AstraZeneca

Clinical Study Protocol

Drug Substance	Dapagliflozin
Study Code	D1691C00004
Edition Number	1.0
Date	
EudraCt No.	2009-014485-26

An open-label, randomised, two-period crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg OD) versus twice a day (5 mg BID) in healthy male and female volunteers

Sponsor: *AstraZeneca AB, 151 85 Södertälje, Sweden*

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment

Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

AstraZeneca

PROTOCOL SYNOPSIS

An open-label, randomised, two-period crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg OD) versus twice a day (5 mg BID) in healthy male and female volunteers

Principal Investigator

Study centre and number of subjects planned

This study will be conducted at . A total of 16 healthy volunteers will be included in order to obtain data on both treatment periods for 14 healthy volunteers.

Study period	Phase of development
Estimated date of first subject enrolled	Phase I
Estimated date of last subject completed	

Objectives

Primary objective

- To assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when dapagliflozin is administered once a day (OD) (10 mg) versus twice a day (5 mg every 12 hours) at steady state (after five days of dosing)

Secondary Objectives

- To assess the effect of dapagliflozin on urine glucose excretion when administered once a day (10 mg) versus twice a day (BID) (5 mg every 12 hours) at steady state (after five days of dosing)

- To examine the safety and tolerability of dapagliflozin dosed once a day versus twice a day
- To evaluate the PK parameters for dapagliflozin dosed twice a day versus once a day at steady-state

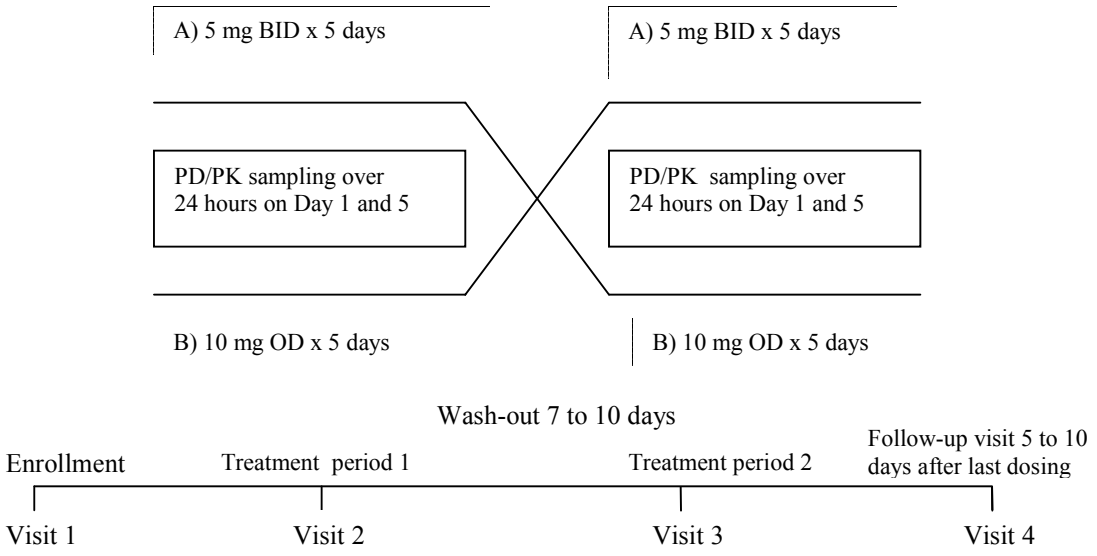
Exploratory Objective

- To indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 (7-36 amide) (glucagon-like peptide) and GIP (glucose-dependent insulinotropic polypeptide) responses to meals accompanied with or without the administration of dapagliflozin

Study design

This is an open-label, randomised, two-period crossover, single-centre study to assess the effect of dapagliflozin dosed once a day versus twice a day. The doses of dapagliflozin will be 10 mg for once daily dosing and 5 mg every 12 hours for twice daily dosing. Each dose will be administered for 5 days, with a 7 to 10 days wash-out between. Up to 16 healthy volunteers will be enrolled (females of non-childbearing potential or being abstinent and males) to obtain 14 completed and evaluable healthy volunteers.

Figure



BID – twice a day; PK – pharmacokinetics; OD– once a day;

Target subject population

Healthy male and female volunteers aged 18 to 45 years both inclusive. Female subjects must be non-fertile or must be abstinent during the study.

Investigational product, dosage and mode of administration

- Dapagliflozin tablets 10 mg, orally administered as once daily dose for 5 days.
- Dapagliflozin tablets 5 mg, orally administered as twice daily dose for 5 days (total daily dose 10 mg).

Comparator, dosage and mode of administration

Not applicable

Duration of treatment

Each healthy volunteer will receive each treatment for 5 days, both treatment periods will be separated by 7 to 10 days. Total duration of the experimental part will be 17 to 20 days.

Outcome variable(s):

Pharmacokinetics

- C_{max}, $C_{ss,max}$, $C_{ss,min}$, AUC_{τ} and AUC_{ss}
- Dose accumulation ratio based on AUC ($R_{ac(AUC)}$) and C_{max} ($R_{ac(Cmax)}$)
- $DF\% = (C_{ss,\,max} - C_{ss,\,min})/AUC_{(0-\tau)/\tau} \times 100$;

For BID dosing, blood PK samples will be collected according to following schedule:

- Day 1: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 12.25, 12.5, 13, 13.5, 14, 15, 16, 18, 20, and 24 hours;
- Day 3 and Day 4: 0 hour (pre-dose of the morning dose);
- Day 5: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 12.25, 12.5, 13, 13.5, 14, 15, 16, 18, 20, and 24 hours.

For OD dosing, blood PK samples will be collected according to following schedule:

- Day 1: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, and 24 hours;
- Day 3 and Day 4: 0 hour (pre-dose of the morning dose);
- Day 5: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, and 24 hours.

Pharmacodynamics

- Percent inhibition of renal glucose re-absorption over 24 hours

  - Blood samples for plasma glucose will be drawn on Day 5 at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 24 hours.

  - Blood samples for creatinine will be drawn at pre-dose of Day 4 and of Day 5.

- Inhibition of rate of gut glucose absorption be determination of plasma glucose, insulin, GLP-1 (7-36 amide) (glucagon-like peptide 1) and GIP (glucose-dependent insulinotropic polypeptide)

  - Blood samples for plasma glucose, insulin, GLP-1 (7-36 amide) and GIP will be drawn on Day 5 at –15, 0 15, 30, 45, 60, 90, 120, 150 and 180 minutes after each meal.

- Total urinary glucose excretion over 24 hour

  - On Day 5, urine will be collected in the following time intervals: from pre-dose to 4 hours post-dose, 4 to 8, hours post-dose, 8 to 12 hours post-dose, 12 to 16 hours post-dose, 16 to 20 hours post-dose and 20 to 24 hours post-dose.

For plasma glucose, insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal ($AUE_{(0-180),}$ AUE= area under the effect time curve) calculated.

Safety

- Safety and tolerability will be assessed by collection of adverse events.

Statistical methods

The primary objective of this study is to assess the effect of twice-daily dosing versus once-daily dosing of the same total daily dose of dapagliflozin on percent inhibition of glucose re-absorption. Given that the goal is one of estimation rather than hypothesis testing, no power calculation has been performed. The choice of a sample size of 14 subjects who complete both periods is based on sufficient precision (expected confidence interval widths) for the dapagliflozin comparisons.

The primary pharmacodynamic endpoint (percent inhibition of renal glucose re-absorption over 24 hours), secondary pharmacodynamic endpoint (total urinary glucose excretion over 24 hours), and the PK parameter $AUC_{ss, (0-24)}$ will be analyzed using an analysis of variance (ANOVA) model, with sequence, period and treatment regimen (10 mg dapagliflozin OD x 5 days versus 5 mg dapagliflozin BID x 5 days) as fixed effects and subject within sequence as a random effect. $AUC_{ss, (0-24)}$ will be log-transformed prior to analysis. The results of the analysis will be presented in terms of estimated means for each treatment regimen (geometric means for PK parameters), estimated difference in treatment regimen means (ratio of geometric means for PK parameters). All estimated means or difference in means will be reported with 95% confidence intervals except for ratios of geometric means, which will be reported with 90% confidence intervals. Geometric means will be obtained by back-

transformation of least squares means from the log-transformed data with adjustment for any imbalances in the design. If there is convincing evidence that one or more of the model assumptions is not valid after inspecting the ANOVA model diagnostics, then a nonparametric analysis of the treatment regimen differences will also be reported using the Hodges-Lehmann estimator of the median difference together with a corresponding exact 95% confidence interval.

For plasma glucose, insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated. Baseline adjusted geometric mean differences between both treatment groups will be estimated by a linear model on the log-transformed AUEs after each meal and retransforming the point estimates and 95% confidence intervals.

Sample Size Calculation

If 14 subjects complete both periods of the study and if the intra-subject variance is one half the total variance observed in the 10 mg group on Day 7 for average percent inhibition of glucose re-absorption over 0-12 hours in Bristol-Myers Squibb Study MB102002 ($mean/SD_{total}$=24.5%/15.4%), then a 95% confidence interval for the difference between regimens in mean percent inhibition of glucose re-absorption over 24 hours (δ) is expected to be approximately $\delta \pm 9.0$ percentage points.

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
AE	Adverse event (see definition in section 6.3.1)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
$AUC_{ss, (0-24)}$	Area under the plasma concentration versus time curve from time zero to 24 hours at steady state [amount•time/volume]
AUC_{τ}	Area under plasma concentration-time curve during a dosing interval [amount· time/volume]
AUC_{ss}	Area under plasma concentration-time curve during any dosing interval at steady state [amount· time/volume]
AZ	AstraZeneca
BID	Twice a day
BMI	Body Mass Index
BMS	Bristol-Myers Squibb
CK	Creatinine kinase
CRF	Case Report Form (electronic/paper)
CSR	Clinical Study Report
$C_{ss,av}$	The average concentration at steady state
C_{max}	Maximum plasma (peak) drug concentration [amount/volume]
$C_{ss,max}$	Maximum (peak) steady state drug concentration in plasma during dosing interval [amount/volume]
$C_{ss,min}$	Minimum (trough) steady state drug concentration in plasma during dosing interval [amount/volume]
DAE	AEs, which leas to discontinuation of the IP
DF%	Degree of fluctuation at steady state
DMP	Data management plan
DVS	Data verification specification
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)

Abbreviation or special term	Explanation
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
FE	Fractional excretion
FSH	Follicle stimulation hormone
Fup	Follow-up examination
GCP	Good Clinical Practice
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide 1
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
ICH	International Conference on Harmonisation
IP	Investigational Product
LIMS	Laboratory Information Management System
LOQ	Limit of Quantification
LSLV	Last Subject Last Visit
max	Maximum
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
min	Minimum
n	Number of subject
OAE	Other Significant Adverse Event (see definition in section 11.1.1)
OD	Once a day
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
q12 hour	Every 12 hours
$R_{ac(Cmax)}$	R_{ac}=Accumulation ratio for C_{max}
$R_{ac(AUC)}$	R_{ac}=Accumulation ratio for AUC
$AUE_{(0-180)}$	Area under the effect time curve until 180 minutes
SAE	Serious adverse event (see definition in section 6.3.2).
SAP	Statistical analysis plan

Abbreviation or special term	Explanation
SAS®	Statistical Analysis System
SD	Standard deviation
SGLT2	Sodium-glucose co-transporter
SUSAR	Suspected Unexpected Serious Adverse Reactions
T2DM	Type 2 diabetes mellitus
$t_{ss,max}$	The time relative to administration to reach C_{max} at steady state [h]
WHO	World Health Organization
β hCG	B-Human chorionic gonadotropin

1. INTRODUCTION

1.1 Background

Dapagliflozin is a rationally designed, potent, highly selective and orally active inhibitor of the human renal sodium-glucose cotransporter (SGLT2), the major transporter responsible for renal glucose re-absorption. Dapagliflozin lowers plasma glucose by inhibiting the renal re-absorption of glucose and thereby by promoting its urinary excretion. Traditionally, the presence of glucose in the urine of diabetes patients has been seen as a sign of poor glycemic control and thus something to be avoided. However, familial renal glucosuria in humans, due to genetic mutations that reduce the function of SGLT2, is associated with life-long glucosuria, which is generally asymptomatic (Hans et al 2008, Komoroski et al 2009, Matthei et al 2000). Results from nonclinical studies and completed clinical studies suggest that dapagliflozin intentionally promotes the urinary excretion of glucose as a safe and effective method of reducing blood glucose levels. Chronic correction of hyperglycaemia by SGLT2 inhibitors has also been shown to improve overall glucose utilization and reduce hepatic glucose production in pre-clinical models (Hans et al 2008, Komoroski et al 2009, Matthei et al 2000).

1.2 Research hypothesis

The research hypotheses are as follows:

- The % inhibition of glucose re-absorption is similar if the same daily dose of dapagliflozin is once a day or twice a day
- The amount of urine glucose excretion at steady state is similar if the same daily dose of dapagliflozin is once a day or twice a day
- Exposure (AUC) of dapagliflozin is similar if dosed once a day versus twice a day

1.3 Rationale for conducting this study

The rationale for this study is

- to demonstrate that dapagliflozin has a similar pharmacodynamic (PD) effect if dosed twice a day as once a day, as this is how dapagliflozin would be given in a fixed-dose combination with metformin IR

1.4 Benefit/risk and ethical assessment

Dapagliflozin's novel mechanism of action, inhibition of SGLT2, can result in lowering plasma glucose regardless of the patient's insulin sensitivity and β-cell functional secretory status. Because the mechanism is independent of insulin secretion or insulin action, this approach to antidiabetic therapy provides an opportunity to achieve clinically important glyceamic efficacy with a relatively low risk of hypoglycaemia. The insulin independent

mechanism of action of SGLT2 inhibitors suggests that this treatment is applicable to a broad spectrum of patients and every stage of T2DM. The risk of hypoglycaemia is inherently low because of the insulin independent mode of action. In addition, increased excretion of glucose may promote weight loss or prevent weight gain, a potential benefit for many patients with T2DM.

The inhibition of SGLT2 results in increased urinary glucose concentrations, which may lead to an increased risk of developing genital myotic infections. Neither UTIs nor genital myotic infections were reported in clinical Phase-I studies, however in clinical Phase-III studies, patients receiving dapagliflozin showed an increased frequency of UTIs and genital myotic infections when compared to placebo.

Based on the mechanism of action of dapagliflozin and results of animal and clinical studies, there may be a potential risk for this compound to cause hypovolemia or electrolyte imbalance. As a precaution, subjects at risk for hypovolemia or electrolyte disturbance should not receive dapagliflozin until more clinical information is available from human studies. In subjects already receiving dapagliflozin who develop conditions that may cause hypovolemia or electrolyte imbalance, decisions to interrupt or discontinue dapagliflozin therapy and management of subjects should be based on clinical judgment.

Imbalances of liver function test parameters in Phase-III studies were observed with diabetic patients in some but not all studies. There were no marked abnormalities reported for alkaline phosphatase or total bilirubin >2x ULN. All elevations have been reversible except a few with a clear non-drug cause, for instance in patients with a pancreatic cancer. The enzyme elevations have not showed any specific pattern with regards to exposure time, but several of them had appeared at more than 3 months of exposure.

The once a day administration of dapagliflozin may enhance the patients compliance during the long-term treatment of T2DM.

The healthy volunteers will not have any medical benefit from this study.

2. STUDY OBJECTIVES

2.1 Primary objective

- To assess the effect of dapagliflozin on % inhibition of glucose re-absorption when dapagliflozin is administered once a day (10 mg) versus twice a day (5 mg q12 hours) at steady state (after five days of dosing).

2.2 Secondary objectives

- To assess the effect of dapagliflozin on urine glucose excretion when administered once a day (10 mg) versus twice a day (5 mg q12 hours) at steady state (after five days of dosing)

- To examine the safety and tolerability of dapagliflozin dosed once a day versus twice a day

- To determine the PK parameters for dapagliflozin dosed twice a day versus once a day at steady-state

2.3 Exploratory objective

- To indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 (7-6 amide) (glucagon-like peptide) and GIP (glucose-dependent insulinotropic polypeptide) responses to meals accompanied with or without the administration of dapagliflozin.

3. STUDY PLAN AND PROCEDURES

This Clinical Study Protocol has been subject to a peer review according to AstraZeneca standard procedures.

3.1 Overall study design and flow chart

This is an open-label, randomised, two-period crossover, single-centre study to assess the effect of dapagliflozin dosed once a day versus twice a day. The doses of dapagliflozin will be 10 mg for once daily dosing and 5 mg every 12 hours for twice daily dosing. Each dose will be administered for 5 days, with a 7 to 10 days wash-out between. Up to 16 healthy volunteers will be enrolled (females of non-childbearing potential or practicing abstinence during trial duration and males) to obtain 14 completed and evaluable healthy volunteers.

Healthy volunteers will be randomised to receive the 2 treatments in a crossover fashion of sequences AB or BA. A wash-out period of 7 to 10 days will occur between the 2 treatments. The treatments are as follows:

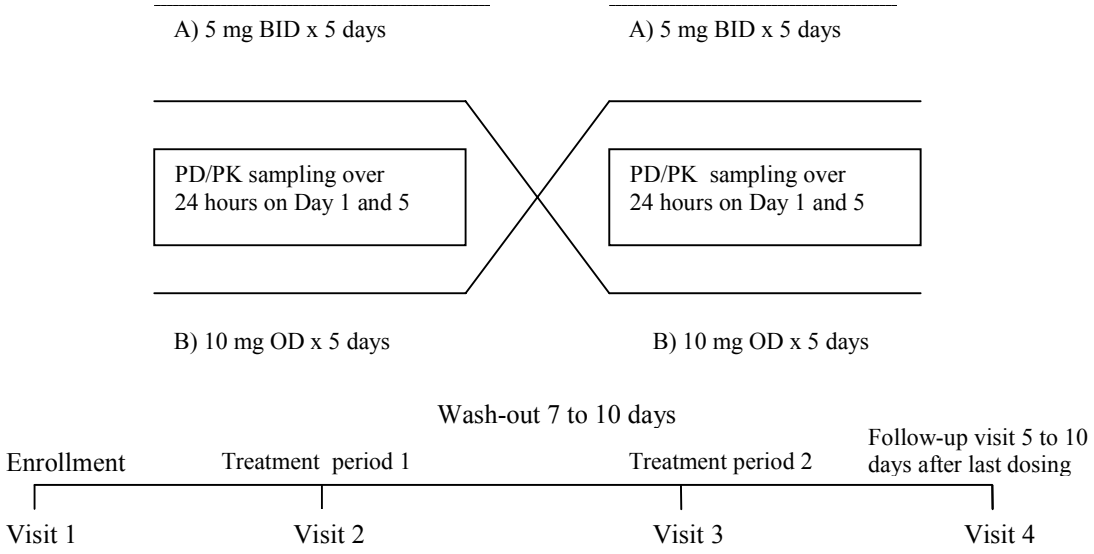
Treatment A: A dose of 5 mg dapagliflozin administered every 12 hours for 5 days following an overnight fast. On Day 1, volunteers will receive the first dose at breakfast at time 0; subjects will receive the second dose of dapagliflozin with dinner 12 hours after time 0 and continue the every 12 hour dosing through Day 5. Lunch will be served 5 hours after time 0. Subjects will receive the second dose of dapagliflozin with dinner 12 hours after time 0. Blood sampling will occur on Day 1, urine and blood sampling on Day 5.

Treatment B: A daily dose of 10 mg dapagliflozin administered daily for 5 days following an overnight fast. On Day 1, volunteers will receive the first dose at breakfast at time 0; subjects will receive the second dose on the morning of Day 2, 24 hours after time 0 and continue the every 24 hour dosing through Day 5. Lunch will be served 5 hours after time 0 and dinner will be served 12 hours after time 0. Blood sampling will occur on Day 1, urine and blood sampling on Day 5.

Breakfast on Day 5 in both periods must be identical; lunch on Day 5 in both periods must be identical; and dinner on Day 5 in both periods must be identical. Healthy volunteers must consume the entire meals within 30 minutes on Day 4 (for practice) and on Day 5. Of each meal on Day 5, approximately 55% of the ingested caloric equivalent should be carbohydrates. The meal schedule for all meals must be identical for all healthy volunteers, and the meal consumption time has to be identical in both periods for each healthy volunteer. Healthy volunteers may not consume caloric beverages between meals or at bedtime, likewise the healthy volunteers are to abstain from liquids with artificial sweeteners' on Day 5.

After having given written informed consent, the healthy volunteers will under go an enrolment examination within 19 days before the first dosing. In the afternoon of Day –1 of each treatment period the subjects will admit to the Early Phase Clinical Unit (EPCU) and a check for inclusion and exclusion criteria and a drug screen will be performed. Following dosing on Day 1 to 5 as randomised, the healthy volunteers will be discharged from the EPCU in the morning of Day 6 after the last PD/PK sampling has been collected and no further safety concern exists. Between both treatment periods there will be a wash-out period of 7 to 10 days, and after 5 to 10 days after the last treatment period, the healthy volunteers will undergo a final examination and discharged from the study (Figure 1).

Figure 1 **Study plan**



BID – twice a day; PK – pharmacokinetics; OD – once a day

Table 1 **Study Schedule**

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Informed consent	✓		
Inclusion/Exclusion criteria	✓	✓	

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Concomitant medication	✓	✓	✓
Medical/Surgical history	✓		
Physical exam	✓	✓[c]	✓[d]
Virology screen	✓		
Alcohol urine test	✓	✓	
Demographics	✓		
Drugs of abuse	✓	✓	
12-lead ECG	✓		
Blood pressure and heart rate (including body temperature [e]	✓	✓	✓
Weight and height [f]	✓		✓
Safety labs (blood and urine) [g]	✓	✓	✓
Randomization [h]		✓	
PK sample collection		✓	
Confinement to unit		✓	
Dapagliflozin dose		✓	
Pregnancy test (serum) [i]	✓	✓	
Adverse event questioning		✓	✓
Serious adverse event	✓	✓	✓
Urine sample collections [k]		✓	
Creatinine measurements		✓	
Plasma glucose, insulin, GLP-1 and GIP		✓	

ECG electrocardiogram, GLP glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide

[a] Beginning of period 1.

[b] Beginning of period 2

[c] Brief history and physical exam

[d] To be performed at end of Visit 4 (follow-up)

[e] Vital signs will be obtained he volunteer in a supine position (resting in bed for at least 10 minutes)

[f] Height will be assessed only at the enrolment visit (Visit 1)

[g] Safety labs (blood and urine) will be performed at Visit 1, and upon admission on Day –1 and pre-dose on Day 5, and at Visit 4

[h] Performed at Visit 2 only after all inclusion and exclusion criteria have been satisfied on Day 1.

[i] Required for female healthy volunteers only. Serum pregnancy test will be required at Visit 1, on Day –1 of Visits 2 and 3. Healthy female volunteers must have confirmed negative serum pregnancy test from Day –1 of each treatment period prior to receiving the IP

[j] AEs will be recorded from first intake of IP in period 1 until follow-up, SAEs will be collected from the time when informed consent is obtained until the end of study (including the follow-up visit).

[k] Urine samples for volume, glucose concentration, creatinine concentration.

Table 2 Study Plan for Each Treatment Period

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
	–1	15:30								Check for inclusion and exclusion criteria, drug screen including alcohol.
		Pre-dose					1	1		
	1	00:00	X							Breakfast[b]
		00:15					2	2		
		00:30					3	3		
		01:00					4	4		
		01:30					5	5		
		02:00					6	6		
		03:00					7	7		
		04:00					8	8		
		06:00					9	9		
		08:00					10	10		
		12:00	X[a]				11	11		Dinner[b]
		12:15						12		
		12:30						13		
		13:00						14		
		13:30						15		
		14:00						16		
		15:00						17		
		16:00					12	18		
		18:00						19		
		20:00					13	20		
	2	24:00	X				14	21		Breakfast[b]

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		12:00	X[a]							Dinner[b]
	3	Pre-dose					15	22		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
	4	Pre-dose			1 (back up sample)		16	23		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
2/3	5	-00:15		1		1				
		Pre-dose			2		17	24	X (start of collec-tion)	Safety lab, vital signs
		00:00	X	2		2			X	Initiation of breakfast[b]
		00:15		3		3	18	25	X	
		00:30		4		4	19	26	X	
		00:45		5		5				
		01:00		6		6	20	27	X	
		01:30		7		7	21	28	X	
		02:00		8		8	22	29	X	
		02:30		9		9				
		03:00		10		10	23	30	X	

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		04:00		11			24	31	X (end of collec-tion period)	
									X (restart of collec-tion)	
		04:45		12		11				
		05:00		13		12			X	Initiation of Lunch[b]
		05:15		14		13				
		05:30		15		14				
		05:45		16		15				
		06:00		17		16	25	32	X	
		06:30		18		17				
		07:00		19		18			X	
		07:30		20		19				
		08:00		21		20	26	33	X (end of collec-tion period)	
									X (restart of collec-tion)	
		09:00		22					X	
		10:00		23					X	
		11:00		24					X	
		11:45		25		21				

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		12:00	X[a]	26		22	27	34	X (end of first collection period) X (restart of collec-tion)	Initiation of dinner[b]
		12:15		27		23		35		
		12:30		28		24		36		
		12:45		29		25				
		13:00		30		26		37	X	
		13:30		31		27		38		
		14:00		32		28		39	X	
		14:30		33		29				
		15:00		34		30		40	X	
		16:00		35			28	41	X (end of collection period) X (restart of collec-tion)	
		17:00							X	
		18:00		36				42	X	
		19:00							X	

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		20:00		37			29	43	X (end of collection period)	
									X (restart of collec-tion)	
		21:00							X	
		22:00							X	
		23:00							X	
	6	24:00		38			30	44	X end of collec-tion period)[b]	

IP investigational product, PK pharmacokinetic, PD pharmacodynamic, GLP glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide

[a] Only in treatment A

[b] The IP will be administered together with the meal, which has to be completed within 30 minutes. Regarding the timing of events at mealtime, the following order of activities should be followed: blood sampling for plasma glucose, insulin, GLP-1 and GIP, with meal initiation occurring within 2 min of blood sampling. If the IP is to be administered the following sequence of events should occur: blood sampling, medication administration then meal initiation within 2 min of blood sampling.

3.2 Rationale for study design, doses and control groups

This is an open-label, randomised, two-period crossover, single-centre study to assess the effect of dapagliflozin dosed once a day versus twice a day. The doses of dapagliflozin will be 10 mg for once daily dosing and 5 mg every 12 hours for twice daily dosing. Up to 16 healthy volunteers will be enrolled (females of non-childbearing potential or practicing abstinence during the study duration and males) to obtain 14 completed and evaluable healthy volunteers.

The dose investigated reflects the doses planned to be used as therapeutic doses in the treatment T2DM.

The dapagliflozin 10 mg OD and 5 mg BID dosing regimens will be evaluated for similarity of pharmacodynamic effect.

4. SUBJECT SELECTION CRITERIA

Investigator(s) should keep a record, the subject enrolment log, of healthy volunteers who entered pre-study enrolment.

Each healthy volunteer should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

4.1 Inclusion criteria

For inclusion in the healthy volunteers should fulfil the following criteria:

1. Provision of informed consent prior to any study specific procedures

2. Healthy male and female volunteers aged 18 to 45 years

3. Female healthy volunteers must be post-menopausal (cessation of menses >1year, be surgically sterile (documented) or have undergone hysterectomy) or be sexually abstinent from enrolment until follow-up examination

4. Have a Body Mass Index (BMI) between 18.5 and 29.9 kg/m^2, inclusive

5. Have normal physical exam, vital signs ECG findings, and laboratory values (unless Investigator considers laboratory abnormality to be not clinically significant)

6. Able to communicate with the Investigator and willing to comply with all study requirements

4.2 Exclusion criteria

Healthy volunteers should not enter the study if any of the following exclusion criteria are fulfilled:

1. Use of prescription medication for a chronic or acute medical condition within 3 weeks of randomization

2. Use of over the counter preparations including herbal remedies, ST. John's Wort, ginseng, gingko, and vitamin preparations within 14 days of randomization unless approved by the sponsor and Investigator

3. History or presence of neurological, haematological, psychiatric, gastrointestinal, hepatic or renal disease, or other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs as determined by the Investigator

4. Symptoms of any clinically relevant illness within 3 weeks prior to randomization

5. History of surgery within 3 months prior to randomization unless approved by sponsor and Investigator

6. History of symptomatic hypoglycaemia

7. History of substance abuse in the last year

8. Use of alcohol, within 3 days prior to admission to the clinical research unit

9. Use of tobacco or history of tobacco use in the 3 months prior to randomization

10. Positive test for Human Immunodeficiency Virus (HIV), Hepatitis B surface Antigen (HBsAg) or hepatitis C antibody

11. Receipt of an investigational drug within 60 days prior to randomization

12. Positive urine drug screen

13. Previous participation in an AstraZeneca (AZ) or Bristol-Myers Sqibb (BMS) dapagliflozin study

14. Involvement in the planning and conduct of the study

15. Blood transfusion within 12 weeks of randomization

16. Strenuous physical exercise within 48 hours prior to admission to the clinical research unit

17. Positive pregnancy test

18. Employees of the clinical research unit conducting the study

19. Vulnerable subjects (eg, kept in detention)

Procedures for withdrawal of incorrectly enrolled healthy volunteers see section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

1. All healthy volunteers will be served standardised meals during the in-house periods at the early phase clinical unit, and no other foods will be permitted. The menus will be identical for Visit 2 and 3. On Day 5, meals should consist of a high carbohydrate diet ie, approximately 55% of the ingested caloric equivalent should be carbohydrates. Copies of the menu will be provided to the sponsor. For details refer to 3.1.

2. On Days 1 to 3 of Visits 2 and 3, respectively, water will be allowed ad lib except from 2 hours before until 2 hours after dose administration. On Day 4 (for practice)

and 5 (related to the urine collection) of Visits 2 and 3, respectively, water consumption will be as follows:

- 250 ml distilled water will be provided for dosing and 250 ml tap water 1h pre-dose.
- 250 ml tap water 4 and 5 hours post-dose respectively
- 250 ml tap water 8 and 9 hours post-dose respectively
- 250 ml tap water 12 hours (in Treatment A, for administration, 250 mL distilled water will be served) and 13 hours post-dose respectively
- 250 ml tap water 16 and 17 hours post-dose respectively
- 250 ml tap water 20 and 21 hours post-dose respectively

3. Eat and drink only the standardised meals and drinks provided (apart from water) during the residential period in the unit.

4. Abstain from consuming any of the following:

- Drugs of abuse during the entire study. In addition, poppy seeds (eg, on bread rolls) can give a positive signal for opiates and should not be ingested 72 hours before enrolment (Visit 1) until the end of the study.
- Consuming grapefruit, grapefruit juice Seville oranges, or orange marmalade (made with Seville oranges) or other products containing grapefruit or Seville oranges beginning 7 days before enrolment (Visit 1) and during the study.
- Liquorice containing products 24 hours prior to admission and during Visit 2 and 3. Alcohol from 72 hours before and during enrolment (Visits 1) and before admission and during Visit 2.
- Caffeine-containing beverages and foods, eg, coffee, tea, chocolate, and soft drinks (eg, Red Bull) for 10 hours before drug administration until completion of study period.
- Abstain from nicotine use and smoking throughout the entire study period until after the final medical examination at the study follow-up.
- Abstain from taking any medication (prescribed or over the counter products, ie, including vitamins, herbal remedies eg, St John´s Wort and mineral supplements) from 2 weeks prior to admission (Visit 2) and during the clinical study except for any occasional use of paracetamol. However, this should not obviate necessary medical treatment. If any medication is necessary, it should

be prescribed by the Investigator and the AstraZeneca Study Team Physician informed (section 5.6).

5. Strenuous physical activity that is not within the subject's normal weekly routine 5 days before enrolment (Visit 1) and after admission during Visit 2 and Visit 3.

6. Blood or plasma donation until 3 months after the final medical examination at the study follow-up.

7. Male healthy volunteers should abstain from unprotected sex and sperm donation from the first administration of the investigational product (IP) until 3 months after the last administration of the IP. As a precaution, healthy volunteers will be advised to use condoms in addition to a reliable form of contraception, for the specified period.

5.2 Subject enrolment and randomisation

The Principal Investigator or designee will:

1. Obtain signed informed consent from the potential healthy volunteer before any study specific procedures are performed.

2. Assign potential healthy volunteer a unique enrolment number, beginning with "E#" after signing the informed consent form.

3. Each healthy volunteer will be assigned a PAREXEL identification number irrespective of eligibility.

4. Determine eligibility of the healthy volunteer. See sections 4.1 and 4.2.

5. Assign eligible healthy volunteer unique randomisation code (subject number), after passing all acceptance criteria and before first dose administration in period 1. The numbers will be sequential and rising starting with number "1".

If a healthy volunteer withdraws from participation in the study, then his/her enrolment/randomisation code cannot be reused and he/she cannot re-enter into the study. Healthy volunteers, who discontinue participation in the study after dosing, will not be replaced.

5.2.1 Procedures for randomisation

A randomisation scheme will be produced by AstraZeneca R&D using the global randomisation system. Randomisation codes will be assigned strictly sequentially as healthy volunteers become eligible for randomisation.

5.3 Procedures for handling incorrectly enrolled healthy volunteers

Healthy volunteers who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be enrolled or randomised. There can be no exceptions to this rule.

Where healthy volunteers that do not meet the selection criteria are randomised in error or incorrectly started on treatment, or where healthy volunteers subsequently fail to meet the study criteria post initiation, a discussion should occur between the AstraZeneca Study Delivery Team Physician and the Investigator regarding whether to continue or discontinue the healthy volunteer from treatment.

The AstraZeneca Study Delivery Team Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the healthy volunteer should have their study therapy stopped.

5.4 Blinding and procedures for unblinding the study (Not applicable)

5.5 Treatments

5.5.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Dapagliflozin	Tablet 5 mg	Bristol-Myers Squibb
Dapagliflozin	Tablet 10 mg	Bristol-Myers Squibb

The investigational products (IP) will be supplied as study specific bulk supply by AstraZeneca, Pharmaceutical and Analytical R&D, Sweden.

The pharmacist of St. Hubertus Pharmacy, Berlin, Germany, will dispense the IPs into individual subject labelled dose cups, according to the randomisation scheme, provided by AstraZeneca. The dose cups will be provided by AstraZeneca.

Primary packaging of Dapagliflozin tablets will be carried out by Bristol-Myers Squibb or their designee in accordance with current Good Manufacturing Practice (GMP).

5.5.2 Doses and treatment regimens

The following doses will be administered in one of two fixed order sequences according to a randomization schedule:

Sequence 1 will administer a dose of 5 mg dapagliflozin administered every 12 hours for 5 days in period 1 and a daily dose of 10 mg dapagliflozin administered daily for 5 days in period 2. Sequence 2 will administer the treatments in the reverse order.

All doses will be orally administered together with 250 mL distilled water, the healthy volunteer in upright position, every 12 hours (treatment A) or in the morning (treatment B).

On Day 5, due to the drinking schedule, the IPs will be administered with 250 mL distilled water. Investigational products administration will be supervised by study staff and will be followed by a mouth check.

5.5.3 Labelling

The IPs will be labelled at Investigational Products (IPS), Pharmaceutical and Analytical R&D AstraZeneca, according to current European Union Good Manufacturing Practice (GMP) guidelines, Annex 13 and local regulatory requirements. The labels will fulfil GMP Annex13 requirements for labelling.

Dose cup labels will be provided by PAREXEL International GmbH. Study site dispensary staff will label the individual dose cups.

5.5.4 Storage

All study drugs should be kept in a secure place under appropriate storage conditions. The IP label on the container specifies the appropriate storage.

5.6 Concomitant and post-study treatment(s)

Apart from paracetamol/acetaminophen and occasional use of nasal anticongestants no concomitant medication or therapy will be allowed. The healthy volunteers should be instructed that no other medication is allowed including herbal remedies, vitamin supplements, minerals and over-the-counter products without the consent of the Investigator.

Medication, which is considered necessary for the healthy volunteer's safety and well-being, may be given at the discretion of the Investigator during the residential period. When any medication is required, it should be prescribed by the Investigator who should inform the AstraZeneca Study Team Physician. Following consultation with the Study Team Physician, the Investigator should determine whether or not the healthy volunteer should continue in the study.

5.7 Treatment compliance

The administration of all medication (including investigational products) should be recorded in the appropriate sections of ClinBase™.

Treatment compliance will be assured by supervised administration of the IP by the Investigator or delegate. The dose, date and time of administration of the IP will be recorded and checked by the monitor at monitoring visits.

5.7.1 Accountability

The study drug provided for this study will be used only as directed in the clinical study protocol.

The study personnel will account for all study drugs dispensed to and returned from the subject.

PAREXEL personnel will account for all study drugs received at the site, unused study drugs and for appropriate destruction of unused study drugs. Certificates of delivery, destruction and return should be signed.

5.8 Discontinuation of investigational product

5.8.1 Criteria for discontinuation – stopping criteria for the individual

Healthy volunteers may be discontinued from investigational product (IP) in the following situations:

- Healthy volunteer decision. The healthy volunteer is at any time free to discontinue treatment, without prejudice to further treatment;
- Adverse Event;
- Severe non-compliance to clinical study protocol as judged by the Investigator and/or AstraZeneca;
- Incorrectly randomised healthy volunteers;
- Healthy volunteer lost to follow-up;
- Risk to healthy volunteers as judged by the Investigator and /or AstraZeneca.

Healthy volunteers who are withdrawn from the study by the Investigator due to Adverse Events (AEs) after dosing will not be replaced. Healthy volunteers who withdraw for reasons other that AEs after dosing will not replaced.

If a healthy volunteer is withdrawn from study, see section 5.9.

5.8.2 Criteria for discontinuation – stopping criteria for the whole study

The trial will be stopped prematurely if:

- Three or more subjects have adverse events of similar type and of moderate or greater severity. Degree of severity will be defined by CTC criteria;
- One serious adverse event (life threatening) occurs in the cohort that is considered to be related to the IMP;
- Two or more subjects show an unexplained increase in liver enzymes (ALT/AST) >/= 3 times ULN in two consecutive samples;
- Data not known before become available and raise concern about the safety of the study drug so that continuation would pose potential risk to the subjects.

5.9 Withdrawal from study

Subjects are at any time free to withdraw from study (investigational product and assessments), without prejudice to further treatment (withdrawal of consent). Such subjects will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an Investigator. Adverse events will be followed up (See sections 6.3.3 and 6.3.4).

6. COLLECTION OF STUDY VARIABLES

6.1 Recording of data

The study assessments are described in the sections below and the timing of these assessments are detailed in the study plan (see Table 1 and Table 2).

6.1.1 Recording of data

Paper Case Report Forms (pCRFs) will be used to record all data not captured electronically at bedside (see Section 10.1.1). Trained study site personnel will perform manual data entry and editing. The data entries will be designed according to the AstraZeneca Case Report Form (CRF) Standard.

In cases where electronic direct data entry is not possible, data will first be recorded on a paper CRF (pCRFs), medical journal or other source documents and thereafter entered into the clinical study database.

The Principal Investigator will provide AstraZeneca with all data produced during the study from the scheduled study assessments. He ensures the accuracy, completeness, legibility, and timelines of the data reported to AstraZeneca in ClinBase™ and in all required reports. For a more detailed description of the data flow, including electronic data capture, refer to section 10.1.1.

6.1.2 Electronic data capture at PAREXEL Early Phase Clinical Unit

During the study, data will be captured electronically at bedside using the electronic data capture (EDC) application ClinBase™.

The Investigator will review and approve the electronically captured data before transferring the data to the clinical study database. Any changes made prior to Investigator approval will be documented within the audit trail of the electronic data capture application. Any missing, impossible, or inconsistent entries will be queried after transfer to the clinical study database, in accordance with PAREXEL practices.

6.2 Data collection and enrolment

At enrolment (Visit 1), each potential healthy volunteer will provide informed consent prior to starting any study specific procedures.

Demographic data and other characteristics will be recorded and will include: year of birth, gender and race and other information.

Each healthy volunteer will undergo enrolment during the 19 days prior to admission to confirm eligibility. This will consist of:

1. A standard medical, medication and surgical history with review of the inclusion and exclusion criteria with the healthy volunteer
2. A complete physical examination
3. Height, weight and calculation of BMI
4. Vital signs – resting supine and standing blood pressure (BP), pulse
5. Recording a resting 12-lead standard ECG
6. A blood sample for routine clinical chemistry, haematology, and screen for hepatitis B surface antigen, antibodies to hepatitis C virus and antibodies to HIV
7. A urine sample for routine urinalysis, drugs of abuse screen, and pregnancy test (for females only)
8. Questions about alcohol, caffeine and nicotine consumption
9. Concomitant medication

6.2.1 Follow-up procedures

At the follow-up examination, a complete physical examination, safety laboratory assessment in blood and urine and questioning of AEs will be performed 5 to 10 days after the end of the 2nd treatment period.

6.3 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

6.3.1 Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the

abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or wash-out periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

6.3.2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, wash-out, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.
- Cancer
- Overdose (defined as the accidental or intentional ingestion of any dose of the investigational product that is considered both excessive and medically important)

For further guidance on the definition of a SAE, see Appendix B to the Clinical Study Protocol.

6.3.3 Recording of adverse events

Time period for collection of adverse events

Adverse events will be collected from the time of obtaining informed consent throughout the treatment periods and including the follow-up period. Before the intake of first dose of study drug only SAEs will be recorded.

Follow-up of unresolved adverse events

Any AEs that are unresolved at the healthy volunteers last visit in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the CRF. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collect for each AE;

- AE (verbatim)
- Date and time when the AE started and stopped
- Maximum intensity
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject's withdrawal from study *(*yes or no*)*
- Outcome.

Additional variables will be collected for all SAEs including treatment given for the event.

The following intensity ratings will be used:

1. Mild (awareness of sign or symptom, but easily tolerated)
2. Moderate (discomfort sufficient to cause interference with normal activities)
3. Severe (incapacitating, with inability to perform normal activities)
4. Very severe (debilitating, significantly incapacitates patient despite symptomatic therapy).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in section 6.3.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Causality collection

The Investigator will assess causal relationship between Investigational Product and each Adverse Event, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

For SAEs causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Appendix B to the Clinical Study Protocol.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the healthy volunteer or reported in response to the open question from the study personnel: "Have you/ had any health problems since the previous visit/you were last asked?", or revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from clinical study protocol mandated laboratory tests and vital signs will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs, and other safety variables should only be reported as AEs if they are clinically significant, fulfil any of the SAE criteria, are the reason for discontinuation or interruption of treatment with the *investigational product*, or require the patient to receive specific corrective therapy.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

6.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

If any SAE occurs in the course of the study, then Investigators or other site personnel inform appropriate study monitor within one day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The designated study monitor works with the Investigator to ensure that all the necessary information is provided to Bristol-Myers Squibb Global Pharmacovigilance and

Epidemiology within **one calendar day** of initial receipt for fatal and life threatening events and **within three calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform study monitor of any follow-up information on a previously reported SAE within one calendar day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The completed report (including all relevant CRF modules) is to be sent by e-mail or fax to Bristol-Myers Squibb Global Pharmacovigilance and Epidemiology.

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca drug.

6.3.5 Laboratory safety assessment

The respective laboratory reference ranges will be provided by the local laboratory and filed in the Investigator Site File.

Each parameter outside the normal range will be assigned as high or low. The Investigator has to interpret each "outside the normal range" value as not clinically significant or clinically significant. In the latter case the Investigator has to give a comment and the deviation is judged as an AE.

These laboratory tests will be performed at the Synlab Clinical Trial GmbH, Turmstr. 21 (Haus M), D 10559 Berlin, Germany, using validated standard methods according to the applicable laboratory standard operating procedure (SOP). Laboratory data will be transmitted electronically from the Synlab Clinical Trial GmbH, Berlin, Germany, to the study site.

For evaluation of haematology, blood (2.7 mL) will be collected into polypropylene tubes containing 15% potassium-Ethylenediaminetetraacetic acid (Monovette 05.1167, Sarstedt).

For evaluation of clinical chemistry, blood (7.5 mL) will be collected into silicone-free polypropylene serum gel tubes (Monovette 01.1602, Sarstedt).

For infectious serology parameters, a blood sample (2.6 mL) will be taken into a serum tube (Monovette 04.1905.001, Sarstedt).

Urinalysis will be performed at the internal laboratory of PAREXEL, Berlin, Germany according to the applicable PAREXEL SOP.

The following laboratory variables will be measured:

Clinical Chemistry	**Haematology**
Serum (S-)Albumin	Blood (B)-Haemoglobin

S-Alanine aminotransferase (ALT)

S- Aspartate aminotransferase (AST)

S-Alkaline phosphatase (ALP)

S-Bilirubin, total

S-Creatinine

S- Glucose

S-Potassium

S-Sodium

S-Chloride

S-Creatinine kinase (CK)

S-Total protein

S-Uric acid

B-Leukocyte (part.conc)

B-Absolute leukocyte differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes

B-Platelet count

Urinalysis

Urine (U)-Glucose (dipstick)

U-Haemoglobin (dipstick)

U-Protein (dipstick)

U-Creatinine

Other samples

S-U-Pregnancy test (females only) (S-βhCG = Human Chorionic Gonadotropin)

S-Follicle stimulation hormone (FSH) (females only and only at enrolment)

Additionally, at enrolment all healthy volunteers will be tested for HIV, hepatitis B surface antigen and antibodies to hepatitis C. Urine will be tested for the following drugs of abuse at enrolment and admission: amphetamines, barbiturates, benzodiazepam, cocaine, methadone, methamphetamine, morphine, phencyclidine, tetrahydrocannabinol, opiates. Tricyclic antidepressant will be analysed in serum. At Visit 2 and 3 the healthy volunteers will be screened for alcohol. A serum pregnancy test will be performed at Visit 1 (enrolment) and on Visit 2 and 3. If a healthy volunteer tests positive to any of these enrolment tests he/she will be excluded from the study.

Serum/plasma samples will be collected to allow for possible post hoc analysis in case of related AEs at time points specified in Table 1.

For blood volume see section 7.1.

6.3.6 Physical examination

The timing of individual examinations is indicated in the Study Plan (Table 1). A complete physical include an assessment of the following: general appearance, skin, head and neck, lymph nodes, thyroid, abdomen, musculoskeletal, cardiovascular, respiratory and neurological systems.

Height will be measured in centimetres and weight in kilograms in underwear. Measurements should be taken without shoes and the same scale used for all measurements. BMI will be calculated from the height and weight.

A medical history will be recorded at the enrolment visit only.

6.3.7 Resting ECG

An ECG recording will be performed at the enrolment visit.

The ECG will be recorded at a paper speed of 25 mm/s and a standard calibration of 1 mV (= 10 mm) for each lead with a standard digital ECG and a Cardiosoft® (CS) version 6.51 ECG device, manufactured by General Electrics Medical Systems (GEMS, Freiburg, Germany).

The 12-lead ECGs will be recorded after the healthy volunteers have rested for at least 10 minutes in supine position. Six limb leads, as specified by Einthoven (I, II and III) and Goldberger (aVR, aVL, aVF), and six pre-cordial leads (V1–V6), according to Wilson, will be used.

Each ECG will include the following information: identification of each lead, study number, subject number, paper speed, voltage calibration as well as date and time of the recording. After conduction, the ECGs will be stored electronically in the CS database and the parameters may be exported to other databases.

The parameters HR, RR, PQ, QRS, QT and QTcB calculated by the Bazett formula ($QTc= QT/RR^{0.5}$) will be assessed. ECG recordings will capture at least four QRS complexes, ie three evaluable RR intervals. The Investigator or a designate will evaluate whether the ECG is normal or abnormal and whether it is clinically significant, if abnormal. Additionally, any occurrence of de- or repolarisation disorders, arrhythmic disorders or other abnormalities will be assessed and any changes compared to the pre-medication record will be commented.

All ECG data are captured and assessed electronically. The electronic version of the ECG is regarded as source data.

ECGs may be repeated for quality reasons and the repeat used for analysis. Additional ECGs may be collected by the Investigator for safety reasons. Clinically relevant abnormal findings will be reported as adverse events.

6.3.8 Vital signs

6.3.8.1 Pulse and blood pressure

Supine blood pressure (BP) and pulse rate will be measured using an oscillometric BP rate monitor (Dynamap Pro Care®), manufactured by General Electrics Medical Systems (GEMS) after 10 minutes rest on a bed. Pulse will be recorded simultaneously with the BP measurements by the same monitor. For timings of assessments refer to the Study Plan Table 1.

6.3.8.2 Body temperature

Oral body temperature will be measured for at least one minute using a digital thermometer (DIGItemp®, LDM Pharma, Wesel, Germany) at the times indicated in the Study Plans Table 1 and Table 2.

6.3.9 Other safety assessments

No further safety assessments will be performed.

6.4 Pharmacokinetics

6.4.1 Collection of samples

Blood samples for determination of dapagliflozin (2 mL) in plasma will be taken at the times presented in the study plan Table 2.

Samples will be collected, labelled stored and shipped as detailed in Laboratory Manual.

For blood volume see section 7.1.

6.4.2 Determination of drug concentration

Samples for determination of concentration of dapagliflozin in plasma will be analysed using appropriate bioanalytical methods on behalf of Clinical Pharmacology & DMPK, AstraZeneca, Mölndal, by Atlanbio, France. The lower limit of quantification LLOQ of dapagliflozin in plasma is 1 ng/mL. Full details of the analytical methods used will be described in a separate bioanalytical report.

6.5 Pharmacodynamics

6.5.1 Collection of pharmacodynamic markers

Blood samples for determination of plasma glucose will be collected into 1.2-mL sodium fluoride tubes. Blood samples for determination of insulin and creatinine each be will be collected in 1.1 mL serum monovette. Samples for determination of plasma glucose, serum insulin and serum creatinine will be transported unfrozen directly for analysis at Synlab Clinical Trial GmbH, Berlin, Germany.

Blood samples for GIP and GLP-1 will be collected in 3.0 mL in K_2-EDTA (sodium ethylenediaminetetraacetic acid) (spray coated) tubes. Samples will be centrifuged at 1500 g for 10 min and the plasma will be stored frozen at –20°C until transport for analysis at Synlab Clinical Trial GmbH, Berlin, Germany.

Blood samples will be collected at the times presented in the study plan Table 2. For blood volume see section 7.1.

For detailed sampling, handling, storage and transport of the samples, refer to the Laboratory Manual.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 3 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry [a]	2.6	6	15.6
	Haematology	2.7	6	16.2
	Tricyclic antidepressants	1.1	3	3.3
	Serology	2.6	1	2.6
Pharmacokinetic		2.0	74	148.0
Pharmacodynamic				
Creatinine		1.1	4	4.4
Glucose		1.2	76	91.2
GLP-1		3.0	60	180.0
Insulin and GIP		1.1	60	66.0
Total				**527.3**

GLP Glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide
a Includes pregnancy testing

The number of samples taken, as well as the volume required for each analysis, may be changed during the study as new data on dapagliflozin become available. However, the maximum volume to be drawn from each healthy volunteer will not exceed 500 mL, ie, the same volume as would be drawn during a regular blood donation.

7.2 Handling, storage and destruction of biological samples

The samples will be used up or disposed of after analyses or retained for further use as described here.

7.2.1 Pharmacokinetic and/or pharmacodynamic samples

Samples will be disposed of after the clinical study report has been finalised.

Blood and urine samples for determination of clinical chemistry, haematology and urinalysis will be disposed of after analysis.

7.3 Labelling and shipment of biohazard samples

The Principal Investigator ensures that samples are labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B Regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C 'IATA 6.2 Guidance Document'.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

7.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The Principal Investigator keeps full traceability of collected biological samples from the subjects while in storage at the centre until shipment or disposal (where appropriate) and keeps documentation of receipt of arrival.

The sample receiver keeps full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps documentation of receipt of arrival.

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca biobank system during the entire life cycle.

7.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, and the action documented. If samples are already analysed, AstraZeneca is not obliged to destroy the results of this research.

As collection of the biological samples is an integral part of the study, then the subject is withdrawn from further study participation.

The Principal Investigator:

- Ensures subjects' withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca
- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed/destroyed, the action documented and the signed document returned to the study site

- Ensures that the subject and AstraZeneca are informed about the sample disposal.

AstraZeneca ensures the central laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed and the action documented and returned to the study site.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

8.3 Ethics and regulatory review

An Ethics Committee should approve the final clinical study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The Investigator will ensure the distribution of these documents to the applicable Ethics Committee, and to the study site staff.

The opinion of the Ethics Committee should be given in writing. The Investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study.

The Ethics Committee should approve all advertising used to recruit subjects for the study.

AstraZeneca should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

If required by local regulations, the clinical study protocol should be re-approved by the Ethics Committee annually.

Before enrolment of any subject into the study, the final clinical study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities.

AstraZeneca will provide Regulatory Authorities, Ethics Committees and Principal Investigator with safety updates/reports according to local requirements, including SUSARs (Suspected Unexpected Serious Adverse Reactions), where relevant.

8.4 Informed consent

The Principal Investigator at each centre will:

- Ensure each healthy volunteer is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each healthy volunteer is notified that they are free to discontinue from the study at any time
- Ensure that each healthy volunteer is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each healthy volunteer provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure one original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure one original, signed Informed Consent Form is given to the healthy volunteer
- Ensure that any incentives for healthy volunteers who participate in the study as well as any provisions for healthy volunteers harmed as a consequence of study participation are described in the informed consent form that is approved by an Ethics Committee.

8.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Principal Investigator and AstraZeneca.

If there are any substantial changes to the clinical study protocol, then these changes will be documented in a clinical study protocol amendment and where required in a new version of the clinical study protocol (Revised Clinical Study Protocol).

The amendment should be approved by each Ethics Committee and if applicable, also the national regulatory authority, before implementation. Local requirements should be followed for revised clinical study protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the clinical study protocol to each Principal Investigator(s). For distribution to Ethics Committee see section 8.3.

If a clinical study protocol amendment requires a change to a centre's Informed Consent Form, AstraZeneca and the centre's Ethics Committee should approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by each Ethics Committee.

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the clinical study protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. The Investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT BY ASTRAZENECA

9.1 Pre-study activities

Before the first subject is entered into the study, it is necessary for a representative of AstraZeneca to visit the investigational study site to:

- Determine the adequacy of the facilities
- Determine availability of appropriate healthy volunteers for the study
- Discuss with the Investigators (and other personnel involved with the study) their responsibilities with regard to clinical study protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the Investigator.

9.2 Training of study site personnel

Before the first healthy volunteer is entered into the study, an AstraZeneca representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures and ClinBase™ system utilised.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

9.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study site, including visits to:

- Provide information and support to the Investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the clinical study protocol, that data are being accurately and timely recorded in the CRFs, that biological samples are handled in accordance with the Laboratory Manual and that investigational product accountability checks are being performed
- Perform source data verification (a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating healthy volunteers. This will require direct access to all original records for each v (eg, clinic charts)
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the healthy volunteer.

The AstraZeneca representative will be available between visits if the Investigators or other staff at the centre needs information and advice about the study conduct.

9.3.1 Source data

Source for all data will be presented in a separate Source Data Document, which will be approved and signed by the Principal Investigator before study start.

Electronic CRF (eCRF), EDC application, medical journal and study specific source documents will act as the source data for all information in the study.

9.4 Study agreements

The Principal Investigator at each/the centre should comply with all the terms, conditions, and obligations of the Clinical Study Agreement, or equivalent, for this study. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the terms of Clinical Study Protocol shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of healthy volunteers, the terms of the Clinical Study Agreement shall prevail.

Agreements between AstraZeneca and the Principal Investigator should be in place before any study-related procedures can take place, or healthy volunteers are enrolled.

9.4.1 Archiving of study documents

Study documents will be archived in the Study Master File (SMF) and the Investigator's Study File.

9.5 Study timetable and end of study

The end of the study is defined as 'the last visit of the last healthy volunteer undergoing the study'.

The study is expected to start in I/2010 and to end by I/2010.

The study may be terminated at individual centres if the study procedures are not being performed according to GCP, or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with the IPs.

10. DATA MANAGEMENT BY ASTRAZENECA OR DELEGATE

10.1 Data collection methods and data validation at study site

10.1.1 Data flow

PAREXEL's ClinBase™ system is an electronic source data capturing and information management system. The system combines all aspects of source data capturing with process control and study management. All clinical and laboratory data, except those which are paper based, will be collected by the eSource system ClinBase™. Only paper-based data will be subject to data entry. For electronic data, no data entry will be performed. Data entered on pCRF should be recorded legibly in blue or black ballpoint pen. Correction fluid or covering labels must not be used.

The responsible monitor will check data at the monitoring visits to the study site. The Investigator will ensure that the data collected are accurate, complete and legible. Data will be validated within ClinBase™ by the Investigator and the study monitor before being exported. Any changes made during validation will be documented with a full audit trail within ClinBase™.

PAREXEL designs and builds a database in iVal* to enter the paper-based data for adverse event and concomitant medication. The database and entry screens will be built and validated following a formal, documented verification plan and the system will be tested against the specification for the entry system. The setup will be controlled by test data entry. A standardised setup of Statistical Analysis System (SAS®) data sets in AstraZeneca specification will be done by the SAS® clinical data programmer. Verification of the setup will be performed with dummy and test data.

*The iVal system is a 21 CFR part 11 compliant application ensuring high data quality. The iVal system is a clinical database management system composed of several modules including, but not limited to, a set-up tool, a database designer (data dictionary editor), an entry screen designer (data entry manager), a query manager (iQue), a validation-programming environment, and a SASTM dataset export module (iSAS). For more information refer to the Data Management Plan.

The PAREXEL clinical data manager will develop a data verification specification (DVS) according to the clinical study protocol, the data dictionary of the iVal database for paper based-data and the description of the SAS® table set for electronic data. Computerised checks to be performed on the data imported or entered into the databases will be defined in this document. The DVS will be sent to AstraZeneca or representative for review and approval.

Paper-based data will be sent to PAREXEL data management and entered into iVal. These data will be exported via the module iSAS later on and merged with the SAS® tables in AstraZeneca format. The SAS® data sets will be used for verification of the electronic study data following the specifications listed in the DVS. Only trained staff will have access to the databases. Every change will be fully audit-trailed.

The ClinBase™ data files will be imported into SAS® data sets and the imported data checked by the related verification programmes specified in the DVS. Data will be changed only in a controlled, audit-trailed, environment. After these data are considered as clean, they will be mapped into AstraZeneca data structure.

All study related electronic source data captured in ClinBase™ will be transferred from ClinBase™ via ClinResult to PAREXEL Data Management after monitoring. These data will be imported into the SAS® data sets. Paper based data will be sent to Data Management after they have been monitored. PAREXEL Data Management reviews, logs and files them.

External PK concentration data will be transferred to Data Management as specified by a Bio analytical data transfer specification. This data will be reconciled against the source ClinBase™ database and any discrepancies will be communicated to the Bio analytical Laboratory.

Any missing, implausible or inconsistent recordings will be referred back to the Investigator using a data query form and be documented for each individual healthy volunteer before clean file status is declared.

The following data will be captured as source electronically using ClinBase™:

- Dates and time of all dosing
- Pulse and BP
- Safety laboratory data

- ECG
- Height
- Weight

Dates and times of all blood and urine samples will be captures on pCRF, entered into ClinBase™ and then evaluated electronically.

10.1.2 Study Data Management Plan (DMP)

The Study Data Management Plan (DMP) will describe the methods used to collect, check and process clinical data in detail. It will also clarify the roles and responsibilities for the different functions and personnel involved in the data management process. Further the DMP will also describe the data flow and timelines within the study.

10.1.3 Coding tools and dictionaries

Adverse events and diagnoses from medical history will be classified according to the terminology of Medical Dictionary for Regulatory Activities (MedDRA). Concomitant medications will be classified according to the WHO Drug Dictionary. All coding will be performed by PAREXEL.

11. EVALUATION AND CALCULATION OF VARIABLES BY ASTRAZENECA OR DELEGATE

11.1 Calculation or derivation of safety variables

The closest observation prior to first administration of investigational product will be regarded as baseline.

Change from baseline variables will be calculated as the value minus the value at baseline.

11.1.1 Other significant adverse events (OAE)

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs and DAE (AEs, which leas to discontinuation of the IP). Based on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered OAEs and reported as such in the Clinical Study Report. A similar review of laboratory/vital signs/ECG data will be performed for identification of OAEs.

Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment.

11.2 Calculation or derivation of pharmacokinetic variables

The pharmacokinetic (PK) analyses of the plasma concentration data for dapagliflozin will be performed by PAREXEL. Derivation of PK parameters will be preformed using the WinNonlin's standard non-compartmental approach (WinNonLin version 5.01, WinNonLin, Pharsight, Mountain View, CA, USA).

The actual sampling times will be used in the PK parameter calculations. Plasma concentrations below limit of quantification (LOQ) will be excluded from the calculations except at time points prior to $C_{ss,\ max}$, where plasma concentrations below LOQ will be taken as zero at clinical study protocol time zero and as missing at all other time points in the calculation.

The secondary objective is to determine the PK parameters for dapagliflozin dosed twice a day versus once a day at steady-state.

The following PK parameters will be determined:

- AUC_{τ} Area under plasma concentration-time curve during a dosing interval [amount• time/volume]
- AUC_{ss} Area under plasma concentration-time curve during any dosing interval at steady state [amount• time/volume]
- $C_{ss,\ av}$ The average concentration at steady state
- $C_{ss,\ max}$ Maximum plasma (peak) drug concentration at steady state [amount/volume]
- $C_{ss,\ min}$ Minimum (trough) steady state drug concentration in plasma during dosing interval [amount/volume]
- DF% Degree of fluctuation at steady state.
  $DF(\%) = (C_{ss,\ max} - C_{ss,\ min})/C_{ss,\ av} \times 100$
- $t_{ss,\ max}$ Time to maximum plasma concentration at steady state [h]

11.3 Calculation or derivation of pharmacodynamic variables

The primary objective of the study is to assess the effect of dapagliflozin on % inhibition of glucose re-absorption when dapagliflozin is administered once a day (10 mg) versus twice a day (5 mg every 12 hours) at steady state (after five days of dosing).

The secondary objectives of the study are to assess the effect of dapagliflozin on 24 hour urine glucose excretion when administered once a day (10 mg) versus twice a day (5 mg every

12 hours) at steady state (after five days of dosing), the exploratory objectives to indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 and GIP responses to meals accompanied with or without the administration of dapagliflozin.

Urine will be evaluated for volume, total glucose and creatinine. At the end of each 4-hour interval, the total urine sample of that period will be mixed thoroughly and the total volume will be recorded. One aliquot will then be taken for measurement of glucose and creatinine. The amount of glucose excreted during each interval and the total glucose excretion over 24 hours (calculated as the sum of the amounts from each 4-hour interval) will be reported.

Inhibition of glucose re-absorption

For each subject, the amount of renally filtered glucose on Day 5 is calculated using the product of the glomerular filtration rate and the mean serum glucose concentration over the 0-4 (inclusive), 4-8 (inclusive), 8-12 (inclusive), 12-16 (inclusive), 16-20 (inclusive), and 20-24 (inclusive) hourly timed intervals from study period start. The glomerular filtration rate is estimated based on the MDRD (Modification of diet in renal disease) method with the pre-dose serum creatinine value on Day 5. The primary PD measure of percent inhibition of renal glucose re-absorption over 24 hours will be calculated as "*100x the sum of the amounts of glucose excreted in the urine from each 4-hour interval / the sum of the amounts of glucose renally filtered from each 4-hour interval*". Only those 4-hour intervals contributing data for both amounts will be included in this calculation,

Inhibition of rate of gut glucose absorption

For plasma glucose, insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated for each subject in each period. The AUE_{0-180} will be calculated using the linear trapezoidal rule.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY ASTRAZENECA OR DELEGATE

12.1 Description of analysis sets

12.1.1 Pharmacodynamic analysis set

The PD population is defined as all subjects who receive at least one dose of IP and for whom any post-dose data are available. The PD population will be used for the analysis of all PD variables and the analysis will be done according to actual treatment received, regardless of randomisation.

12.1.2 Pharmacokinetic analysis set

All subjects who have evaluable pharmacokinetic data without any protocol deviations/violations deemed to effect the pharmacokinetics of Dapagliflozin will be included in the summaries and listings of the pharmacokinetic data.

12.1.3 Safety analysis set

The safety population is defined as all subjects who receive at least one dose of IP and for whom any post-dose data are available. The safety population will be used for the analysis of all safety variables and the analysis will be done according to actual treatment received, regardless of randomisation. Post-dose data imply that there was a contact during which an opportunity was given to report any health problems.

12.2 Methods of statistical analyses

12.2.1 General principles

All statistical summaries and analyses will be performed by PAREXEL International GmbH using SAS® 9.1.3 (SAS® Institute, Cary NC, USA) or the statistical package StatXact®, version 6. Statistical and analytical procedures will be detailed in a Statistical Analysis Plan (SAP).

12.2.2 Subject characteristics

Continuous variables will be summarised using descriptive statistics (n, mean, standard deviation [SD], min, median, max). Categorical variables will be summarised in frequency tables (frequency and proportion) by treatment.

12.2.3 Pharmacokinetics

$AUC_{ss, (0-24)}$, $C_{ss, max}$ and $C_{ss, min}$ will be analyzed using an analysis of variance (ANOVA) model, with sequence, period and treatment regimen (10 mg dapagliflozin OD x 5 days versus 5 mg dapagliflozin BID x 5 days) as fixed effects and subject within sequence as a random effect. $AUC_{ss, (0-24)}$, $C_{ss, max}$ and $C_{ss, min}$ will be log-transformed prior to analysis. Least squares means and differences in least squares means together with corresponding confidence intervals will be back-transformed to provide estimates of geometric means (with 95% confidence intervals) and the ratio of geometric means (with 90% confidence intervals), following adjustment for other factors in the model.

Graphical presentations will be used as appropriate. Examples may include line graphs showing individual or mean development over time.

12.2.4 Pharmacodynamics

The primary objective of this study is to assess the effect of twice-daily dosing versus once-daily dosing of the same total daily dose of dapagliflozin on percent inhibition of glucose re-absorption. The primary pharmacodynamic endpoint (percent inhibition of renal glucose re-absorption over 24 hours), and the secondary pharmacodynamic endpoint (total urinary glucose excretion over 24 hours) will be analyzed using an ANOVA model, with sequence, period and treatment regimen (10 mg dapagliflozin OD x 5 days versus 5 mg dapagliflozin BID x 5 days) as fixed effects and subject within sequence as a random effect. Estimated least square means and difference between treatments least squares means will be reported with 95% confidence intervals, with adjustment for other factors in the model done.

If there is after inspecting ANOVA model diagnostics convincing evidence that one or more of the model assumptions are not valid, a nonparametric analysis of the treatment regimen differences will also be reported using the Hodges-Lehmann estimator of the median difference together with a corresponding exact 95% confidence interval.

Graphical presentations will be used as appropriate. Examples may include line graphs showing individual or mean development over time.

For plasma glucose, insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated. Baseline adjusted geometric mean differences between both treatment groups will be estimated by a linear model on the log-transformed AUEs after each meal and retransforming the point estimates and 95% confidence intervals. The linear model will include treatment, sequence, period as fixed effects, subjects nested within sequence as random effect and baseline concentration as continuous covariate. AUEs after each meal will be analyzed in a separate model.

Results of the analysis after dinner will be used to determine if dapagliflozin inhibits the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 and GIP responses to meals accompanied with (5 mg BID) or without the administration of dapagliflozin (10 mg OD).

12.2.5 Safety and tolerability

Continuous variables will be summarised using descriptive statistics (n, mean, SD, min, median, max) by treatment group. Categorical variables will be summarised in frequency tables (frequency and proportion) by treatment group. Graphical presentations will be used as appropriate. Examples include shift plots showing pre-treatment values on the horizontal axis and post-treatment values on the vertical axis.

12.2.5.1 Adverse events

Adverse events will be summarised by preferred term and system organ class using MedDRA vocabulary. Furthermore, listings of serious adverse events and adverse events that led to withdrawal will be made and the number of subjects who had any AEs, SAEs, AEs that led to withdrawal, and adverse events with severe intensity will be summarised. AEs that occur before dosing will be reported separately.

12.2.5.2 ECG

Results from ECG assessments will be provided in listings.

12.2.5.3 Laboratory variables

Clinical chemistry, haematology and urinalysis variables will be described by shift plots (for numerical data), descriptive statistics, subject listings and frequency tables of values outside project specific reference ranges, details of which will be presented in a separate comprehensive SAP.

12.2.5.4 Vital Signs

All vital signs data will be summarised with descriptive statistics and will include mean, standard deviation, min, max, details of which will be presented in a separate comprehensive SAP.

12.2.6 Interim analyses (Not applicable)

12.3 Determination of sample size

If 14 subjects complete both periods of the study and if the intra-subject variance is one half the total variance observed in the 10 mg group on Day 7 for average percent inhibition of glucose re-absorption over 0-12 hours in Bristol-Myers Squibb Study MB102002 (mean/SDtotal=24.5%/15.4%), then a 95% confidence interval for the difference between regimens in mean percent inhibition of glucose re-absorption over 24 hours (δ) is expected to be approximately δ ± 9.0 percentage points.

The sample size for this study was selected to be consistent with the research hypothesis as described in section 1.2.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

Reporting of SAE, pregnancy, overdose and all other safety related reports to Bristol-Myers Squibb is to be performed by e-mail (primary route): worldwide.safety@BMS.com and fax (secondary): +16098183804.

13.1 Medical emergencies and AstraZeneca contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see section 6.3.4**

In the case of a medical emergency the Investigator may contact the Study Team Physician and the Medical Science Lead at AstraZeneca. If the Study Team Physician is not available, contact the Senior Project Manager at PAREXEL.

Name	Role in the study	Address & telephone number
	SDT Physician responsible	
Medical Science Lead	Medical Science Lead Clinical Pharmacology EPMT	
	Senior Project Manager at PAREXEL	

13.2 Overdose

An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be

reported as an SAE (see section 6.3.4). If an overdose is suspected, monitoring of vital functions as well as treatment as appropriate should be performed.

13.3 Pregnancy

If a healthy volunteer becomes pregnant, the investigational product should be stopped and the study monitor should be informed. The pregnancy report module in the CRF should be completed by the Investigator and the study monitor will forward the information to Bristol-Myers Squibb using the same procedure as for SAE reporting (see section 6.3.4). The outcome of each pregnancy will also be collected once this information is available.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication.

However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

14. LIST OF REFERENCES

Hans et al 2008
Hans S. et al, Dapagliflozin, a selective SGLT2 inhibitor improves glucose homeostasis in normal and diabetic rats, Diabetes, 57,1723-1729, 2008

Komoroski et al 2009
Komoroski B., et al, Dapagliflozin, a novel, selective SGLT2 inhibitor, improved glycemic control over 2 weeks in patients with type 2 diabetes mellitus, Clin. Pharmacology and Therapeutics, 85; 520-526, 2009.

Matthei et al 2000
Matthaei S, Stumvoll M, Kellerer M, Haring HU. Pathophysiology and Pharmacological treatment of insulin resistance. Endocr Rev 2000;21: 585–618.

Clinical Study Protocol Appendix A

Drug Substance	Dapagliflozin
Study Code	D1691C00004
Edition Number	1.0
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURES

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representative

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol.

AstraZeneca Research and Development
site representative

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol.

PAREXEL
Projectmanagement

SIGNATURE OF PRINCIPAL INVESTIGATOR

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice and local regulations, and I ensure that all relevant site staff follows the instructions given in the latest version of the Laboratory Manual for Investigators

Centre No.:

Signature:

Clinical Study Protocol Appendix B

Drug Substance	Dapagliflozin
Study Code	D1691C00004
Edition Number	1.0
Date	

Appendix B
Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

<<Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse.>>

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Clinical Study Protocol Appendix C

Drug Substance	Dapagliflozin
Study Code	D1691C00004
Edition Number	1.0

Appendix C
International Airline Transportation Association (IATA) 6.2 Guidance Document

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).** For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are eg, Ebola, Lassa fever virus:

- are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are eg, Hepatitis A, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)**
- **Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content**
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable

- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

Clinical Study Protocol Amendment	
Amendment Number	1
Drug Substance	Dapagliflozin
Study Code	D1691C00004
Date	
Protocol Dated	

An open-label, randomised, two-period crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg OD) versus twice a day (5 mg BID) in healthy male and female volunteers

Sponsor:

AstraZeneca AB, SE-151 85 Södertälje, Sweden

Centres affected by the Amendment:

The protocol for the study is to be amended as follows:

Changes and additions are highlighted in **bold and underlined**, deleted text as ~~strikethrough~~.

Overview of the changes in this Amendment

Section in the protocol affected	Action	Protocol page
Synopsis, Exploratory objectives	New objective added	3
Synopsis, Outcome variables, pharmacokinetics:	Additional PK variable added ($C_{ss, av, t-t+4}$) Deletion of time points in BID dosing	4
Synopsis, Outcome variables, Pharmacodynamics	Deletion of GLP-1 and time points for blood sampling for determination of GIP	4
Synopsis, Statistical methods, last paragraph	Changes in analysis of PD parameter GIP	6
Abbreviation list	New abbreviations added	11
Section 1.3, Rationale for conduction this study	Corrected	14
Section 2.3, Exploratory objectives	New objective added	16
Section 3.1, Overall study design and flowchart, Table 1	Adjustment according to the deletion of PD Parameter and sampling times of PK time points in BID dosing and GIP	17
Section 3.1, Overall study design and flowchart, Table2	Adjustment according to the deletion of PD Parameter and sampling times of PK time points in BID dosing and GIP	19
Section 6.3.5, Laboratory assessments, 8th paragraph and following	Deletion of urine	36
Section 6.5.1, Collection of pharmacodynamic markers	Deletion of GLP-1 as PD parameter	39
Section 7.1, Volume of blood, Table 3	Adjustment of blood volume due to deletion of sampling times points for PK and GLP-1	40
Section 11.3, Calculation or derivation of pharmacokinetic variable, 4th paragraph and following	Additional PK variable added ($C_{ss, av, t-t+4}$).	50
Section 12.2.4, Pharmacodynamics, 4th paragraph	Adjustment of method of calculation due to deletion of PD parameter in several subheadings	51
Section 12.2, Methods of statistical analyses	A section for analysis of PJ/PD relationship added	52
Section 12.2.5, Methods of statistical analyses ff	Adjustment of numbering of table headings for safety and tolerability	52f

Section of protocol affected:

Synopsis, Explorative objectives:

Previous text:

Exploratory Objective

- To indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 (7-36 amide) (glucagon-like peptide) and GIP (glucose-dependent insulinotropic polypeptide) responses to meals accompanied with or without the administration of dapagliflozin

Revised text:

Exploratory Objectives

- To indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin~~, GLP-1 (7-36 amide) (glucagon-like peptide)~~ and GIP (glucose-dependent insulinotropic polypeptide) responses to meals accompanied with or without the administration of dapagliflozin

- **To explore plasma dapagliflozin concentration vs urine glucose excretion relationship**

Section of protocol affected:

Synopsis, Outcome variables, pharmacokinetics:

Previous text:

- C_{max}, $C_{ss,max}$, $C_{ss,min}$, AUC_{τ} and AUC_{ss}

[…]

For BID dosing, blood PK samples will be collected according to following schedule:

  - Day 1: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 12.25, 12.5, 13, 13.5, 14, 15, 16, 18, 20, and 24 hours;

  - Day 3 and Day 4: 0 hour (pre-dose of the morning dose);

[…]

Revised text:

Synopsis, Outcome variables, pharmacokinetics:

- C_{max}, $C_{ss,max}$, $C_{ss,min}$, **$C_{ss, av, t-t+4}$**, AUC_{τ} and AUC_{ss}

[…]

For BID dosing, blood PK samples will be collected according to following schedule:

- Day 1: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12~~, 12.25, 12.5, 13, 13.5, 14, 15, 16, 18, 20, and 24~~ hours;

- Day 3 and Day 4: 0 hour (pre-dose of the morning dose);

[…]

Section of protocol affected:

Synopsis, Outcome variables, Pharmacodynamics

Previous text:

- Percent inhibition of renal glucose re-absorption over 24 hours

  - Blood samples for plasma glucose will be drawn on Day 5 at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 24 hours.

  - Blood samples for creatinine will be drawn at pre-dose of Day 4 and of Day 5.

- Inhibition of rate of gut glucose absorption be determination of plasma glucose, insulin, GLP-1 (7-36 amide) (glucagon-like peptide 1) and GIP (glucose-dependent insulinotropic polypeptide)

  - Blood samples for plasma glucose and insulin will be drawn on Day 5 at –15, 0 15, 30, 45, 60, 90, 120, 150 and 180 minutes after each meal, for GLP-1 (7-36 amide) and GIP after dinner beginning at 11:45.

- Total urinary glucose excretion over 24 hour

  - On Day 5, urine will be collected in the following time intervals: from pre-dose to 4 hours post-dose, 4 to 8, hours post-dose, 8 to 12 hours post-dose, 12 to 16 hours post-dose, 16 to 20 hours post-dose and 20 to 24 hours post-dose.

- Percent inhibition of renal glucose re-absorption over 24 hours

Revised text:

- Percent inhibition of renal glucose re-absorption **at each urine collection interval and** over 24 hours

  - Blood samples for plasma glucose will be drawn on Day 5 at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 24 hours.

  - Blood samples for creatinine will be drawn at pre-dose of Day 4 and of Day 5.

- Inhibition of rate of gut glucose absorption be determination of plasma glucose, insulin~~, GLP-1 (7-36 amide) (glucagon-like peptide 1)~~ and GIP (glucose-dependent insulinotropic polypeptide)

  - ~~Blood samples for plasma glucose, insulin, GLP-1 (7-36 amide) and GIP will be drawn on Day 5 at –15, 0 15, 30, 45, 60, 90, 120, 150 and 180 minutes after each meal~~ **Blood samples for plasma glucose and insulin will be drawn on Day 5 at –15, 0 15, 30, 45, 60, 90, 120, 150 and 180 minutes after each meal, for GIP 11:45 (before dinner) and 13.30 (after dinner).**

- Total urinary glucose excretion **at each urine collection interval and** over 24 hour

  - On Day 5, urine will be collected in the following time intervals: from pre-dose to 4 hours post-dose, 4 to 8, hours post-dose, 8 to 12 hours post-dose, 12 to 16 hours post-dose, 16 to 20 hours post-dose and 20 to 24 hours post-dose.

~~For plasma glucose, insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal ($AUE_{(0-180),}$ AUE= area under the effect time curve) calculated.~~ **For plasma glucose and insulin, the area under the effect time curve until 180 minutes after each meal ($AUE_{(0-90),}$ AUE= area under the effect time curve) will be calculated. Differences between pre- and post-meal GIP measurements will be determined.**

Section of protocol affected:

Synopsis, Statistical methods, last paragraph

Previous text:

For plasma glucose, insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated. Baseline adjusted geometric mean differences between both treatment groups will be estimated by a linear model on the log-transformed AUEs after each meal and retransforming the point estimates and 95% confidence intervals.

Revised text:

For plasma glucose **and** insulin~~, GLP-1 (7-36 amide) and GIP~~ the area under the effect time curve until 180 minutes after each meal (AUE_{0-180})will be calculated. Baseline adjusted geometric mean differences between both treatment groups will be estimated by a linear model on the log-transformed AUEs after each meal and retransforming the point estimates and 95% confidence intervals**. Differences between pre- and post-meal GIP measurements will be determined at dinner only. The change in GIP between the two study periods for each subject will be compared and statistically analyzed.**

The relationship of plasma dapagliflozin concentration vs. percent inhibition of renal glucose re-absorption or urine glucose excretion will be explored.

Section of protocol affected:

Abbreviation list

Previous text:

[…]

AST	Aspartate aminotransferase
$AUC_{ss, (0-24)}$	Area under the plasma concentration versus time curve from time zero to 24 hours at steady state [amount•time/volume]

[…]

$C_{ss,av}$	The average concentration at steady state
C_{max}	Maximum plasma (peak) drug concentration [amount/volume]

[…]

GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide 1
HBsAg	Hepatitis B surface antigen

[…]

Revised text

[…]

AST	Aspartate aminotransferase
$AUC_{ss, t-t+4}$	**Area under plasma concentration-time curve at steady state during each urine collection interval [amount.time/volume]**

$AUC_{ss, (0-24)}$	Area under the plasma concentration versus time curve from time zero to 24 hours at steady state [amount•time/volume]
[…]	
$C_{ss,av}$	The average concentration at steady state
$C_{ss, av, t-t+4}$	**The average plasma concentration at steady state during each urine collection interval**
C_{max}	Maximum plasma (peak) drug concentration [amount/volume]
[…]	
GIP	Glucose-dependent insulinotropic polypeptide
~~GLP-1~~	~~Glucagon-like peptide 1~~
HBsAg	Hepatitis B surface antigen
[…]	

Section of protocol affected:

Section 1.3, Rationale for conduction this study

Previous text:

- To demonstrate that dapagliflozin has a similar pharmacodynamic (PD) effect if dosed twice a day as once a day, as this is how dapagliflozin would be given in a fixed-dose combination with metformin IR

Revised text

- To demonstrate that dapagliflozin has a similar pharmacodynamic (PD) effect if dosed twice a day as once a day~~, as this is how dapagliflozin would be given in a fixed-dose combination with metformin IR~~

Section of protocol affected:

Section 2.3, Exploratory objectives

Previous text:

Exploratory Objective

- To indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 (7-36 amide) (glucagon-like peptide) and GIP (glucose-dependent insulinotropic polypeptide) responses to meals accompanied with or without the administration of dapagliflozin

Revised text:

Exploratory Objectives

- To indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin~~, GLP-1 (7-36 amide) (glucagon-like peptide)~~ and GIP (glucose-dependent insulinotropic polypeptide) responses to meals accompanied with or without the administration of dapagliflozin

- **To explore plasma dapagliflozin concentration vs urine glucose excretion relationship**

Section of protocol affected:

Section 3.1, Overall study design and flowchart, Table 1

Previous table:

Table 1 Study Schedule

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Informed consent	✓		
Inclusion/Exclusion criteria	✓	✓	
Concomitant medication	✓	✓	✓
Medical/Surgical history	✓		
Physical exam	✓	✓[c]	✓[d]
Virology screen	✓		
Alcohol urine test	✓	✓	
Demographics	✓		
Drugs of abuse	✓	✓	
12-lead ECG	✓		
Blood pressure and heart rate (including body temperature [e]	✓	✓	✓
Weight and height [f]	✓		✓
Safety labs (blood and urine) [g]	✓	✓	✓
Randomization [h]		✓	
PK sample collection		✓	
Confinement to unit		✓	
Dapagliflozin dose		✓	

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Pregnancy test (serum) [i]	✓	✓	
Adverse event questioning		✓	✓
Serious adverse event	✓	✓	✓
Urine sample collections [k]		✓	
Creatinine measurements		✓	
Plasma glucose, insulin, GLP-1 and GIP		✓	

ECG electrocardiogram, GLP glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide

[a] Beginning of period 1.
[b] Beginning of period 2
[c] Brief history and physical exam
[d] To be performed at end of Visit 4 (follow-up)
[e] Vital signs will be obtained he volunteer in a supine position (resting in bed for at least 10 minutes)
[f] Height will be assessed only at the enrolment visit (Visit 1)
[g] Safety labs (blood and urine) will be performed at Visit 1, and upon admission on Day –1 and pre-dose on Day 5, and at Visit 4
[h] Performed at Visit 2 only after all inclusion and exclusion criteria have been satisfied on Day 1.
[i] Required for female healthy volunteers only. Serum pregnancy test will be required at Visit 1, on Day –1 of Visits 2 and 3. Healthy female volunteers must have confirmed negative serum pregnancy test from Day –1 of each treatment period prior to receiving the IP
[j] AEs will be recorded from first intake of IP in period 1 until follow-up, SAEs will be collected from the time when informed consent is obtained until the end of study (including the follow-up visit).
[k] Urine samples for volume, glucose concentration, creatinine concentration.

Table 1 Study Schedule

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Informed consent	✓		
Inclusion/Exclusion criteria	✓	✓	
Concomitant medication	✓	✓	✓
Medical/Surgical history	✓		
Physical exam	✓	✓[c]	✓[d]
Virology screen	✓		
Alcohol urine test	✓	✓	
Demographics	✓		
Drugs of abuse	✓	✓	
12-lead ECG	✓		
Blood pressure and heart rate (including body temperature [e]	✓	✓	✓

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Weight and height [f]	✓		✓
Safety labs (blood and urine) [g]	✓	✓	✓
Randomization [h]		✓	
PK sample collection		✓	
Confinement to unit		✓	
Dapagliflozin dose		✓	
Pregnancy test (serum) [i]	✓	✓	
Adverse event questioning		✓	✓
Serious adverse event	✓	✓	✓
Urine sample collections [k]		✓	
Creatinine measurements		✓	
Plasma glucose, insulin~~, GLP-1~~ and GIP		✓	

ECG electrocardiogram~~, GLP glucagon-like peptide~~, GIP glucose-dependent insulinotropic polypeptide

[a] Beginning of period 1.

[b] Beginning of period 2

[c] Brief history and physical exam

[d] To be performed at end of Visit 4 (follow-up)

[e] Vital signs will be obtained he volunteer in a supine position (resting in bed for at least 10 minutes)

[f] Height will be assessed only at the enrolment visit (Visit 1)

[g] Safety labs (blood and urine) will be performed at Visit 1, and upon admission on Day –1 and pre-dose on Day 5, and at Visit 4

[h] Performed at Visit 2 only after all inclusion and exclusion criteria have been satisfied on Day 1.

[i] Required for female healthy volunteers only. Serum pregnancy test will be required at Visit 1, on Day –1 of Visits 2 and 3. Healthy female volunteers must have confirmed negative serum pregnancy test from Day –1 of each treatment period prior to receiving the IP

[j] AEs will be recorded from first intake of IP in period 1 until follow-up, SAEs will be collected from the time when informed consent is obtained until the end of study (including the follow-up visit).

[k] Urine samples for volume, glucose concentration, creatinine concentration.

Revised table:

Section of protocol affected:

Section 3.1, Overall study design and flowchart, Table2

Previous table:

Table 2 Study Plan for Each Treatment Period

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
	–1	15:30								Check for inclusion and exclusion criteria, drug screen including alcohol.
		Pre-dose					1	1		
	1	00:00	X							Breakfast[b]
		00:15					2	2		
		00:30					3	3		
		01:00					4	4		
		01:30					5	5		
		02:00					6	6		
		03:00					7	7		
		04:00					8	8		
		06:00					9	9		
		08:00					10	10		
		12:00	X[a]				11	11		Dinner[b]
		12:15						12		
		12:30						13		
		13:00						14		
		13:30						15		
		14:00						16		
		15:00						17		

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		16:00					12	18		
		18:00						19		
		20:00					13	20		
	2	24:00	X				14	21		Breakfast[b]
		12:00	X[a]							Dinner[b]
	3	Pre-dose					15	22		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
	4	Pre-dose			1 (back up sample)		16	23		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
2/3	5	-00:15		1		1				
		Pre-dose			2		17	24	X (start of collec-tion)	Safety lab, vital signs
		00:00	X	2		2			X	Initiation of breakfast[b]
		00:15		3		3	18	25	X	
		00:30		4		4	19	26	X	
		00:45		5		5				
		01:00		6		6	20	27	X	
		01:30		7		7	21	28	X	
		02:00		8		8	22	29	X	
		02:30		9		9				
		03:00		10		10	23	30	X	

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		04:00	11			24	31	X (end of collec-tion period)	
								X (restart of collec-tion)	
		04:45	12		11				
		05:00	13		12			X	Initiation of Lunch[b]
		05:15	14		13				
		05:30	15		14				
		05:45	16		15				
		06:00	17		16	25	32	X	
		06:30	18		17				
		07:00	19		18			X	
		07:30	20		19				
		08:00	21		20	26	33	X (end of collec-tion period)	
								X (restart of collec-tion)	
		09:00	22					X	
		10:00	23					X	
		11:00	24					X	
		11:45	25		21				

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		12:00	X[a]	26		22	27	34	X (end of first collection period)	Initiation of dinner[b]
									X (restart of collec-tion)	
		12:15		27		23		35		
		12:30		28		24		36		
		12:45		29		25				
		13:00		30		26		37	X	
		13:30		31		27		38		
		14:00		32		28		39	X	
		14:30		33		29				
		15:00		34		30		40	X	
		16:00		35			28	41	X (end of collection period)	
									X (restart of collec-tion)	
		17:00							X	
		18:00		36				42	X	
		19:00							X	

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		20:00	37			29	43	X (end of collection period)	
								X (restart of collec-tion)	
		21:00						X	
		22:00						X	
		23:00						X	
	6	24:00	38			30	44	X end of collec-tion period)[b]	

IP investigational product, PK pharmacokinetic, PD pharmacodynamic, GLP glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide

[a] Only in treatment A

[b] The IP will be administered together with the meal, which has to be completed within 30 minutes. Regarding the timing of events at mealtime, the following order of activities should be followed: blood sampling for plasma glucose, insulin, GLP-1 and GIP, with meal initiation occurring within 2 min of blood sampling. If the IP is to be administered the following sequence of events should occur: blood sampling, medication administration then meal initiation within 2 min of blood sampling.

Revised table:

Table 2 Study Plan for Each Treatment Period

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
	–1	15:30								Check for inclusion and exclusion criteria, drug screen including alcohol.
		Pre-dose					1	1		
	1	00:00	X							Breakfast[b]
		00:15					2	2		
		00:30					3	3		
		01:00					4	4		
		01:30					5	5		
		02:00					6	6		
		03:00					7	7		
		04:00					8	8		
		06:00					9	9		
		08:00					10	10		
		12:00	X[a]				11	11		Dinner[b]
		12:15						~~12~~		
		12:30						~~13~~		
		13:00						~~14~~		
		13:30						~~15~~		
		14:00						~~16~~		
		15:00						~~17~~		
		16:00					12	~~18~~		

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		18:00						~~19~~		
		20:00					13	~~20~~		
	2	24:00	X				14	~~21~~		Breakfast[b]
		12:00	X[a]							Dinner[b]
	3	Pre-dose					15	~~22~~ **12**		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
	4	Pre-dose			1 (back up sample)		16			
		00:00	X					~~23~~ **13**		Breakfast[b]
		12:00	X[a]							Dinner[b]
2/3	5	-00:15		1		~~1~~				
		Pre-dose			2		17		X (start of collec-tion)	Safety lab, vital signs
		00:00	X	2		~~2~~		~~24~~ **14**	X	Initiation of breakfast[b]
		00:15		3		~~3~~	18		X	
		00:30		4		~~4~~	19	~~25~~ **15**	X	
		00:45		5		~~5~~		~~26~~ **16**		
		01:00		6		~~6~~	20		X	
		01:30		7		~~7~~	21	~~27~~ **17**	X	
		02:00		8		~~8~~	22	~~28~~ **18**	X	
		02:30		9		~~9~~		~~29~~ **19**		
		03:00		10		~~10~~	23		X	

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose, ~~Insulin~~	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		04:00	11			24	~~30~~ **20**	X (end of collec-tion period) X (restart of collec-tion)	
		04:45	12		~~11~~		~~31~~ **21**		
		05:00	13		~~12~~			X	Initiation of Lunch[b]
		05:15	14		~~13~~				
		05:30	15		~~14~~				
		05:45	16		~~15~~				
		06:00	17		~~16~~	25		X	
		06:30	18		~~17~~		~~32~~ **22**		
		07:00	19		~~18~~			X	
		07:30	20		~~19~~				
		08:00	21		~~20~~	26		X (end of collec-tion period) X (restart of collec-tion)	
		09:00	22				~~33~~ **23**	X	
		10:00	23					X	
		11:00	24					X	
		11:45	25		~~21~~ **1**				

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		12:00	X[a]	26		~~22~~	27		X (end of first collection period) X (restart of collec-tion)	Initiation of dinner[b]
		12:15		27		~~23~~		~~34~~ **24**		
		12:30		28		~~24~~		~~35~~ **25**		
		12:45		29		~~25~~		~~36~~ **~~26~~**		
		13:00		30		~~26~~			X	
		13:30		31		~~27~~ **2**		~~37~~ **27**		
		14:00		32		~~28~~		~~38~~ **28**	X	
		14:30		33		~~29~~		~~39~~ **29**		
		15:00		34		~~30~~			X	
		16:00		35			28	~~40~~ **30**	X (end of collection period) X (restart of collec-tion)	
		17:00						~~41~~ **31**	X	
		18:00		36					X	
		19:00						~~42~~ **32**	X	

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		20:00	37			29		X (end of collection period)	
								X (restart of collec-tion)	
		21:00					~~43~~ **33**	X	
		22:00						X	
		23:00						X	
	6	24:00	38			30		X end of collec-tion period)[b]	

IP investigational product, PK pharmacokinetic, PD pharmacodynamic, ~~GLP glucagon-like peptide,~~ GIP glucose-dependent insulinotropic polypeptide

[a] Only in treatment A

[b] The IP will be administered together with the meal, which has to be completed within 30 minutes. Regarding the timing of events at mealtime, the following order of activities should be followed: blood sampling for plasma glucose, insulin~~, GLP-1~~ and GIP **(GIP only at dinner)** , with meal initiation occurring within 2 min of blood sampling. If the IP is to be administered the following sequence of events should occur: blood sampling, medication administration then meal initiation within 2 min of blood sampling.

Section of protocol affected:

Section 6.3.5, Laboratory assessments, 8th paragraph and following

Previous text:

[…]

The following laboratory variables will be measured:

Clinical Chemistry	**Haematology**
Serum (S-)Albumin	Blood (B)-Haemoglobin
S-Alanine aminotransferase (ALT)	B-Leukocyte (part.conc)
S- Aspartate aminotransferase (AST)	B-Absolute leukocyte differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes

S-Alkaline phosphatase (ALP)

S-Bilirubin, total

S-Creatinine

S- Glucose

S-Potassium

S-Sodium

S-Chloride

S-Creatinine kinase (CK)

S-Total protein

S-Uric acid

B-Platelet count

Urinalysis

Urine (U)-Glucose (dipstick)

U-Haemoglobin (dipstick)

U-Protein (dipstick)

U-Creatinine

Other samples

S-U-Pregnancy test (females only) (S-βhCG = Human Chorionic Gonadotropin)

S-Follicle stimulation hormone (FSH) (females only and only at enrolment)

[…]

Revised text:

[…]

The following laboratory variables will be measured:

Clinical Chemistry

Serum (S-)Albumin

S-Alanine aminotransferase (ALT)

S- Aspartate aminotransferase (AST)

S-Alkaline phosphatase (ALP)

S-Bilirubin, total

S-Creatinine

S- Glucose

S-Potassium

S-Sodium

S-Chloride

S-Creatinine kinase (CK)

S-Total protein

S-Uric acid

Haematology

Blood (B)-Haemoglobin

B-Leukocyte (part.conc)

B-Absolute leukocyte differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes

B-Platelet count

Urinalysis

Urine (U)-Glucose (dipstick)

U-Haemoglobin (dipstick)

U-Protein (dipstick)

~~U-Creatinine~~

Other samples

S-U-Pregnancy test (females only) (S-βhCG = Human Chorionic Gonadotropin)

S-Follicle stimulation hormone (FSH) (females only and only at enrolment)

[…]

Section of protocol affected:

Section 6.5.1, Collection of pharmacodynamic markers

Previous text:

Blood samples for GIP and GLP-1 will be collected in 3.0 mL in K2-EDTA (sodium ethylenediaminetetraacetic acid) (spray coated) tubes. Samples will be centrifuged at 1500 g for 10 min and the plasma will be stored frozen at –20°C until transport for analysis at Synlab Clinical Trial GmbH, Berlin, Germany.

Blood samples will be collected at the times presented in the study plan Table 2. For blood volume see section 7.1.

Revised text:

Blood samples for GIP ~~and GLP-1~~ will be collected in 3.0 mL in K2-EDTA (sodium ethylenediaminetetraacetic acid) (spray coated) tubes. Samples will be centrifuged at 1500 g for 10 min and the plasma will be stored frozen at –20°C until transport for analysis at Synlab Clinical Trial GmbH, Berlin, Germany.

Blood samples will be collected at the times presented in the study plan Table 2. For blood volume see section 7.1.

Section of protocol affected:

Section 7.1, Volume of blood, Table 3

Previous text:

Table 3 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry [a]	2.6	6	15.6
	Haematology	2.7	6	16.2
	Tricyclic antidepressants	1.1	3	3.3
	Serology	2.6	1	2.6
Pharmacokinetic		2.0	74	148.0
Pharmacodynamic				

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry [a]	2.6	6	15.6
	Haematology	2.7	6	16.2
Creatinine		1.1	4	4.4
Glucose		1.2	76	91.2
GLP-1		3.0	60	180.0
Insulin and GIP		1.1	60	66.0
Total				**527.3**

GLP Glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide
a Includes pregnancy testing

Revised text:

Table 3 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry [a]	2.6	6	15.6
	Haematology	2.7	6	16.2
	Tricyclic antidepressants	1.1	3	3.3
	Serology	2.6	1	2.6
Pharmacokinetic		2.0	~~74~~ **64**	~~148.0~~ **128.0**
Pharmacodynamic				
Creatinine		1.1	4	4.4
Glucose		1.2	76	91.2
~~GLP-1~~**GIP**		3.0	**4**	**12.0** ~~180.0~~
Insulin ~~and GIP~~		1.1	60	66.0
Total				**339.3** ~~**527.3**~~

~~GLP Glucagon-like peptide,~~ GIP glucose-dependent insulinotropic polypeptide
a Includes pregnancy testing

Section of protocol affected:

Section 11.3, Calculation or derivation of pharmacokinetic variable, 4th paragraph and following

Previous text:

[…]

The following PK parameters will be determined:

- AUC_{τ} Area under plasma concentration-time curve during a dosing interval [amount• time/volume]
- AUC_{ss} Area under plasma concentration-time curve during any dosing interval at steady state [amount• time/volume]
- $C_{ss, av}$ The average concentration at steady state
- $C_{ss, max}$ Maximum plasma (peak) drug concentration at steady state [amount/volume]
- $C_{ss, min}$ Minimum (trough) steady state drug concentration in plasma during dosing interval [amount/volume]
- DF% Degree of fluctuation at steady state.
  $DF(\%) = (C_{ss, max} - C_{ss, min})/C_{ss, av} \times 100$
- $t_{ss, max}$ Time to maximum plasma concentration at steady state [h]

Revised text:

[…]

The following PK parameters will be determined:

- AUC_{τ} Area under plasma concentration-time curve during a dosing interval [amount• time/volume]
- AUC_{ss} Area under plasma concentration-time curve during any dosing interval at steady state [amount• time/volume]
- $C_{ss, av}$ The average concentration at steady state
- $C_{ss, max}$ Maximum plasma (peak) drug concentration at steady state

[amount/volume]

- $C_{ss, min}$ Minimum (trough) steady state drug concentration in plasma during dosing interval [amount/volume]
- DF% Degree of fluctuation at steady state.
  $DF(\%) = (C_{ss, max} - C_{ss, min})/C_{ss, av} \times 100$
- $t_{ss, max}$ Time to maximum plasma concentration at steady state [h]
- **$AUC_{ss, t-t+4}$ Area under plasma concentration-time curve at steady state during each urine collection interval [amount.time/volume]**
- **$C_{ss, av, t-t+4}$ The average plasma concentration at steady state during each urine collection interval. Css, av, t-t+4 = AUCss, t-t+4/4**

Section of protocol affected:

Section 11.3, Calculation or derivation of pharmacodynamic variables, second paragraph

Previous text:

[…]

The secondary objectives of the study are to assess the effect of dapagliflozin on 24 hour urine glucose excretion when administered once a day (10 mg) versus twice a day (5 mg every 12 hours) at steady state (after five days of dosing), the exploratory objectives to indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 and GIP responses to meals accompanied with or without the administration of dapagliflozin.

[…]

Revised text:

[…]

The secondary objectives of the study are to assess the effect of dapagliflozin on 24 hour urine glucose excretion when administered once a day (10 mg) versus twice a day (5 mg every 12 hours) at steady state (after five days of dosing), the exploratory objectives to indirectly determine if dapagliflozin may inhibit the rate of gut glucose absorption by comparing the plasma glucose, insulin~~, GLP-1~~ and GIP responses to meals accompanied with or without the administration of dapagliflozin.

[…]

Section of protocol affected:

Section 11.3, Calculation or derivation of pharmacodynamic variables, subheading Inhibition of glucose re-absorption

The following was added following the paragraph of this section:

The percent inhibition of renal glucose re-absorption during each urine collection interval will be calculated as 100 X amount of glucose excreted in the urine from each 4-our interval/the amount of glucose renally filtrated from each 4-hour interval.

Section of protocol affected:

Section 11.3, Calculation or derivation of pharmacodynamic variables, subheading Inhibition of rate of gut glucose absorption

Previous text:

Inhibition of rate of gut glucose absorption

For plasma glucose insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated for each subject in each period. The AUE_{0-180} will be calculated using the linear trapezoidal rule.

Revised text:

Inhibition of rate of gut glucose absorption

For plasma glucose **and** insulin~~, GLP-1 (7-36 amide) and GIP~~ the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated for each subject in each period. The AUE_{0-180} will be calculated using the linear trapezoidal rule.

Section of protocol affected:

Section 12.2.4, Pharmacodynamics, 4th paragraph

Previous text:

[…]

For plasma glucose, insulin, GLP-1 (7-36 amide) and GIP the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated. Baseline adjusted geometric mean differences between both treatment groups will be estimated by a linear model on the log-transformed AUEs after each meal**,** and retransforming the point estimates and 95% confidence intervals. The linear model will include treatment, sequence, period as fixed effects, subjects nested within sequence as random effect and baseline concentration as continuous covariate. AUEs after each meal will be analyzed in a separate model.

Results of the analysis after dinner will be used to determine if dapagliflozin inhibits the rate of gut glucose absorption by comparing the plasma glucose, insulin, GLP-1 and GIP responses to meals accompanied with (5 mg BID) or without the administration of dapagliflozin (10 mg OD).

Revised text:

[…]

For plasma glucose **and** insulin~~, GLP-1 (7-36 amide) and GIP~~ the area under the effect time curve until 180 minutes after each meal (AUE_{0-180}) will be calculated. Baseline adjusted geometric mean differences between both treatment groups will be estimated by a linear model on the log-transformed AUEs after each meal and retransforming the point estimates and 95% confidence intervals. The linear model will include treatment, sequence, period as fixed effects, subjects nested within sequence as random effect and baseline concentration as continuous covariate. AUEs after each meal will be analyzed in a separate model. **Differences between pre- and post-meal GIP measurements will be determined at dinner only. The change in GIP between the two study periods for each subject will be compared and statistically analyzed.**

Results of the analysis after dinner will be used to determine if dapagliflozin inhibits the rate of gut glucose absorption by comparing the plasma glucose, insulin~~, GLP-1~~ and GIP responses to meals accompanied with (5 mg BID) or without the administration of dapagliflozin (10 mg OD).

Section of protocol affected:

Section 12.2, Methods of statistical analyses

The following section was added:

12.2.5 PK/PD relationship

The average plasma concentration vs percent inhibition of glucose re-absorption or the amount of glucose excreted will be explored and the results will be reported separately if any relationship can be determined.

Section of protocol affected:

Section 12.2, Methods of statistical analyses

Previous heading number:

12.2.5, Safety and tolerability

Revised heading number:

~~12.2.5~~, **12.2.6** Safety and tolerability

Section of protocol affected:

Section 12.2, Methods of statistical analyses

Previous heading number:

12.2.5.1 Adverse events

Revised heading number:

~~12.2.5.1~~, **12.2.6.1** Adverse events

Section of protocol affected:

Section 12.2, Methods of statistical analyses

Previous heading number:

12.2.5.2 ECG

Revised heading number:

~~12.2.5.2~~, **12.2.6.2** ECG

Section of protocol affected:

Section 12.2, Methods of statistical analyses

Previous heading number:

12.2.5.3 Laboratory variables

Revised heading number:

~~12.2.5.3~~, **12.2.6.3** Laboratory variables

Section of protocol affected:

Section 12.2, Methods of statistical analyses

Previous heading number:

12.2.5.4 Vital signs

Revised heading number:

~~12.2.5.4~~, **12.2.6.4** Vital signs

Section of protocol affected:

Section 12.2, Methods of statistical analyses

Previous heading number:

12.2.6 Interim analyses (Not applicable)

Revised heading number:

~~12.2.6~~, **12.2.7** Interim analyses (Not applicable)

Reasons for Amendment:

- To explore the relationship between dapagliflozin plasma concentrations and urine glucose excretion
- Deletion of GLP-1 as PD parameter
- Restricting blood samples for GIP to be taken to only after dinner on Day 5
- Deletion of PK samples Nr. 12 to 21 for BID dosing
- Harmonizing of Section 6.5.1 and Table 3 with regard to blood sampling of GIP and Insulin
- Corrections and harmonisations

Persons who initiated the Amendment:

Astra Zeneca Study Delivery Team Leader

Clinical Study Protocol Amendment No 1
Appendix A

Drug Substance	Dapagliflozin
Study Code	D1691C00004
Edition Number	1
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of amendment.

AstraZeneca Research and Development site representative

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

---

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of this amendment.

PAREXEL
Projectmanagement

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of this amendm

AstraZeneca Research and Development site representative

SIGNATURE OF PRINCIPAL INVESTIGATOR

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of this amendment.

Signature:

Clinical Study Protocol Amendment

Amendment Number	2
Drug Substance	Dapagliflozin
Study Code	D1691C00004
Protocol Dated	

An open-label, randomised, two-period crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg OD) versus twice a day (5 mg BID) in healthy male and female volunteers

Sponsor:

AstraZeneca AB, SE-151 85 Södertälje, Sweden

Centres affected by the Amendment:

The protocol for the study is to be amended as follows:

Changes and additions are highlighted in **bold and underlined**, deleted text as ~~strikethrough~~.

Overview of the changes in this Amendment

Section in the protocol affected	Action	Protocol page
Section 3.1, Overall study design and flowchart, 5th paragraph	Note to type and amount of fluid intake during for Day 1 and Day 5	17
Section 3.1, Overall study design and flowchart, Table 1	Cotinine test added and fasting time before safety lab defined	17f
Section 3.1, Overall study design and flowchart, Table 2	Adjustment of PK sampling times and numbering in BID dosing Day 5, adding safety lab on Day –1	19
Section 4.2, Exclusion criteria 12	Positive cotinine test at enrolment added	25
Section 5.1, Restrictions during the study, bullet 2	Subjects may drink either tap water or non-caloric and non-caffeinated beverages during the study, however at each time interval of both periods the same type of beverage	25f
Section 6.2, Data collection and enrolment	Subjects to be in fasted state for at least 8 hours prior examinations All pregnancy tests will be performed in serum	32
Section 6.21, Follow-up	Subjects to be in fasted state for at least 8 hours prior examinations.	32
Section 6.3.5	Adjustment to reduced blood amount for clinical chemistry Cotinine test added	36 37
Section 7.1	Volume of blood	40

Section of protocol affected:

Section 3.1, Overall study design and flowchart, 5th paragraph

Previous text:

[…]

Breakfast on Day 5 in both periods must be identical; lunch on Day 5 in both periods must be identical; and dinner on Day 5 in both periods must be identical. Healthy volunteers must consume the entire meals within 30 minutes on Day 4 (for practice) and on Day 5. Of each meal on Day 5, approximately 55% of the ingested caloric equivalent should be carbohydrates. The meal schedule for all meals must be identical for all healthy volunteers, and the meal consumption time has to be identical in both periods for each healthy volunteer. Healthy volunteers may not consume caloric beverages between meals or at bedtime, likewise the healthy volunteers are to abstain from liquids with artificial sweeteners' on Day 5.

[…]

Revised text:

[…]

Breakfast on Day 5 in both periods must be identical; lunch on Day 5 in both periods must be identical; and dinner on Day 5 in both periods must be identical. Healthy volunteers must consume the entire meals within 30 minutes on Day 4 (for practice) and on Day 5. Of each meal on Day 5, approximately 55% of the ingested caloric equivalent should be carbohydrates. The meal schedule for all meals must be identical for all healthy volunteers, and the meal consumption time has to be identical in both periods for each healthy volunteer. **On Day 1 and Day 5, the subjects should drink the same liquid and amount with meals.** Healthy volunteers may not consume caloric beverages between meals or at bedtime, likewise the healthy volunteers are to abstain from liquids with artificial sweeteners' on Day 1 and Day 5.

[…]

Section of protocol affected:

Section 3.1, Overall study design and flowchart, Table1

Previous table:

Table 1 Study Schedule

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Informed consent	✓		
Inclusion/Exclusion criteria	✓	✓	

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Concomitant medication	✓	✓	✓
Medical/Surgical history	✓		
Physical exam	✓	✓[c]	✓[d]
Virology screen	✓		
Alcohol urine test	✓	✓	
Demographics	✓		
Drugs of abuse	✓	✓	
12-lead ECG	✓		
Blood pressure and heart rate (including body temperature [e]	✓	✓	✓
Weight and height [f]	✓		✓
Safety labs (blood and urine) [g]	✓	✓	✓
Randomization [h]		✓	
PK sample collection		✓	
Confinement to unit		✓	
Dapagliflozin dose		✓	
Pregnancy test (serum) [i]	✓	✓	
Adverse event questioning		✓	✓
Serious adverse event	✓	✓	✓
Urine sample collections [k]		✓	
Creatinine measurements		✓	
Plasma glucose, insulin~~, GLP-1~~ and GIP		✓	

ECG electrocardiogram, GLP glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide

[a] Beginning of period 1.
[b] Beginning of period 2
[c] Brief history and physical exam
[d] To be performed at end of Visit 4 (follow-up)
[e] Vital signs will be obtained he volunteer in a supine position (resting in bed for at least 10 minutes)
[f] Height will be assessed only at the enrolment visit (Visit 1)
[g] Safety labs (blood and urine) will be performed at Visit 1, and upon admission on Day –1 and pre-dose on Day 5, and at Visit 4.
[h] Performed at Visit 2 only after all inclusion and exclusion criteria have been satisfied on Day 1.
[i] Required for female healthy volunteers only. Serum pregnancy test will be required at Visit 1, on Day –1 of Visits 2 and 3. Healthy female volunteers must have confirmed negative serum pregnancy test from Day –1 of each treatment period prior to receiving the IP
[j] AEs will be recorded from first intake of IP in period 1 until follow-up, SAEs will be collected from the time when informed consent is obtained until the end of study (including the follow-up visit).
[k] Urine samples for volume, glucose concentration, creatinine concentration.

Revised table

Table 2 Study Schedule

Assessment	Visit 1 Enrolment	Visits 2[a] and 3[b] Treatment	Visit 4 Follow- up
Informed consent	✓		
Inclusion/Exclusion criteria	✓	✓	
Concomitant medication	✓	✓	✓
Medical/Surgical history	✓		
Physical exam	✓	✓[c]	✓[d]
Virology screen	✓		
Alcohol urine test	✓	✓	
Demographics	✓		
Drugs of abuse **including cotinine test**	✓	✓	
12-lead ECG	✓		
Blood pressure and heart rate (including body temperature [e]	✓	✓	✓
Weight and height [f]	✓		✓
Safety labs (blood and urine) [g]	✓	✓	✓
Randomization [h]		✓	
PK sample collection		✓	
Confinement to unit		✓	
Dapagliflozin dose		✓	
Pregnancy test (serum) [i]	✓	✓	
Adverse event questioning		✓	✓
Serious adverse event	✓	✓	✓
Urine sample collections [k]		✓	
Creatinine measurements		✓	
Plasma glucose, insulin~~, GLP-1~~ and GIP		✓	

ECG electrocardiogram~~, GLP glucagon-like peptide~~, GIP glucose-dependent insulinotropic polypeptide

[a] Beginning of period 1.
[b] Beginning of period 2
[c] Brief history and physical exam
[d] To be performed at end of Visit 4 (follow-up)
[e] Vital signs will be obtained the volunteer in a supine position (resting in bed for at least 10 minutes)
[f] Height will be assessed only at the enrolment visit (Visit 1)

[g] Safety labs (blood and urine) will be performed at Visit 1, and upon admission on Day –1 and pre-dose on Day 5, and at Visit 4**. At enrolment and follow-up examination, the subjects should be in fasted state for at least 8 hours, on Day –1 of each period for at least 4 hours. Non-caloric beverages are allowed for the fasting period.**

[h] Performed at Visit 2 only after all inclusion and exclusion criteria have been satisfied on Day 1.

[i] Required for female healthy volunteers only. Serum pregnancy test will be required at Visit 1, on Day –1 of Visits 2 and 3. Healthy female volunteers must have confirmed negative serum pregnancy test from Day –1 of each treatment period prior to receiving the IP

[j] AEs will be recorded from first intake of IP in period 1 until follow-up, SAEs will be collected from the time when informed consent is obtained until the end of study (including the follow-up visit).

[k] Urine samples for volume, glucose concentration, creatinine concentration.

Section of protocol affected:

Section 3.1, Overall study design and flowchart, Table2

Previous table:

Table 2 Study Plan for Each Treatment Period

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
	–1	15:30								Check for inclusion and exclusion criteria, drug screen including alcohol.
		Pre-dose					1	1		
	1	00:00	X							Breakfast[b]
		00:15					2	2		
		00:30					3	3		
		01:00					4	4		
		01:30					5	5		
		02:00					6	6		
		03:00					7	7		
		04:00					8	8		
		06:00					9	9		
		08:00					10	10		

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		12:00	X[a]				11	11		Dinner[b]
		12:15						12		
		12:30						13		
		13:00						14		
		13:30						15		
		14:00						16		
		15:00						17		
		16:00					12	18		
		18:00						19		
		20:00					13	20		
	2	24:00	X				14	21		Breakfast[b]
		12:00	X[a]							Dinner[b]
	3	Pre-dose					15	22		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
	4	Pre-dose			1 (back up sample)		16	23		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
2/3	5	-00:15		1		1				
		Pre-dose			2		17	24	X (start of collec-tion)	Safety lab, vital signs
		00:00	X	2		2			X	Initiation of breakfast[b]
		00:15		3		3	18	25	X	
		00:30		4		4	19	26	X	
		00:45		5		5				

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		01:00	6		6	20	27	X	
		01:30	7		7	21	28	X	
		02:00	8		8	22	29	X	
		02:30	9		9				
		03:00	10		10	23	30	X	
		04:00	11			24	31	X (end of collection period) X (restart of collec-tion)	
		04:45	12		11				
		05:00	13		12			X	Initiation of Lunch[b]
		05:15	14		13				
		05:30	15		14				
		05:45	16		15				
		06:00	17		16	25	32	X	
		06:30	18		17				
		07:00	19		18			X	
		07:30	20		19				
		08:00	21		20	26	33	X (end of collec-tion period) X (restart of collec-tion)	

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		09:00		22					X	
		10:00		23					X	
		11:00		24					X	
		11:45		25		21				
		12:00	X[a]	26		22	27	34	X (end of first collection period)	Initiation of dinner[b]
									X (restart of collec-tion)	
		12:15		27		23		35		
		12:30		28		24		36		
		12:45		29		25				
		13:00		30		26		37	X	
		13:30		31		27		38		
		14:00		32		28		39	X	
		14:30		33		29				
		15:00		34		30		40	X	
		16:00		35			28	41	X (end of collection period)	
									X (restart of collec-tion)	
		17:00							X	
		18:00		36				42	X	
		19:00							X	

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose	Plasma crea-tinine	Insulin, GLP-1, GIP	OD dosing	BID dosing		
		20:00	37			29	43	X (end of collection period)	
								X (restart of collec-tion)	
		21:00						X	
		22:00						X	
		23:00						X	
	6	24:00	38			30	44	X end of collec-tion period)[b]	

IP investigational product, PK pharmacokinetic, PD pharmacodynamic, GLP glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide

[a] Only in treatment A

[b] The IP will be administered together with the meal, which has to be completed within 30 minutes. Regarding the timing of events at mealtime, the following order of activities should be followed: blood sampling for plasma glucose, insulin, GLP-1 and GIP, with meal initiation occurring within 2 min of blood sampling. If the IP is to be administered the following sequence of events should occur: blood sampling, medication administration then meal initiation within 2 min of blood sampling.

Revised table:

Table 2 Study Plan for Each Treatment Period

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose, **Insulin,**	Plasma crea-tinine	**~~Insulin, GLP-1~~, GIP,**	OD dosing	BID dosing		
	–1	15:30								Check for inclusion and exclusion criteria, **safety lab,** drug screen including alcohol.
		Pre-dose					1	1		
	1	00:00	X							Breakfast[b]
		00:15					2	2		
		00:30					3	3		
		01:00					4	4		
		01:30					5	5		
		02:00					6	6		
		03:00					7	7		
		04:00					8	8		
		06:00					9	9		
		08:00					10	10		
		12:00	X[a]				11	11		Dinner[b]
		12:15						~~12~~		
		12:30						~~13~~		
		13:00						~~14~~		
		13:30						~~15~~		
		14:00						~~16~~		
		15:00						~~17~~		
		16:00					12	~~18~~		

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		18:00						~~19~~		
		20:00					13	~~20~~		
	2	24:00	X				14	~~21~~		Breakfast[b]
		12:00	X[a]							Dinner[b]
	3	Pre-dose					15	~~22~~ **12**		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
	4	Pre-dose			1 (back up sample)		16	~~23~~ **13**		
		00:00	X							Breakfast[b]
		12:00	X[a]							Dinner[b]
2/3	5	-00:15		1		~~1~~				
		Pre-dose			2		17	~~24~~ **14**	X (start of collec-tion)	Safety lab, vital signs
		00:00	X	2		~~2~~			X	Initiation of breakfast[b]
		00:15		3		~~3~~	18	~~25~~ **15**	X	
		00:30		~~4~~		~~4~~	19	~~26~~ **16**	X	
		00:45		5		~~5~~				
		01:00		6		~~6~~	20	~~27~~ **17**	X	
		01:30		7		~~7~~	21	~~28~~ **18**	X	
		02:00		8		~~8~~	22	~~29~~ **19**	X	
		02:30		9		~~9~~				
		03:00		10		~~10~~	23	~~30~~ **20**	X	

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		04:00	11			24	~~31~~ **21**	X (end of collec-tion period) X (restart of collec-tion)	
		04:45	12		~~11~~				
		05:00	13		~~12~~			X	Initiation of Lunch[b]
		05:15	14		~~13~~				
		05:30	15		~~14~~				
		05:45	16		~~15~~				
		06:00	17		~~16~~	25	~~32~~ **22**	X	
		06:30	18		~~17~~				
		07:00	19		~~18~~			X	
		07:30	20		~~19~~				
		08:00	21		~~20~~	26	~~33~~ **23**	X (end of collec-tion period) X (restart of collec-tion)	
		09:00	22					X	
		10:00	23					X	
		11:00	24					X	
		11:45	25		~~21~~ **1**				

Visit	Day	Nominal protocol time	IP	Blood for PD			Blood for PK		Urine for PD	Other
				Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		12:00	X[a]	26		~~22~~	27	~~34~~ **24**	X (end of first collection period)	Initiation of dinner[b]
									X (restart of collec-tion)	
		12:15		27		~~23~~		~~35~~ **25**		
		12:30		28		~~24~~		~~36~~ **26**		
		12:45		29		~~25~~				
		13:00		30		~~26~~		~~37~~ **27**	X	
		13:30		31		~~27~~ **2**		~~38~~ **28**		
		14:00		32		~~28~~		~~39~~ **29**	X	
		14:30		33		~~29~~				
		15:00		34		~~30~~		~~40~~ **30**	X	
		16:00		35			28	~~41~~ **31**	X (end of collection period)	
									X (restart of collec-tion)	
		17:00							X	
		18:00		36				~~42~~ **32**	X	
		19:00							X	

Visit	Day	Nominal IP protocol time	Blood for PD			Blood for PK		Urine for PD	Other
			Plasma glucose, Insulin,	Plasma crea-tinine	~~Insulin, GLP-1~~, GIP,	OD dosing	BID dosing		
		20:00	37			29	~~43~~ **33**	X (end of collection period)	
								X (restart of collec-tion)	
		21:00						X	
		22:00						X	
		23:00						X	
	6	24:00	38			30	~~44~~ **34**	X end of collec-tion period)[b]	

IP investigational product, PK pharmacokinetic, PD pharmacodynamic, GIP glucose-dependent insulinotropic polypeptide

[a] Only in treatment A

[b] The IP will be administered together with the meal, which has to be completed within 30 minutes. Regarding the timing of events at mealtime, the following order of activities should be followed: blood sampling for plasma glucose, insulin~~, GLP-1 and GIP~~ **(GIP only at dinner)** , with meal initiation occurring within 2 min of blood sampling. If the IP is to be administered the following sequence of events should occur: blood sampling, medication administration then meal initiation within 2 min of blood sampling.

Section of protocol affected:

Section 4.2, Exclusion criteria, Exclusion criteria No. 12

Previous text:

[…]

12. Positive urine drug screen

[…]

Revised text:

[…]

12. Positive urine drug screen **including cotinine**

[…]

Section of protocol affected:

Section5.1, Restrictions during the study, 2nd bullet

Previous text:

[…]

2. On Days 1 to 3 of Visits 2 and 3, respectively, water will be allowed ad lib except from 2 hours before until 2 hours after dose administration. On Day 4 (for practice) and 5 (related to the urine collection) of Visits 2 and 3, respectively, water consumption will be as follows:

   - 250 mL distilled water will be provided for dosing and 250 mL tap water 1h pre-dose.
   - 250 mL tap water 4 and 5 hours post-dose respectively
   - 250 mL tap water 8 and 9 hours post-dose respectively
   - 250 mL tap water 12 hours (in Treatment A, for administration, 250 mL distilled water will be served) and 13 hours post-dose respectively
   - 250 mL tap water 16 and 17 hours post-dose respectively
   - 250 mL tap water 20 and 21 hours post-dose respectively

[…]

Revised text:

[…]

2. On Days 1 to 3 of Visits 2 and 3, respectively, water will be allowed ad lib except from 2 hours before until 2 hours after dose administration.
   On Day 4 (for practice) and 5 (related to the urine collection) of Visits 2 and 3, respectively, **liquid** consumption **apart from beverages with meals,** will be as follows:

- 250 mL distilled water will be provided for dosing and 250 mL tap water***** **1 hour post-dose.**
- 250 mL tap water***** 4 and 5 hours post-dose respectively
- 250 mL tap water***** 8 and 9 hours post-dose respectively
- 250 mL tap water***** 12 hours (in Treatment A, for administration, 250 mL distilled water will be served) and 13 hours post-dose respectively
- 250 mL tap water***** 16 and 17 hours post-dose respectively
- 250 mL tap water***** 20 and 21 hours post-dose respectively

*** Instead of tap water subjects may also drink non-caffeinated, non-caloric beverages without artificial sweetener**

However they should receive the same beverage at the same time for the duration of the study, i.e. Day 1 to 5, in both periods.

[…]

Section of protocol affected:

Section 6.2, Data collection and enrolment

Previous text:

[…]

Each healthy volunteer will undergo enrolment during the 19 days prior to admission to confirm eligibility.

[…]

6. A blood sample for routine clinical chemistry, haematology, and screen for hepatitis B surface antigen, antibodies to hepatitis C virus and antibodies to HIV

7. A urine sample for routine urinalysis, drugs of abuse screen, and pregnancy test (for females only)

[…]

Revised text:

[…]

Each healthy volunteer will undergo enrolment during the 19 days prior to admission to confirm eligibility**, the healthy volunteers being in fasted state for at least 8 hours. Only non-caloric beverages are allowed for this time.**

[…]

6. A blood sample for routine clinical chemistry **(including pregnancy test for females)**, haematology, and screen for hepatitis B surface antigen, antibodies to hepatitis C virus and antibodies to HIV

7. A urine sample for routine urinalysis, drugs of abuse screen, **and cotinine test**~~, and pregnancy test (for females only)~~

[…]

Section of protocol affected:

Section 6.2.1, Follow-up procedures

Previous text:

[…]

At the follow-up examination, a complete physical examination, safety laboratory assessment in blood and urine and questioning of AEs will be performed 5 to 10 days after the end of the 2nd treatment period.

[…]

Revised text:

[…]

At the follow-up examination, a complete physical examination, safety laboratory assessment in blood and urine and questioning of AEs will be performed 5 to 10 days after the end of the 2nd treatment period**. For this follow-up visit, the subjects should be in fasted state for at least 8 hours. Only non-caloric beverages are allowed.**

[…]

Section of protocol affected:

Section 6.3.5, Laboratory safety assessment

Previous text:

[…]

For evaluation of clinical chemistry, blood (7.5 mL) will be collected into silicone-free polypropylene serum gel tubes (Monovette 01.1602, Sarstedt).

[…]

Additionally, at enrolment all healthy volunteers will be tested for HIV, hepatitis B surface antigen and antibodies to hepatitis C. Urine will be tested for the following drugs of abuse at enrolment and admission: amphetamines, barbiturates, benzodiazepam, cocaine, methadone, methamphetamine, morphine, phencyclidine, tetrahydrocannabinol, opiates. Tricyclic antidepressant will be analysed in serum. At Visit 2 and 3 the healthy volunteers will be screened for alcohol. A serum pregnancy test will be performed at Visit 1 (enrolment) and on Visit 2 and 3. If a healthy volunteer tests positive to any of these enrolment tests he/she will be excluded from the study.

[…]

Revised text:

[…]

For evaluation of clinical chemistry, blood (**2.6** mL) will be collected into silicone-free polypropylene serum gel tubes (Monovette 01.1602, Sarstedt).

[…]

Additionally, at enrolment all healthy volunteers will be tested for HIV, hepatitis B surface antigen and antibodies to hepatitis C. Urine will be tested for the following drugs of abuse at enrolment and admission: amphetamines, barbiturates, benzodiazepam, cocaine, methadone, methamphetamine, morphine, phencyclidine, tetrahydrocannabinol, opiates. Tricyclic antidepressant will be analysed in serum. At Visit 2 and 3 the healthy volunteers will be screened for alcohol. **A cotinine test will be performed together with the drug screen.** A serum pregnancy test will be performed at Visit 1 (enrolment) and on Visit 2 and 3. If a healthy volunteer tests positive to any of these enrolment tests he/she will be excluded from the study.

[…]

Section of protocol affected:

Section 7.1, Volume of blood

Previous text:

Table 3 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry [a]	2.6	6	15.6
	Haematology	2.7	6	16.2
	Tricyclic antidepressants	1.1	3	3.3
	Serology	2.6	1	2.6
Pharmacokinetic		2.0	74	148.0
Pharmacodynamic				
Creatinine		1.1	4	4.4
Glucose		1.2	76	91.2
GLP-1		3.0	60	180.0
Insulin and GIP		1.1	60	66.0
Total				**527.3**

GLP Glucagon-like peptide, GIP glucose-dependent insulinotropic polypeptide
a Includes pregnancy testing

Revised text:

Table 3 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry [a]	2.6	6	15.6
	Haematology	2.7	6	16.2
	Tricyclic antidepressants	1.1	3	3.3
	Serology	2.6	1	2.6
Pharmacokinetic		2.0	~~74~~ **64**	~~148.0~~ **128.0**
Pharmacodynamic				
Creatinine		1.1	4	4.4

Glucose	1.2	76	91.2
~~GLP-1~~**GIP**	3.0	**4**	~~180.0~~ **12.0**
Insulin ~~and GIP~~	1.1	~~60~~ **76**	~~66.0~~ **83.6**
Total			~~**527.3**~~ **356.9**

~~GLP Glucagon-like peptide,~~ GIP glucose-dependent insulinotropic polypeptide
[a] Includes pregnancy testing

Reasons for Amendment:

- Error in Amendment No. 1 (and thus changes in Table 2 in relation to the original protocol are given) in timing and numbering of PK sampling time points on Day 5 of BID dosing in Table 2, Study Plan for Each Treatment Period.
- Inconsistencies within the original protocol
- Adding the cotinine test.
- Definition of allowed drinks, clarifying of drinking schedule in relation to beverages to meal
- Adding the requirement the subjects in fasted state prior safety lab at enrolment, follow-up and Day –1 of both periods.

Persons who initiated the Amendment:

Astra Zeneca Study Delivery Team Leader; study physicians

Clinical Study Protocol Amendment No 2
Appendix A

Drug Substance	Dapagliflozin
Study Code	D1691C00004
Edition Number	1
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of amendment.

AstraZeneca Research and Development site representative

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of this amendment.

PAREXEL
Projectmanagement

ASTRAZENECA SIGNATURE(S)

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of this amendment.

AstraZeneca Research and Development site representative

SIGNATURE OF PRINCIPAL INVESTIGATOR

An open-label, randomized, two-period, crossover study to assess the effect of dapagliflozin on percent inhibition of glucose re-absorption when administered once a day (10 mg QD) versus twice a day (5 mg BID) in healthy male and female volunteers

This Clinical Study Protocol and all Amendments to the CSP have been subjected to an internal AstraZeneca peer review.

I agree to the terms of this amendment.

Signature: