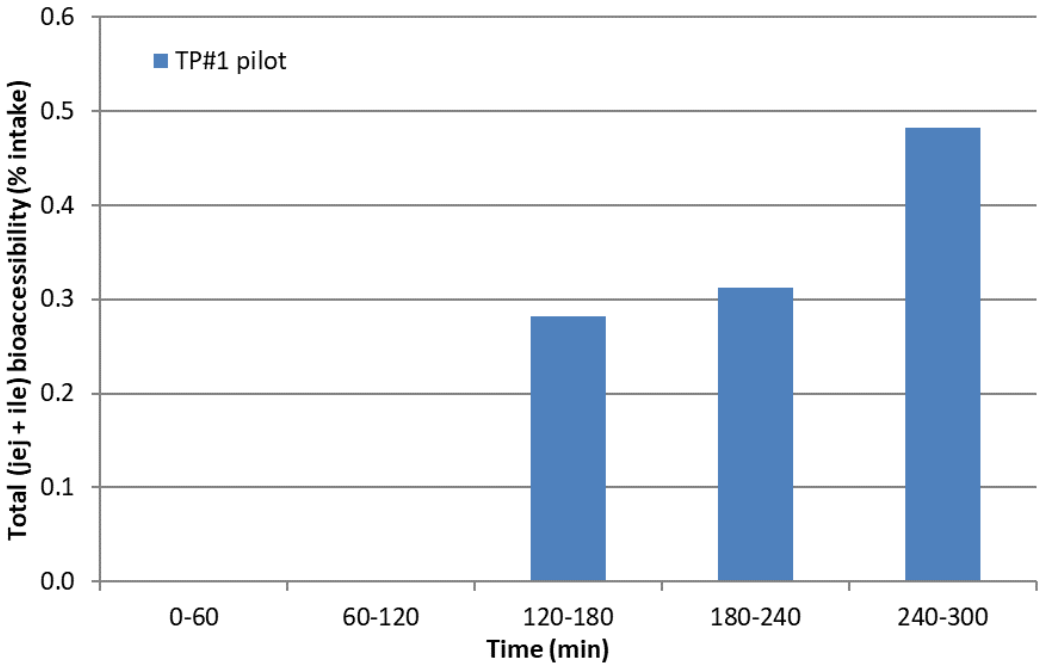

Figure 6 Bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 (n=1) at 25 mg dose expressed as percentage of intake.

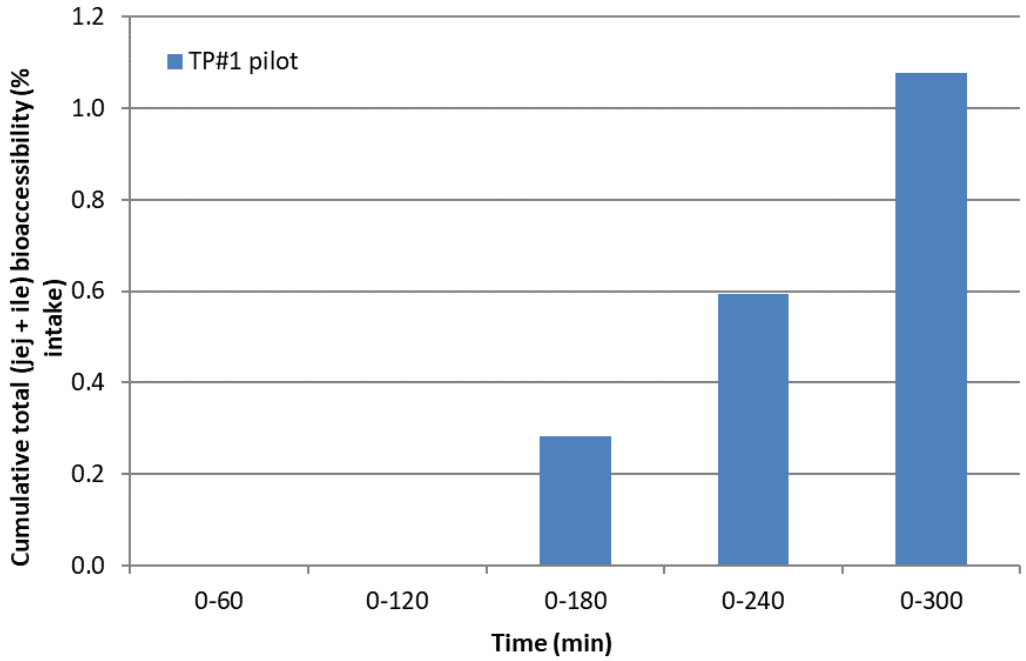

Figure 7 Cumulative bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 (n=1) at 25 mg dose expressed as percentage of intake

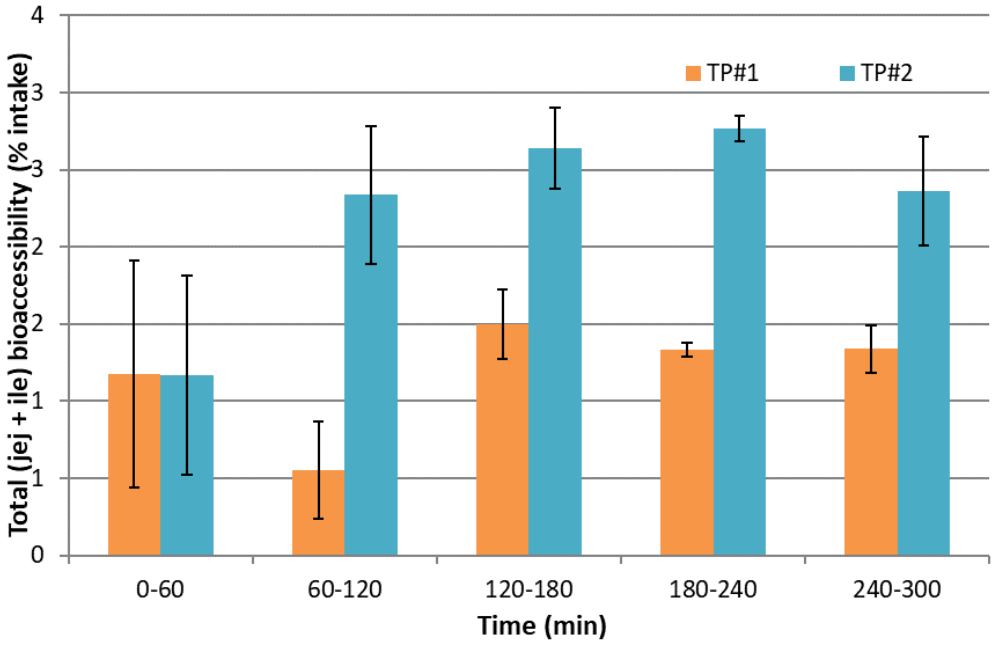

Figure 8 Bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 and TP#2 SD051718-01 at 25 mg Cannabidiol dose expressed as percentage of intake (n=2), mean ±range

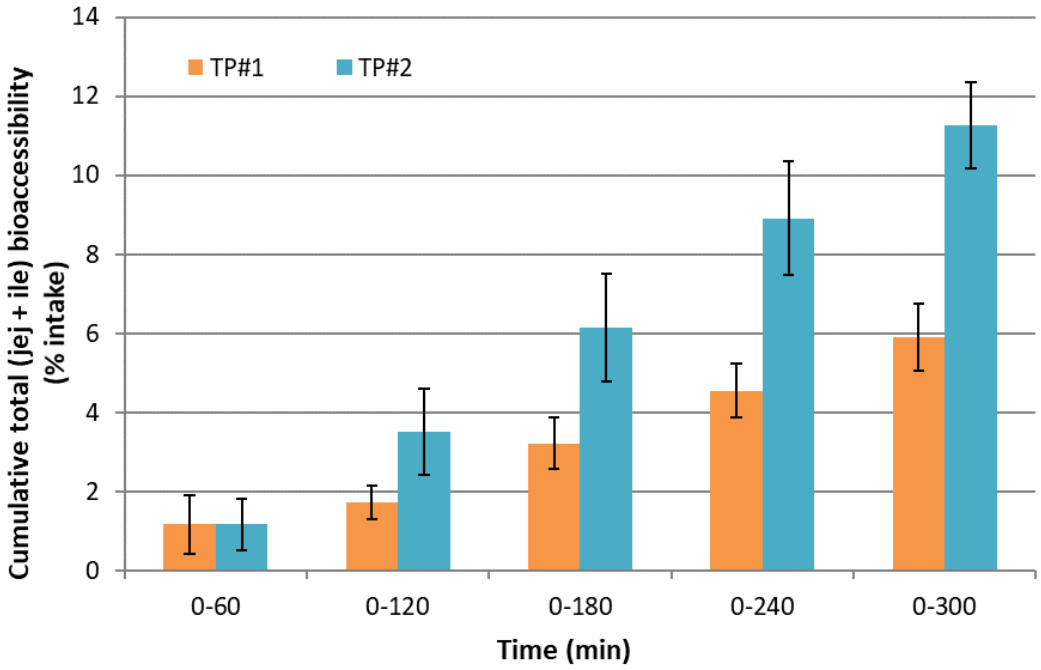

Figure 9 Cumulative bioaccessibility of Cannabidiol in TIM-1 (0-5 hours) from TP#1 VS051718-01 and TP#2 SD051718-01 at 25 mg Cannabidiol dose expressed as percentage of intake (n=2), mean ±range