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## ColoPulse tablets in inflammatory bowel disease

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*  
2017

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Maurer, M. (2017). *ColoPulse tablets in inflammatory bowel disease: Formulation, potential application and evaluation*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen.

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ColoPulse tablets perform comparably in healthy volunteers and crohn's patients and show no influence of food and time of food intake on bioavailability

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## Abstract

ColoPulse tablets are an innovative development in the field of oral drug delivery and are characterized by a colon-specific release. Until now ColoPulse dosage forms (only capsules) have been studied in healthy volunteers having a standardized breakfast three hours after administration but not in specific patient groups and not with a shorter interval between administration and breakfast. Information on bioavailability and release characteristics of ColoPulse tablets in Crohn's patients and the influence of food and time of food intake is a prerequisite to properly design future clinical studies with active substances in these patients.

In the current cross-over study bioavailability and drug release characteristics of ColoPulse tablets were compared in healthy volunteers and in Crohn's patients in remission. Furthermore the influence of food and time of food intake on the *in vivo* drug release behaviour of ColoPulse tablets was investigated.

In this study the dual label isotope strategy was used which means that a ColoPulse tablet containing  $^{13}\text{C}$ -urea and an uncoated, immediate release tablet containing  $^{15}\text{N}_2$ -urea were taken simultaneously. Breath and urine samples were collected during the test day for isotope analysis. The appearance of the stable isotopes in breath and/or urine provides information on the site of release from the dosage form, release characteristics and bioavailability.

Both tablets were administered on two different days in a cross-over design: the first day with a breakfast (non-standardized) one hour after administration and the second day with a standardized breakfast three hours after administration of the tablets. There was no difference in instructions for administration between both days.

Results of 16 healthy volunteers and 14 Crohn's patients were evaluated. At least 86% (51 out of 59) of all ColoPulse tablets administered in this study released their contents at the desired intestinal region. There was no significant difference in bioavailability between healthy volunteers and Crohn's patients on both days (day 1 75.8% vs 90.2%,  $p = 0.070$  and day 2 83.4% vs 91.4%,  $p = 0.265$ ). There was also no significant influence of food and time of food intake on bioavailability in healthy volunteers (75.8% and 83.4%,  $p = 0.077$ ) and in Crohn's patients (90.2% and 91.4%,  $p = 0.618$ ) when day 1 and day 2 were compared. Release characteristics did not significantly differ between healthy volunteers and Crohn's patients. However, food and

time of food intake had some, clinically non-relevant, influence on the release characteristics within both groups which is in line with the fact that food affects gastro-intestinal transit times.

This study shows that ColoPulse tablets enable the site-specific delivery of drugs or other compounds (e.g. diagnostics) deep in the ileo-colonic region of the intestine of Crohn's patients in a comparable amount and rate as in healthy volunteers. Food and time of food intake had no relevant influence on bioavailability. In conclusion ColoPulse delivery systems are promising and deserve further research for local therapy with immunosuppressive drugs in Crohn's patients in the near future.



## 1. Introduction

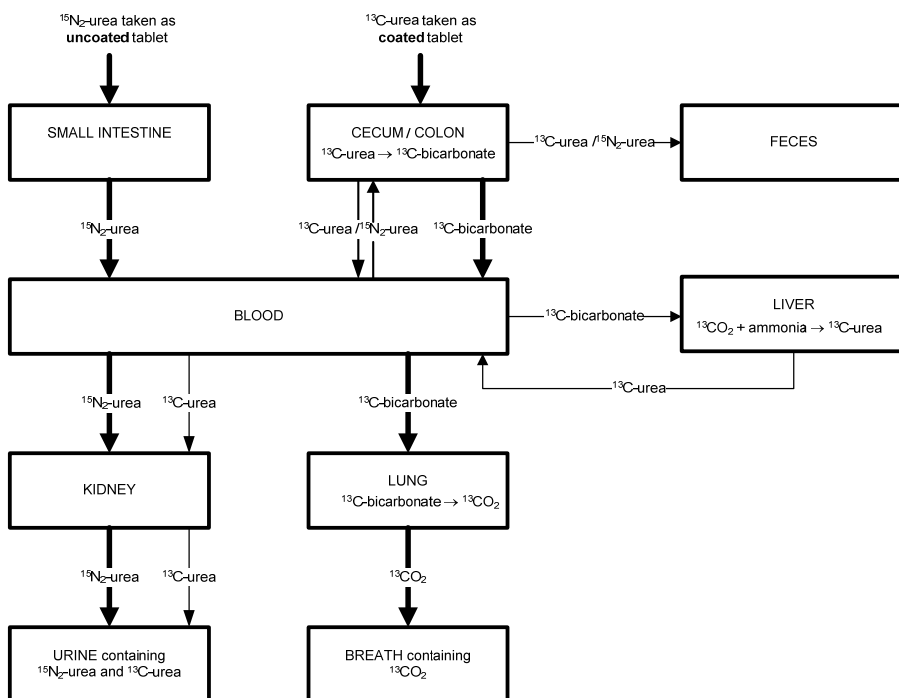
Oral dosage forms with a site-specific drug release in the colon are of interest because they have the potential to improve the drugs efficacy or to minimize side effects of both locally or systemically acting drugs especially in the case of systemic administration. This may have advantages in the treatment of for instance inflammatory bowel diseases. Furthermore, colon-specific dosage forms may be used to improve the bioavailability of drugs that are poorly absorbed in the higher parts of the small intestine or are metabolized in the upper intestinal tract, such as peptide-based drugs [1].

In the literature different strategies for colon-targeting have been described. They include pH-responsive systems, time-based systems and systems triggered by the colon flora, as well as combinations of such systems [1,2]. The ColoPulse technology is a typical example of a pH-responsive system that delivers a drug to the ileo-colonic region. Release from the coated ColoPulse system is triggered by a physiologically occurring variation in the gastrointestinal pH in the terminal ileum and occurs at  $\text{pH} > 7.0$ . It differs from other pH responsive systems because of the non-percolating incorporation of a disintegrant in the coating, yielding a highly reliable and pulsatile release pattern in the targeted region [3].

In two previous studies [4,5] we determined the bioavailability and release profile from a ColoPulse capsule in healthy volunteers using the stable isotope  $^{13}\text{C}$ -urea. Release of  $^{13}\text{C}$ -urea in the ileocolonic intestinal region (urease-rich) from a ColoPulse capsule leads to *in situ* fermentation of  $^{13}\text{C}$ -urea into  $^{13}\text{CO}_2$  which is subsequently exhaled in breath. The delivery of the isotope in the colon was established by measuring the  $^{13}\text{CO}_2$  response in breath. The release characteristics could also be derived from the breath  $^{13}\text{C}$ -measurements and correlated well with the release characteristics derived from blood. Unfermented urea (i.e. release in the small intestine, urease-poor) was measured in blood and urine. Bioavailability was defined as the sum of the recovery of fermented  $^{13}\text{C}$ -urea in breath and the amount of unfermented urea in blood or urine and ranged from 93 to 99%. A strong correlation ( $r = 0.943$ ) was found between blood and urine kinetics, indicating that non-invasive urine sampling could replace blood sampling. In these studies the release of  $^{13}\text{C}$ -urea from a ColoPulse capsule was compared with the release from an uncoated, immediate release capsule containing  $^{13}\text{C}$ -urea administered on a second test day as a reference.

We recently improved the study design by using an uncoated capsule with a second stable isotope of urea,  $^{15}\text{N}_2$ -urea, as a reference on the same day in stead of a  $^{13}\text{C}$ -urea uncoated capsule on a different day [6]. This simplifies the study

design, reduces costs and eliminates day-to-day variation in urea metabolism. Release of  $^{15}\text{N}_2$ -urea in the small intestine from an uncoated capsule leads to recovery of  $^{15}\text{N}_2$ -urea in urine. Bioavailability can be described by the difference between kinetics of  $^{13}\text{C}$ - and  $^{15}\text{N}_2$ -urea (figure 1, a modified version of this figure was published before [6]). The difference in kinetics of these urea isotopes reflects release characteristics, can be used to determine the gastrointestinal region of release and can be helpful in determination of the cause of a failure in release from a ColoPulse dosage form.



**Figure 1:** Absorption, metabolism and elimination of  $^{13}\text{C}$ -urea and  $^{15}\text{N}_2$ -urea. The weight of the arrow symbolizes the importance of the kinetic step.  $^{15}\text{N}_2$ -urea administered as an uncoated tablet is mainly excreted in urine.  $^{13}\text{C}$ -urea administered as coated tablet is converted to  $^{13}\text{C}$ -bicarbonate by bacterial fermentation in the colon followed by absorption in blood and exhalation as  $^{13}\text{CO}_2$  in breath.

Reliable delivery in the targeted intestinal region of the contents from ColoPulse coated capsules has been shown in 18 healthy volunteers [4-6]. Driven by this success we are currently developing ColoPulse tablet formulations for colon-specific delivery and subsequent local therapy of the intestinal mucosa with

immunosuppressive drugs, like TNF- $\alpha$  inhibitors, for Crohn's patients. Currently these types of drugs are only available as parenteral formulations. This requires, depending on the type of drug, parenteral self-administration or frequent visits to the out-patient clinic for intravenous administration. Parenteral administration of immunosuppressive drugs always harbors the risk of infusion reactions, i.e for Infliximab in 10 - 40% of the cases [7]. Furthermore parenteral administration can lead to unwanted systemic side effects.

We believe that administration of such drugs in a ColoPulse tablet may overcome the mentioned problems. However, before we can test this hypothesis and studies with an active substance can be carried out in a specific patient group, it is necessary to determine the performance of the ColoPulse system in this group of patients and to compare it with the performance in healthy volunteers. This is especially of importance, since it is so far unknown whether the conditions of the diseased intestine might affect the performance of the ColoPulse system.

In this paper we describe a cross-over study in healthy volunteers and in Crohn's patients in remission with the aim to determine drug release characteristics in terms of lag- and pulse time and bioequivalence (marker substance bioavailability) of ColoPulse tablets between both groups. Furthermore, the influence of food and time of food intake on the *in vivo* drug release behaviour of ColoPulse tablets is investigated both in healthy volunteers and in Crohn's patients, a prerequisite to properly design future clinical studies with immunosuppressive drugs.

## **2. Material and methods**

### **2.1. Study design**

The bioavailability study design was based on a previous feasibility study in which two stable isotopes of urea are administered simultaneously [6]. The current study was performed on two different test days with at least one week wash-out between both test days. On the respective test days, further designated as 'day 1' and 'day 2', an uncoated tablet containing 50 mg  $^{15}\text{N}_2$ -urea and a ColoPulse tablet containing 50 mg  $^{13}\text{C}$ -urea were taken simultaneously at around 8 a.m. On day 1 a non-standardized breakfast chosen by the subjects was taken one hour later, on day 2 a standardized breakfast three hours later, this to investigate the influence of food and the time of food intake on bioavailability. Food and time of food intake were varied simultaneously to mimic realistic conditions for further clinical studies in which subjects have to take the tablets



every day for a period of several weeks. We decided to investigate only the administration schedules which we planned to use in future clinical studies.

During the experiments the subjects' food and liquid intake were standardized as described before [6] except for the breakfast on day 1. This breakfast was chosen by the subjects and varied largely between the subjects; from two crackers with marmalade to six slices of bread with various types of filling. This represents the variety of breakfasts among different people on a normal day. The breakfast of day 2 consisted of a standardized double sandwich and 200 ml (unsweetened) tea. The subjects were fasted from 8 p.m. the day before both test days. Only water, apple juice (until 11 p.m.) and tea without sugar were allowed. On the test day the tablets were swallowed with 200 ml of apple juice. Approximately 5 and 10 hours after tablet intake, lunch and dinner were taken. There were no food-restrictions for lunch and dinner except foods enriched in  $^{13}\text{C}$ , like corn products, cane sugar and pineapple. During the test day, (that ended at 8 a.m. the next morning) water, apple juice and tea without sugar were the only drinks allowed.

The study was approved by the ethical committee of the University Medical Center Groningen (EudraCT 2009-01347121) and the study was performed according to the principles of the Declaration of Helsinki.

## **2.2. Subjects**

16 healthy volunteers (10 male, 6 female, age 19-63) and 16 Crohn's patients (7 male, 9 female, age 22 - 64) in remission (Harvey Bradshaw  $\leq 3$ , no intestinal stenosis, no ileocecal resection or other intestinal surgery) were initially included in the study. The healthy volunteers had neither a history of gastrointestinal diseases (ulcerative colitis, Crohn's disease, spastic colon, colon cancer, ileus, stoma, stomach- and/or gastrointestinal infection) nor of gastrointestinal surgery. None of the subjects used antibiotics or drugs influencing the gastrointestinal transit time for at least three months prior to the start of the study. A possible *Helicobacter pylori* infection was excluded with a  $^{13}\text{C}$ -urea breath test (INFAI, Germany).

## **2.3. Chemicals, drug substances and drug products**

Polyethylene glycol 6000, acetone, caffeine, colloidal anhydrous silica, sodium stearyl fumarate, talc (BUFA, The Netherlands), microcrystalline cellulose (Avicel PH102, FMC Biopolymer, USA), croscarmellose sodium (Ac-di-sol, FMC Biopolymer, USA), methacrylic acid-methyl methacrylate copolymer 1:2 (Eudragit S100, Röhm, Germany), were obtained via a certified wholesaler (Spruyt-Hillen, The Netherlands). Sucrose was obtained from VWR (the

Netherlands). Inulin 4.0 kD was obtained from Sensus (the Netherlands) and water for injections was obtained from Fresenius Kabi (Germany). All ingredients were of pharmacopoeial grade (Ph Eur or USP). The stable isotope labelled  $^{13}\text{C}$ -urea and  $^{15}\text{N}_2$ -urea (AP 99%) was obtained from a FDA-controlled facility (Isotec, USA). Tablet cores containing 50 mg  $^{13}\text{C}$ - or 50 mg  $^{15}\text{N}_2$ -urea and 25 mg caffeine were compounded in the Department of Hospital and Clinical Pharmacy of the University Medical Center Groningen and analysed according to the European Pharmacopoeia 7<sup>th</sup> edition (table 1). A coating was applied on the tablets containing  $^{13}\text{C}$ -urea using the ColoPulse technology. The coating of ColoPulse tablets was composed of a mixture of Eudragit S-100:PEG 6000:Ac-di-sol:talc in a ratio of 7:1:3:2 (w/w). The solvent was an acetone/water 97:3 mixture (w/w). Coating thickness was determined and expressed as the amount of Eudragit S100 applied per  $\text{cm}^2$  [3]. Caffeine was added to the  $^{13}\text{C}$ -urea tablet cores for quality control purposes and was used as a marker substance for the *in vitro* determination of the release characteristics lag- and pulse time in the *in vitro* dissolution test. Caffeine was also added to the  $^{15}\text{N}_2$ -urea tablet cores to obtain comparable tablet cores, with no particular function in this tablet. All tablets, coated and uncoated, met established quality control criteria (table 1).

**Table 1:** Quality control data of 50 mg  $^{15}\text{N}_2$ -urea tablets and ColoPulse 50 mg  $^{13}\text{C}$ -urea tablets

Parameter	Specification	Result $^{15}\text{N}_2$ -urea (uncoated)	Result $^{13}\text{C}$ -urea (coated)
Crushing strength (cores)	150-200 N	160 N	175 N
Friability (cores)	< 1% (Ph Eur)	0.03%	0.03%
Disintegration (cores)	< 15 min (Ph Eur)	1.5 min	3.5 min
Uniformity of dosage units (urea, n = 10)	AV < 15 (Ph Eur)	14.8	13.1
Uniformity of dosage units (caffeine, n = 10)	AV < 15 (Ph Eur)	11.1	11.6
Content (urea, n = 10)	90-110%	104.3%	106.3%
Coat thickness (n = 20)	13-17 $\text{mg}/\text{cm}^2$	n.a. <sup>a</sup>	15.1 $\text{mg}/\text{cm}^2$
Bursts or cracks in coating (n = 6)	none	n.a.	none
Lag time (n = 6)	> 240 min	n.a.	244 min
Pulse time (n = 6)	< 60 min	n.a.	21 min
Release at t360 min (n = 6)	> 80%	n.a.	106.3%

<sup>a</sup>n.a. = not applicable

The pulsatile release properties are reflected by the lag time ( $t_{5\% \text{ release}}$ ) and the pulse time. The lag time is the time point at which the tablets start to release the

active substance and was defined as the time at which 5% of 25 mg caffeine was released. The pulse time reflects the pulsatile release characteristics and was defined as the period between the lag time ( $t_{5\% \text{ release}}$ ) and  $t_{70\% \text{ release}}$ . These parameters were established in a modified dissolution test with a total duration of 360 minutes in which the pH was varied in time to simulate the different stages of the gastrointestinal tract as described before [8].

## 2.4. Sample collections and analysis

Breath samples were collected every 0.5-1 h up to 15 h after intake of the tablets and were analyzed as described before [4]. Briefly,  $^{13}\text{C}/^{12}\text{C}$  isotope ratios in the  $\text{CO}_2$  of breath samples were analyzed by using a validated breath  $^{13}\text{C}$ -analyser (Thermo Fisher Scientific, Bremen, Germany) based on isotope ratio mass spectrometry (IRMS).

Urine samples were collected during 24 h after intake of the tablets at prescribed intervals in 500 or 1000 ml containers containing an aliquot of 6M HCl. Urine volumes were recorded and 20 ml samples were stored at  $-80^\circ\text{C}$  until analysis. Concentrations of total N and C were determined based on element analysis. Urine aliquots of 25  $\mu\text{l}$  were combusted in an elemental analyzer  $\text{SL}^{\text{TM}}$  (SerCon, Crewe, United Kingdom) using chromium(VI)-oxide at  $900^\circ\text{C}$  to  $\text{N}_2$ ,  $\text{NO}_x$ ,  $\text{H}_2\text{O}$  and  $\text{CO}_2$ .  $\text{NO}_x$  was subsequently reduced to nitrogen gas over copper at  $600^\circ\text{C}$ . Thereafter, the  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichments were measured online by IRMS (Tracer mass 20-20 $^{\text{TM}}$ , SerCon, Crewe, UK).

## 2.5. Calculations

The Percentage of the administered Dose Recovered (PDR) of  $^{13}\text{C}$  and  $^{15}\text{N}$  in each urine sample, the ratio of the PDRs from  $^{13}\text{C}$  versus  $^{15}\text{N}$ -ratio (the  $^{13}\text{C}/^{15}\text{N}$ -ratio), the fermented ( $F_{\text{fermented}}$ ) and not-fermented ( $F_{\text{not-fermented}}$ ) fraction of  $^{13}\text{C}$  urea were calculated as described before [4]. In short, the fermented fraction was calculated as the cumulative (c)PDR of  $^{13}\text{C}$  in breath over a 15 h time period. The not-fermented fraction was calculated as the ratio of the cPDR  $^{13}\text{C}$  and  $^{15}\text{N}$  in a 24 h urine collection. Bioavailability was expressed as the sum of  $F_{\text{fermented}}$  and  $F_{\text{not-fermented}}$ .

The lag time was derived from the cPDR of  $^{13}\text{C}$  in breath and was defined as the time between administration of the tablets and the time the cPDR reached the value of 5% of cPDR at  $t = 15$  h. The pulse time, reflecting the *in vivo* pulsatile characteristics, was calculated as the difference between cPDR $_{70\%}$  (70% of cPDR at  $t = 15$  h) and cPDR $_{5\%}$ .

When the cPDR in breath was  $< 10\%$  at  $t = 15$  h, the results of the corresponding test day for that subject were excluded from further analysis because in that case

the observed small amount of  $^{13}\text{C}$  is probably caused by diffusion of  $^{13}\text{C}$ -urea from the blood to the colon. This was observed in a previous study [5] in which it was found that on average 7.5% of the administered dose of  $^{13}\text{C}$ -urea was recovered in breath 12 h after administration.

All data were corrected for baseline-concentrations of  $^{13}\text{C}$  and  $^{15}\text{N}$  in breath and /or urine. Furthermore, breath data were corrected for  $\text{CO}_2$ -retention as described before [4].

## 2.6. Bioequivalence

For bioequivalence the 90% confidence interval of the ratio of the population means for bioavailability should lie within an acceptance interval of 0.80 - 1.25 [9]. Lag- and pulse time were used to characterize the system.

## 2.7. Statistical procedures

The results were evaluated by descriptive statistics with SPSS version 18.0. Normal distribution of the data was confirmed with the ShapiroWilk test. The center was characterized by the mean and the dispersion by the standard deviation (SD). A paired-samples t-test and an independent samples t-test (both two tailed,  $\alpha = 0.05$ ) were used to compare the results within and between groups, respectively. The 90% confidence interval of the ratio of the population means for bioavailability was calculated to evaluate bioequivalence.

## 2.8. Endpoints

The first endpoint of the study was to determine bioequivalence and drug release characteristics of ColoPulse tablets in healthy volunteers and Crohn's patients. A second endpoint was to investigate the influence of food and time of food intake on the *in vivo* drug (marker substance) release from a ColoPulse tablet.

## 3. Results

The results of 16 healthy volunteers and 14 of the 16 Crohn's patients initially included were evaluated. One patient was withdrawn just before the start of the study because of a urinary tract infection treated with antibiotics which was one of the exclusion criteria and one patient withdrew permission just before start and could not be replaced in time. One patient (subject 27) completed only the first day and the results of this day were used for further analysis.

Based on a first evaluation of the results from the 30 remaining subjects the data of eight test days (~14%) were excluded from further analysis for several reasons. In one healthy volunteer (subject 5) and in one patient (subject 25) (both on day 1) the coating appeared to be defect as the release pattern in urine

of the coated tablet  $^{13}\text{C}$ -urea tablet was equal to that of the uncoated  $^{15}\text{N}_2$ -urea tablet. One patient (subject 29) appeared unable to ferment urea and the data of both test days were therefore excluded from further analysis. Finally, two healthy volunteers (subject 8 and 5, respectively day 1 and 2) and two Crohn's patients (subject 23 and 24, day 1) had a release of  $^{13}\text{C}$  in breath  $< 10.0\%$  being a reason for exclusion as explained in the methods section. For these subjects only the mentioned test day was excluded. The results of the other test day (if applicable) were still used in the analysis. In one healthy volunteer (subject 3, day 1) no lag- and pulse time could be calculated due to the absence of a sigmoid release profile, however bioavailability was available for further analysis. This test day was therefore not excluded.

### 3.1. Bioavailability

Bioavailability ( $F_{\text{fermented}}+F_{\text{not-fermented}}$ ) was used to determine bioequivalence between healthy volunteers and Crohn's patients and to determine the influence of food and time of administration on bioavailability. The results clearly indicate release in or nearby the ileo-colonic region (cPDR  $^{13}\text{C}$  in breath  $> 10\%$ , lag time  $> 4$  h) in 91% and 89% of the tablets administered to healthy volunteers and Crohn's patients, respectively. The mean course of the exhalation of  $^{13}\text{C}$  in breath in healthy volunteers and in Crohn's patients on both days is shown in figures 2a and b. The lag time in Crohn's patients on day 2 in figure 1b is  $< 4$  h. This is due to the results of 2 subjects (18 and 25).

The mean course of the excretion of  $^{13}\text{C}$  and  $^{15}\text{N}$  in urine (used to determine  $F_{\text{not-fermented}}$ ) in healthy volunteers and in Crohn's patients on both days is shown in figure 3a and b. On all test days  $F_{\text{fermented}}$  was 3 - 4 times higher than  $F_{\text{not-fermented}}$ . There was no difference in excretion of  $^{13}\text{C}$  in urine between both days and this was similar for  $^{15}\text{N}$ .

### 3.2. Bioequivalence and influence of food and time of food intake

Bioavailability, lag- and pulse time were evaluated to determine whether there is any difference between healthy volunteers and Crohn's patients in the *in vivo* drug release characteristics of the ColoPulse tablet and whether there is an effect of food and time of food intake on release and bioavailability. A summary of the results is shown in table 2A - C. More detailed results are presented in figures 4A and B. There was no significant difference in mean bioavailability between healthy volunteers and patients who took a non-standardized breakfast after 1 h (75.8 vs 90.2%,  $p = 0.070$ ) neither was there a difference between both groups taking the standardized breakfast after 3 h (83.4 vs 91.4%,  $p = 0.265$ ). The 90% confidence intervals (CI) around the ratio of the means were 0.72 - 0.99

(breakfast after 1 h) and 0.80 - 1.04 (breakfast after 3 h), respectively. The lower CI of day 1 is just outside 0.80 - 1.25.

To evaluate the influence of food and time of food intake the results for day 1 and day 2 were compared within both groups. There were no significant differences within the group of healthy volunteers ( $p = 0.077$ , 90% CI ratio 0.87 - 0.99) and within the group of Crohn's patients ( $p = 0.618$ , 90% CI 0.89 - 1.19).

The release pattern of a ColoPulse tablet is characterized by the parameters lag- and pulse time. There was no significant difference in mean lag time between volunteers and patients who took breakfast after 1 h (278 min vs 332 min,  $p = 0.111$ ) as well as breakfast after 3 h (340 min vs 309 min,  $p = 0.333$ ). The pulse time was not significantly different between both groups either. With breakfast at 1 h after administration of the tablets the pulse time was 224 minutes in healthy volunteers vs 274 minutes in Crohn's patients ( $p = 0.343$ ). With breakfast 3 h after administration the pulse time was 218 and 225 minutes, respectively ( $p = 0.825$ ).

There was an effect of food and time of food intake on both release characteristics within both groups. There was a significant difference for the lag time within the group of healthy volunteers ( $p = 0.021$ ) and for the pulse time within the group of Crohn's patients ( $p = 0.015$ ) when the results of day 1 and day 2 were compared. However food and time of food intake had no significant influence on the lag time for Crohn's patients ( $p = 0.780$ ) and the pulse time in healthy volunteers ( $p = 0.917$ ).

**Table 2:** Summary of parameter results in healthy volunteers and Crohn's patients.  
Data are means and (SD) for 14 (day 1) + 15 (day 2) healthy volunteers and 10 (day 1) + 12 (day 2) Crohn's patients  
Day 1: breakfast (non-standardized) after 1 h  
Day 2: standardized breakfast after 3 h

Parameter	Day	Healthy volunteers	Crohn's patients	P-value <sup>a</sup>	90% CI of ratio
<b>A: Bioavailability</b>					
Bioavailability	1	75.8% (20.1)	90.2% (15.4)	0.070	0.72-0.99
Bioavailability	2	83.4% (14.1)	91.4% (22.2)	0.265	0.80-1.04
P-value <sup>b</sup>	n.a. <sup>c</sup>	0.077	0.618	n.a.	n.a.
90% CI of ratio	n.a.	0.87-0.99	0.89-1.19	n.a.	n.a.
<b>B: Lag time</b>					
Lag time	1	278 min <sup>d</sup> (81)	332 min (72)	0.111	
Lag time	2	340 min (57)	309 min (97)	0.333	
P-value <sup>b</sup>	n.a. <sup>c</sup>	0.021 <sup>e</sup>	0.780	n.a.	
<b>C: Pulse time</b>					
Pulse time	1	224 min <sup>d</sup> (135)	274 min (103)	0.343	
Pulse time	2	218 min (94)	225 min (61)	0.825	
P-value <sup>b</sup>	n.a. <sup>c</sup>	0.917	0.015 <sup>e</sup>	n.a.	

<sup>a</sup> determined with an independent samples t-test

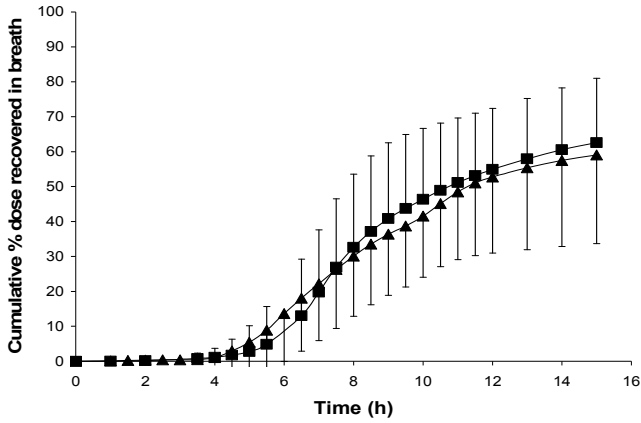
<sup>b</sup> determined with a paired samples t-test

<sup>c</sup> n.a. = not applicable

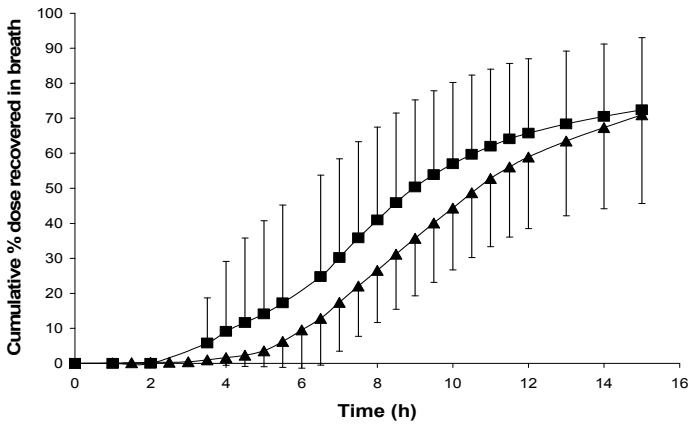
<sup>d</sup> results of 13 healthy volunteers

<sup>e</sup> significant difference between day 1 and day 2

A: Healthy volunteers



B: Crohn's patients



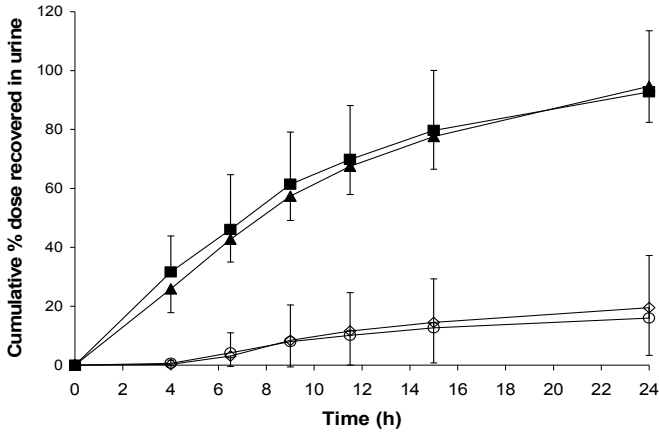
**Figure 2:** Appearance of  $^{13}\text{C}$  in breath in healthy volunteers (mean of 16 subjects) and in Crohn's patients (mean of 14 (day 1) and 13 (day 2) subjects). The error bars represent the standard deviation.

▲ day 1: breakfast (non-standardized) after 1 h

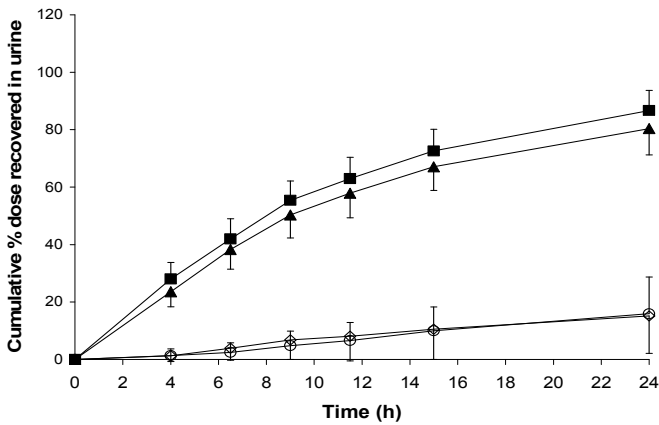
■ day 2: standardized breakfast after 3 h



A: Healthy volunteers



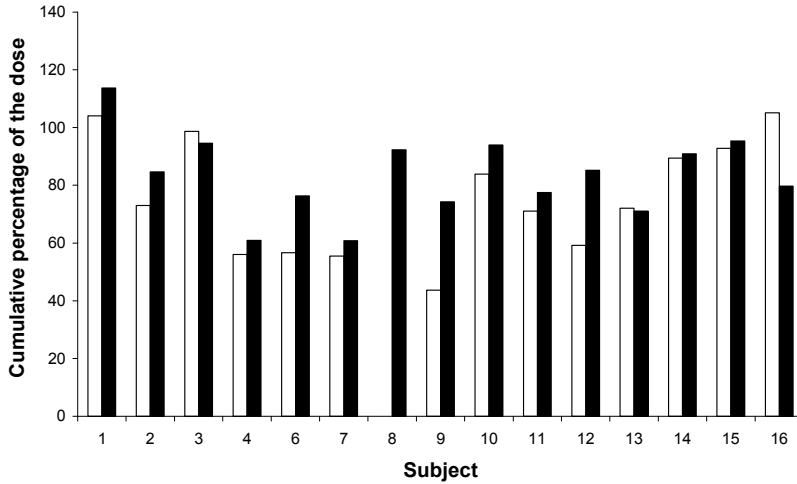
B: Crohn's patients



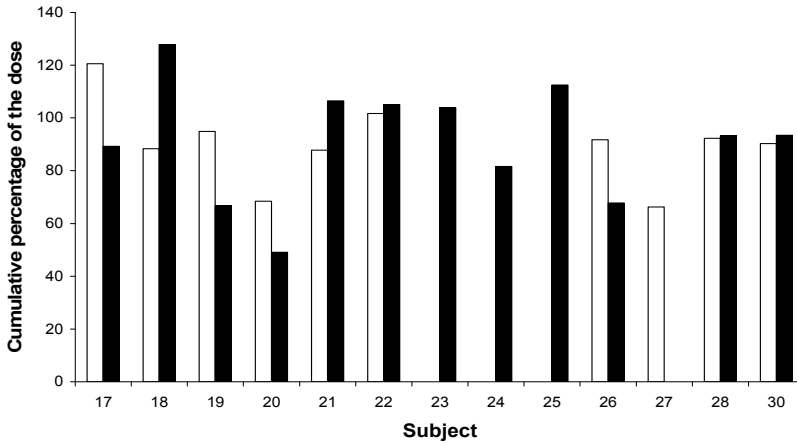
**Figure 3:** Appearance of  $^{13}\text{C}$  and  $^{15}\text{N}$  in urine in healthy volunteers (mean of 16 subjects) and Crohn's patients (mean of 14 (day 1) and 13 (day 2) subjects). The error bars represent the standard deviation.

- $^{13}\text{C}$  day 1: breakfast (non-standardized) after 1 h
- ◇  $^{13}\text{C}$  day 2: standardized breakfast after 3 h
- $^{15}\text{N}$  day 1: breakfast (non-standardized) after 1 h
- ▲  $^{15}\text{N}$  day 2: standardized breakfast after 3 h

A: Healthy volunteers (subject 5 was excluded for both test days)



B: Crohn's patients (subject 29 was excluded for both test days)



**Figure 4:** Bioavailability in healthy volunteers and Crohn's patients

- day 1 : breakfast (non-standardized) after 1 h
- day 2 : standardized breakfast after 3 h

## 4. Discussion

This is the first study in which the ColoPulse technology is tested in Crohn's patients. From the results obtained it can be concluded that the ColoPulse tablet delivers its content effectively in the ileo-colonic region of the intestine in both healthy volunteers and Crohn's patients. This study delivers clear proof that the behaviour of a ColoPulse tablet in Crohn's patients and in healthy volunteers is comparable. Furthermore, only small differences in bioavailability were found when the results from a non-standardized breakfast 1 h after intake of the tablets mimicking real life conditions were compared with a standardized breakfast after 3 h (both in healthy volunteers as well as in patients). Despite the fact that the amount and type of food taken by the subjects on the first day differed substantially, the mean bioavailability ranged between and 75.8 and 91.4% and criteria for bioequivalence (as used by the EMA [9]) were met in three out of four 90% confidence intervals of the ratio of population means for bioavailability.

At least 86% (51 out of 59) of all tablets administered in this study released the marker substance at the desired site of delivery. As described above, the results of eight test days out of 59 were excluded from further analysis due to low or altered bioavailability. On four of these test days  $F_{\text{fermented}}$  was below 10%. This phenomenon was also observed in a previous study in healthy volunteers [4]. In that study two subjects out of 12 showed a low  $F_{\text{fermented}}$  (4.5 and 7.5%).

There are several possible reasons for a low or altered bioavailability: no or unusual bacterial flora in the colon, too early release in a region with low urease activity (terminal ileum), a coating defect and incomplete release from the tablet or insufficient mass transport in the lumen due to a viscous fecal mass. The dual-label stable-isotope technique offers the possibility to discriminate between these possible causes of low or altered bioavailability and the results obtained from these eight patients can all be explained adequately.

In one Crohn's patient (subject 29)  $F_{\text{fermented}}$  was relatively low on both test days (3.7 and 11.1%). Urinary excretion was around 75% at  $t = 24$  h. However, appearance of  $^{13}\text{C}$  in urine was  $\geq 4$  h delayed compared to  $^{15}\text{N}$ . This probably means that the content was released in the right region but that no fermentation occurred. Because this happened on both days it is most likely that this subject had an unusual low urease activity in the ileocolonic region. Therefore we considered this patient not suitable for evaluation of the ColoPulse technology using  $^{13}\text{C}$ -urea. One healthy volunteer (subject 5, day 2) showed a similar profile, low  $F_{\text{fermented}}$  and delayed excretion of  $^{13}\text{C}$  in urine compared to  $^{15}\text{N}$ .

Total bioavailability was  $> 78\%$ . The most likely explanation again is that the content of the tablets was released in a urease-poor region nearby the colon, most likely the terminal ileum.

For subjects 5 (day 1) and 25 the urinary excretion profile of  $^{13}\text{C}$  from a ColoPulse tablet was equal to that of  $^{15}\text{N}$  from an uncoated tablet. This is a clear indication of a coating defect with early release of the contents. Finally in subjects 8, 23 and 24 the content of the tablets was released incompletely, maybe due to the fact that pH 7.0 was not reached or that the tablet became trapped in the viscous fecal mass with no further systemic absorption. In this group excretion of  $^{13}\text{C}$  in urine was delayed compared to  $^{15}\text{N}$ , but total bioavailability was also relatively low indicating an incomplete release of the marker substance from the tablets.

The Eudragit S polymer used for the ColoPulse coating has a dissolution threshold at pH = 7.0. It is known from the literature that the intraluminal pH in healthy volunteers gradually changes from about 6.6 to 7.5 (from jejunum to terminal ileum). A pH above 7 is encountered in a short intestinal region where the ColoPulse tablet resides only 0.5 to 1 h [10]. It is not known whether the gastrointestinal pH-profile of Crohn's patients differs from that of healthy volunteers. In the literature there is only scarce information available on the intestinal pH profiles in Crohn's patients. One study describes that the median pH value in healthy controls compared to that of Crohn's patients was significantly lower in the stomach (1.55 vs 2.4) but not in the terminal ileum, cecum and right colon [11]. In another study it was found that there was no difference in intraluminal pH of the gastrointestinal tract between controls and patients with active Crohn's disease [12]. Based on this, we assumed for our study that the pH in the intestinal lumen of Crohn's patients would not differ compared to that of healthy volunteers. Therefore we expected no difference in the performance of the ColoPulse system between healthy volunteers and Crohn's patients (meaning bioequivalence). The results from this study support our hypothesis.

In the development of new oral dosage forms, especially controlled release formulations, and their applications, food-interaction studies are of great importance [13]. The previous studies with the ColoPulse technology in healthy volunteers were done with a standardized breakfast 3 h after intake of a ColoPulse coated capsule. This schedule is not feasible in daily (clinical) practice. From the perspective of the patient, non-restricted food and beverage intake are preferred. However, it cannot be excluded that effects on the drug release profile will occur due to a temporary high rise (pH  $> 7.0$ ) in gastric pH caused by the intake of certain food and fluids. We considered the intake of a

ColoPulse coated tablet 1 h before breakfast in this stage of development an acceptable and feasible option for patients.

The used study design with almost no restrictions in food and drinks differs from commonly conducted food-interaction studies. This was done to come as close as possible to a setting with daily use of medication. The results show that even in this setting, there was no difference in bioavailability and in the location of drug release within the groups of healthy volunteers and Crohn's patients when a non-standardized breakfast 1 h after intake of the tablets was compared with a standardized breakfast after 3 h. This is an important precondition for the further development of ColoPulse tablets to be used in this particular patient group.

This design gives only information for active substances administered once daily. If other dosage regimens are used in future studies the absence of food influence has to be verified for the particular conditions applied. Furthermore, the influence of food on the bioavailability of a specific drug substance has to be investigated using the applicable EMA guidelines [9,14].

The drug release characteristics of the ColoPulse system were assessed by the lag- and pulse time. Although there appeared to be no significant differences when the results of healthy volunteers and Crohn's patients were compared, differences were found within both groups when food effects were studied. In healthy volunteers the lag time was significantly shorter with administration 1 h before breakfast compared to 3 h before breakfast (278 vs 340 minutes). This could be explained by the principle that after a period of fasting a subsequent meal activates the gastrointestinal motility. This causes the dosage form to pass the ileo-cecal junction. The two hours difference in breakfast after intake of the tablets could explain the shorter lag time with breakfast after 1 h. The fact that there is no difference in bioavailability between both days supports this [15]. In the literature an altered gastrointestinal motility was observed in Crohn's patients compared to healthy controls which can be an explanation of the fact that the interval between administration and breakfast does not have an effect on the lag time in Crohn's patients [16]. However, there is no literature available about the influence of a subsequent meal on gastrointestinal motility.

In Crohn's patients the pulse time was longer with administration of the tablet 1 h before breakfast compared to 3 h before breakfast (274 versus 225 minutes). The pulse time is influenced by the rate of disintegration of the tablet coating and core, dissolution, metabolism and kinetics of  $^{13}\text{C}$ -urea. However the observed difference cannot be explained by one of these aspects. The clinical relevance of this difference seems limited, especially for chronic therapy and the type of medications Crohn's patients' use. This is supported by the fact that the

difference in bioavailability within both groups was not found to be significant and to be within the 90% confidence intervals for bioequivalence. Furthermore, it should be kept in mind that the residence time in the first part of the colon is several hours. This makes a difference of approximately 1 hour in lag- and pulse time relatively short and less meaningful. In particular the lag time is mainly determined by the small intestinal transit time. This transit time is determined solely by the variability of the intestinal motility of the subject.

Compared to previous studies in healthy volunteers with ColoPulse capsules, the ColoPulse tablets exhibited longer pulse times (median around 200 minutes) than the capsules (median 99 minutes) [5]. However, a large range of 276 minutes was noticed in the study with capsules. A possible explanation could be the fact that the tablet cores are relatively dense compared to the contents of a capsule. The dissolution process when little fluid is available takes probably more time for the tablets. The clinical relevance of this phenomenon seems to be negligible since the pulse time is less than the residence time in the proximal colon and the active substance will arrive at the desired site of delivery.

## **5. Conclusions**

This clinical cross-over study in healthy volunteers and Crohn's patients shows that the ColoPulse technology enables the site-specific delivery of drugs or other compounds (e.g. diagnostics) deep in the ileo-colonic region of the intestine of Crohn's patients with a mean bioavailability of around 90%. Bioavailability was similar in healthy volunteers and in Crohn's patients and met in most tests the criteria for bioequivalence. Food had no relevant effect on bioavailability and drug release characteristics of the ColoPulse system. We conclude that the ColoPulse system is a delivery system that deserves further research for its application in local therapy with immunosuppressive drugs in Crohn's patients in the near future.

## **Acknowledgements**

The authors thank Theo de Boer for analyzing the breath samples.

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