Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.4 Summary of pharmacokinetic study

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Terms and abbreviations used in this section

Term / Abbreviation	Not abbreviated expressions or definitions
ALC-0159	PEG lipid added to this drug
ALC-0315	Amino lipid added to this drug
[3 H]-CHE	Radiolabeled [cholesteryl-1,2- 3 H (N)] - cholesteryl hexadecyl Ether: radiolabeled [cholesteryl Lil-1, 2-3 H (N)] Hexadecyl ether
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine: 1,2-distearoyl-sn-glycero-3-phosphocholine Rin
GLP	Good Laboratory Practice: Criteria for conducting non-clinical studies on drug safety
LNP	Lipid-nanoparticle: Lipid nanoparticle
modRNA	Nucleoside-modified mRNA: Modified nucleoside mRNA
mRNA	Messenger RNA: Messenger RNA
m / z	m / z (m over z): Obtained by dividing the mass of an ion by the unified atomic mass unit (= Dalton). The dimensionless quantity obtained by dividing the obtained dimensionless quantity by the absolute value of the number of charges of the ion.
PEG	Polyethylene glycol: Polyethylene glycol
PK	Pharmacokinetics: Pharmacokinetics
RNA	Ribonucleic acid: Ribonucleic acid
S9	Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g: liver homogenate Supernatant fraction centrifuged at 9000 g
WHO	World Health Organization: World Health Organization

Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.4 Summary of pharmacokinetic study

1. Summary

BNT162b2 (BioNTech code number: BNT162, Pfizer code number: PF-07302048) is a severe acute call.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike glycoprotein (S protein) full length

It is a modified nucleoside mRNA (modRNA) that encodes against SARS-CoV-2 infection.
Development is underway as the essence of the mRNA vaccine. When formulating BNT162b2, there are two

Functional lipids ALC-0315 (aminolipid) and ALC-0159 (PEG lipid) and two structural lipids

By mixing with DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) and cholesterol

Lipid nanoparticles (LNP) that encapsulate BNT162b2 are formed (hereinafter, "BNT162b2-encapsulated LNP").
ALC-0315 and ALC-0315 contained in LNP to evaluate the nonclinical pharmacokinetics of BNT162b2 encapsulated LNP
In vivo and in vitro studies assessing absorption (PK), metabolism and excretion of ALC-0159 and BNT162b2
Biodistribution studies using luciferase or radiolabeled lipids as an alternative reporter for
Was carried out.

Based on the fact that the development of vaccines aimed at preventing infectious diseases does not require evaluation of systemic exposure.
(WHO, 2005; Non-clinical study guidelines for infectious disease preventive vaccines) 1 , 2 , BNT162b2 Encapsulated LNP muscle
No internal PK study was performed. In addition, two other types of lipids (choleste) contained in this drug
Rolls and DSPCs) are naturally occurring lipids that are thought to be metabolized and excreted in the same way as endogenous lipids.
available. In addition, BNT162b2 is degraded by ribonucleases in the cells that have taken it up, resulting in nucleic acid charges.
Apologize, the S protein from BNT162b2 is expected to undergo proteolysis. From the above,
It was considered unnecessary to evaluate the metabolism and excretion of these components again.

LNP (Luciferase) encapsulating RNA encoding luciferase as an alternative reporter for BNT162b2
Lase RNA is encapsulated in an LNP having the same lipid composition as the BNT162b2-encapsulated LNP:
In a PK study in which ZeRNA-encapsulated LNP ") was intravenously administered to Wistar Han rats, plasma, urine, feces and
Liver samples were collected over time and the concentrations of ALC-0315 and ALC-0159 in each sample were measured. The conclusion
As a result, ALC-0315 and ALC-0159 were shown to be rapidly distributed from the blood to the liver. Also,
About 1% and about 50% of the doses of ALC-0315 and ALC-0159 are excreted in feces as unchanged drug, respectively.
All of them were below the detection limit in urine.

In the biodistribution test, luciferase RNA-encapsulated LNP was intramuscularly administered to BALB / c mice. That
As a result, the expression of luciferase was observed at the administration site, and the expression level was lower than that in the liver.
Was also recognized. Expression at the administration site of luciferase was observed from 6 hours after administration, and 9 days after administration.
Disappeared. Expression in the liver was also observed 6 hours after administration and disappeared by 48 hours after administration. Also,
Intramuscular administration of radiolabeled LNP containing luciferase RNA to rats to quantify biodistribution
Upon evaluation, the radioactivity concentration was the highest at the administration site. Liver is highest except at the administration site
It was good (up to 18% of the dose).

Metabolism of ALC-0315 and ALC-0159 in CD-1 / ICR mice, Wistar Han or Sprague Dawley rats,
In vitro using cynomolgus monkey or human blood, liver microsomes, liver S9 fraction and hepatocytes
evaluated. In addition, plasma, urine, feces and liver samples collected in the above rat intravenous administration PK test were used.
We also examined in vivo metabolism. From these in vitro and in vivo studies, ALC-0315 and
ALC-0159 was added to ester and amide bonds in all animal species tested.
The solution showed that it was slowly metabolized.

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From the above nonclinical pharmacokinetic evaluation, it was shown that LNP that reached the circulating blood is distributed in the liver.
In addition, metabolism and fecal excretion may be involved in the disappearance of ALC-0315 and ALC-0159, respectively.
It was suggested.

2. Analytical method

Report number: PF-07302048_06 _072424

Intravenous administration of rats without GLP PK test (M2.6.4.3), ALC-0315, which is a constituent lipid of LNP, and
ALC-0159 We have developed an LC / MS method with appropriate performance for quantifying the concentration. That is, 20 µL
Plasma, liver homogenate (homogenates are prepared using sections collected from three parts of the liver, and they are used.
Dilute with a blank matrix as appropriate), urine and fecal homogenate (as appropriate, bran)
Dilute with kumatrix) Divide each sample with acetonitrile containing an internal standard substance (PEG-2000)
After protein, it was centrifuged and the supernatant was subjected to LC-MS / MS measurement.

3. Absorption

Report number: PF-07302048_06 _072424 , Summary table: 2.6.5.3

Male luciferase RNA-encapsulated LNP to study the pharmacokinetics of ALC-0315 and ALC-0159
A single intravenous dose of 1 mg RNA / kg was administered to Wistar Han rats over time (pre-dose, post-dose 0.1, 0.25,

Sparse plasma and liver 0.5, 1, 3, 6 and 24 hours and 2, 4, 8 and 14 days after dosing)

Sampling was performed (3 animals / time point). ALC-0315 and ALC-0159 in plasma and liver

The concentration was measured and the PK parameters were calculated (Table 1). ALC-0315 and ALC-0159 in the blood are thrown

It was promptly distributed to the liver by 24 hours after administration. In addition, the plasma concentration 24 hours after administration is the highest in plasma.

It was less than 1% of the concentration (Figure 1). The apparent terminal phase elimination half-life (t½) is in plasma and liver

At the same level, ALC-0315 took 6 to 8 days and ALC-0159 took 2 to 3 days. From the results of this test, the liver is in the blood

It was suggested that it is one of the major organizations that take up ALC-0315 and ALC-0159 from.

Results of examination of urinary and fecal concentrations of ALC-0315 and ALC-0159 conducted in this study

Is M2.6.4.Described in Section 6 .

Table 1 Intravenous injection of luciferase **RNA-** encapsulated **LNP** into **Wistar Han** rats at a dose of **1 mg RNA / kg**

Pharmacokinetics of **ALC-0315** and **ALC-0159** when given

Analytical material	Dosage of analyte **(mg / kg)**	Gender / **N**	**t½ (h)**	**AUC inf (μg • h / mL)**	**AUC last (Mg • h / mL)**	To the liver Distribution ratio **(%) a**
ALC-0315	15.3	Male / 3 b	139	1030	1020	60
ALC-0159	1.96	Male / 3 b	72.7	99.2	98.6	20

Calculated as [maximum liver distribution (μg)] / [dose (μg)].

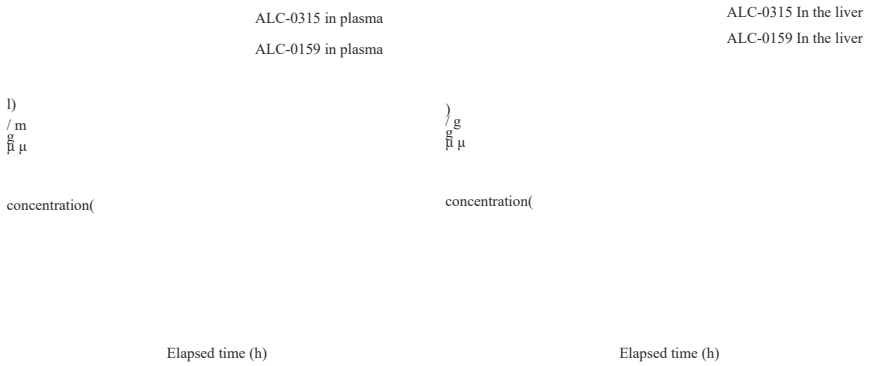
b. 3 animals at each time point. Sparse sampling.

Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.4 Summary of pharmacokinetic study

Figure 1 Intravenous injection of luciferase **RNA-** encapsulated **LNP** into **Wistar Han** rats at a dose of **1 mg RNA / kg**

Plasma and liver concentrations of **ALC-0315** and **ALC-0159** when given



4. Distribution

Report number: R- -0072 , 185350, Summary table: 2.6.5.5A, 2.6.5.5B

Female BALB / c mice (3 mice) were administered luciferase RNA-encapsulated LNP to emit luciferase luminescence.

The biodistribution of BNT162b2 was examined as an alternative marker. That is, luciferase RNA inclusion
LNP was intramuscularly administered to the left and right hind limbs of mice at a dose of 1 μg RNA (2 μg RNA in total). After that, Le

Intraperitoneal administration of luciferin, a luminescent substrate, 5 minutes before detection of cipherase luminescence, isoflurane hemp

Intoxication, in vivo luminescence 6 and 24 hours after administration using Xenogen IVIS Spectrum and 2,

By measuring on days 3, 6 and 9, the expression of luciferase protein in the same individual was estimated over time.

Evaluated the transfer. As a result, expression of luciferase at the administration site was observed from 6 hours after administration, and it was administered.

It disappeared 9 days after giving. Expression in the liver was also observed 6 hours after administration and disappeared by 48 hours after administration. It was. Regarding the distribution to the liver, a part of locally administered luciferase RNA-encapsulated LNP reaches the circulating blood, and the liver

It was thought to indicate that it was taken up by the viscera. M2.6.4.Lucife in rats, as detailed in Section 3.

When intravenously administered with Lase RNA-encapsulated LNP, the liver is the major ALC-0315 and ALC-0159.

It has been suggested that it is a distributed organ, which is the finding of the results of this study, which was intramuscularly administered to mice.

It was a match. Toxicity findings indicating liver damage were observed in the rat repeated-dose toxicity test.

Not available (M2.6.6.3).

Figure 2 In vivo luminescence in **BALB / c** mice intramuscularly administered with luciferase **RNA-** encapsulated **LNP**

Male and female Wistar Han rats labeled with [3 H] -cholesteryl hexadecyl ether ([3 H] -CHE) LNP

Luciferase RNA-encapsulated LNP using luciferase RNA was intramuscularly administered at a dose of 50 μg RNA, and 15 minutes after administration.

Blood, plasma and tissue were collected from 3 males and 3 females at 1, 2, 4, 8, 24 and 48 hours each.

The biodistribution of LNP is evaluated by measuring the radioactivity concentration by the liquid scintillation counting method.

Worth it. In both males and females, the radioactivity concentration was highest at the administration site at all measurement points.

The radioactivity concentration in plasma was the highest 1 to 4 hours after administration. Also, mainly the liver, spleen, adrenal glands and

Distribution to the ovaries was observed, and the highest radioactivity concentration in these tissues was 8 to 48 after administration.

It was time. The total radioactivity recovery rate for doses other than the administration site is the highest in the liver (up to 18%).

Significantly lower in the spleen (1.0% or less), adrenal gland (0.11% or less) and ovary (0.095% or less) compared to the liver

won. In addition, the average concentration of radioactivity and the tissue distribution pattern were generally similar between males and females.

The in vivo expression distribution of the antigen encoded by BNT162b2 is considered to depend on the LNP distribution. For this test

Is the lipid composition of the luciferase RNA-encapsulated LNP the same as that of the submitted preparation of BNT162b2?

Therefore, the results of this test are considered to indicate the distribution of BNT162b2-encapsulated LNP.

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2.6.4 Summary of pharmacokinetic study

5. Metabolism

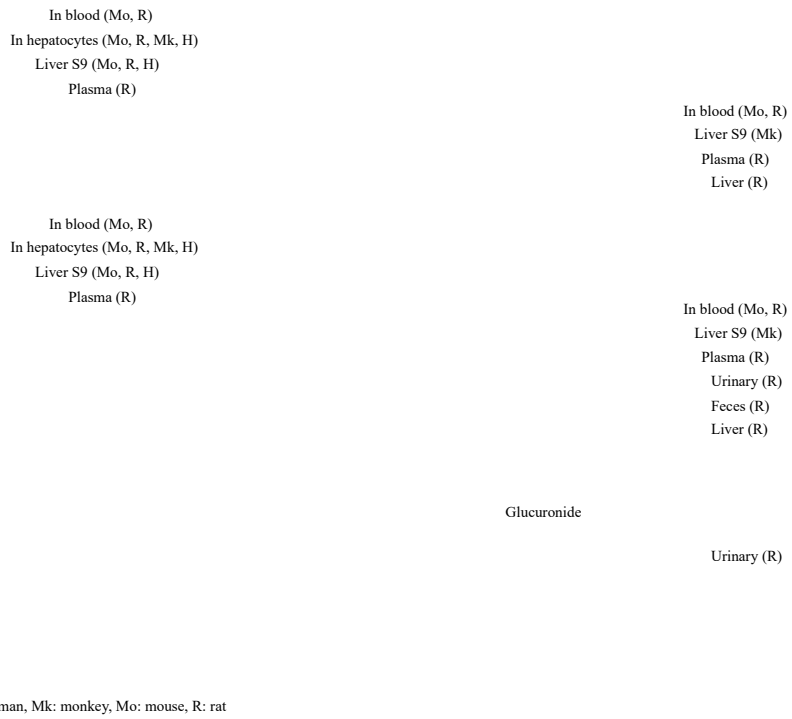
Report number: 01049-008 , 01049-009 , 01049- 010 , 01049- 020 , 01049-021 , 01049-022 , PF-07302048_05 _043725 , Summary table: 2.6.5.10A , 2.6.5.10B , 2.6.5.10C , 2.6.5.10D

CD-1 / ICR mouse, Wistar Han or Sprague Dawley rat, cynomolgus monkey and human liver mi

In vitro metabolic stabilization of ALC-0315 and ALC-0159 using crosome, liver S9 fraction and hepatocytes
Gender was evaluated. Liver microsomes or liver S9 fractions of each animal species with ALC-0315 or ALC-0159 (120)
Incubate) or add to hepatocytes (240 minutes incubation) and incubate
The proportion of unchanged drug after vation was measured. As a result, which of ALC-0315 and ALC-0159
It was also metabolically stable in animal species and test systems, with the final proportion of unchanged drug being over 82%.

Furthermore, the metabolic pathways of ALC-0315 and ALC-0159 were evaluated in vitro and in vivo. this
In these studies, CD-1 mouse, Wistar Han rat, cynomolgus monkey and human blood, liver S9 fractions
And hepatocytes were used to evaluate metabolism in vitro. In addition, plasma, urine, and feces collected in the rat PK test.
And liver samples were used to evaluate metabolism in vivo (M2.6.4.Item 3). From the test results, ALC-0315
And ALC-0159 are both slowly metabolized, with hydrolysis of ester and amide bonds, respectively.
It was revealed that it was metabolized by.Hydrolytic metabolism shown in Figures 3 and 4
Was found in all the animal species evaluated.

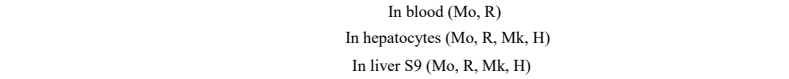
Figure 3 Estimated in vivo metabolic pathway of **ALC-0315** in various animal species



H: human, Mk: monkey, Mo: mouse, R: rat

ALC-0315 is metabolized by undergoing ester hydrolysis twice in a row. These two hydrolysiss
First produces a monoester metabolite (*m / z* 528) and then a double deesterified metabolite (*m / z* 290).
Will be done. This double deesterified metabolite is further metabolized to the glucuronide conjugate (*m / z* 466).
However, this glucuronic acid conjugate was detected only in urine in the rat PK test. Also, two hydrolysiss
It was also confirmed that all of the acidic products of were 6-hexyldecanoic acid (*m / z* 255).

Figure 4 Estimated in vivo metabolic pathway of **ALC-0159** in various animal species



N, N-ditetradecylamine
m / z 410

H: human, Mk: monkey, Mo: mouse, R: rat

In ALC-0159, *N* , *N* -ditetradecylamine (*m / z* 410) is produced by hydrolysis of the amide bond.

The pathway was the main metabolic pathway. This metabolite is found in mouse and rat blood as well as in mouse and rat.

It was detected in monkey and human hepatocytes and liver S9 fractions. Metabolites of ALC-0159 from in vivo samples

Not confirmed.

6. Excretion

PK study of intravenous luciferase RNA-encapsulated LNP in rats at a dose of 1 mg RNA / kg

(M2.6.4.The concentrations of ALC-0315 and ALC-0159 in urine and feces collected over time were measured in (3).

Neither ALC-0315 nor ALC-0159 unchanged form was detected in urine. On the other hand, in the feces

Unaltered forms of ALC-0315 and ALC-0159 were detected, at a rate of approximately 1% per dose, respectively.

It was about 50%. Also,Figure 3 As shown in, a metabolite of ALC-0315 was detected in urine.

7. Pharmacokinetic drug interactions

No pharmacokinetic drug interaction studies have been conducted with this vaccine.

8. Other pharmacokinetic studies

No other pharmacokinetic studies of this vaccine have been conducted.

9. Discussion and conclusion

Plasma and liver ALC-0315 levels were highest in rat PK studies by 2 weeks post-dose

It is reduced to about 1/7000 and about 1/4, respectively, and the ALC-0159 concentration is about 1/8000, respectively.

And reduced to about 1/250. t½ is comparable in plasma and liver, ALC-0315 is 6-8 days,

ALC-0159 was 2-3 days. The plasma t½ value is that each lipid is distributed in the tissue as LNP.

After that, it is considered to indicate that it was redistributed in plasma during the disappearance process.

Little unchanged form of ALC-0315 was detected in either urine or feces, but in the rat PK study

Monoester metabolites, double deesterified metabolites and 6-hexy from fecal and plasma samples collected in

Ludecanoic acid was detected in urine, and a glucuronic acid conjugate, a double deesterified metabolite, was detected in urine. This metabolism

The process is thought to be the major disappearance mechanism of ALC-0315, but quantitative data have been obtained to test this hypothesis.

Absent. On the other hand, about 50% of the dose of ALC-0159 was excreted in feces as unchanged drug. In vitro metabolism experiment

In, it was slowly metabolized by hydrolysis of the amide bond.

Since the in vivo expression distribution of the antigen encoded by BNT162b2 is considered to depend on the LNP distribution,

Intramuscularly administered luciferase RNA-encapsulated LNP to BALB / c mice as an alternative reporter protein

The biodistribution was examined. As a result, expression of luciferase was observed at the administration site, and more than that.

Although the expression level was low, it was also observed in the liver. Expression at the administration site of luciferase is post-administration

It was observed from 6 hours and disappeared 9 days after administration. Expression in the liver was observed from 6 hours after administration, and it was administered.

It disappeared by 48 hours after giving. Locally administered luciferase RNA-encapsulated LNP circulates in the liver

It was considered to indicate that it reached the ring blood and was taken up by the liver. Also, Luciferer on rats

When the radioactivity-labeled body of ZeRNA-encapsulated LNP was intramuscularly administered, the radioactivity concentration was the highest at the administration site.

Indicated. Other than the site of administration, it was highest in the liver, followed by the spleen, adrenal glands and ovaries.

Total radioactivity recovery for doses in these tissues was significantly lower than in the liver. This result is

This was consistent with the expression of luciferase in the liver in the mouse biodistribution test. In addition, it should be noted.

No toxic findings indicating liver damage were found in the rat repeated-dose toxicity test (M2.6.6.3).

From the above nonclinical pharmacokinetic evaluation, it was shown that LNP that reached the circulating blood is distributed in the liver.

In addition, metabolism and fecal excretion may be involved in the disappearance of ALC-0315 and ALC-0159, respectively.

It was suggested.

10. Chart

Charts are shown in the text and in the summary table.

References

1 World Health Organization. Annex 1. Guidelines on the nonclinical evaluation of vaccines. In: WHO Technical Report Series No. 927, Geneva, Switzerland. World Health Organization; 2005: 31-63.

2 Non-clinical study guidelines for infectious disease preventive vaccines (No. 0527 from Yaksik Examination) No. 1, May 27, 2010)

Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.5 Pharmacokinetic study summary table

2.6.5.1. PHARMACOKINETICS OVERVIEW

Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number	
Single Dose Pharmacokinetics						
Single Dose Pharmacokinetics and Excretion in Urine and Feces of ALC-0159 and ALC-0315	Rat (Wistar Han)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IV bolus	Pfizer Inc **a**	PF-07302048_06	_072424
Distribution						
In Vivo Distribution	Mice BALB / c	modRNA encoding luciferase formulated in LNP comparable to BNT162b2	IM Injection	b b	R- -0072	
In Vivo Distribution	Rat (Wistar Han)	modRNA encoding luciferase formulated in LNP comparable to BNT162b2 with trace amounts of [3 H] -CHE as non-diffusible label	IM Injection	c	185350	
Metabolism						
In Vitro and In Vivo Metabolism						
In Vitro Metabolic Stability of ALC-0315 in Liver Microsomes	Mouse (CD-1 / ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human liver microsomes	ALC-0315	In vitro	d	01049- 008	
In Vitro Metabolic Stability	Mouse (CD-1 / ICR), rat	ALC-0315	In vitro		01049-009	

of ALC-0315 in Liver S9	(Sprague Dawley), monkey (Cynomolgus), and human S9 liver fractions			d	

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2.6.5.1. PHARMACOKINETICS OVERVIEW — Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number	
In Vitro Metabolic Stability of ALC-0315 in Hepatocytes	Mouse (CD-1 / ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human hepatocytes	ALC-0315	In vitro	d	01049- 010	
In Vitro Metabolic Stability of ALC-0159 in Liver Microsomes	Mouse (CD-1 / ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human liver microsomes	ALC-0159	In vitro	d	01049- 020	
In Vitro Metabolic Stability of ALC-0159 in Liver S9	Mouse (CD-1 / ICR), rat (Sprague Dawley), monkey (Cynomolgus), and human S9 fractions	ALC-0159	In vitro	d	01049-021	
In Vitro Metabolic Stability of ALC-0159 in Hepatocytes	Mouse (CD-1 / ICR), rat (Sprague Dawley and Wistar Han), monkey (Cynomolgus), and human hepatocytes	ALC-0159	In vitro	d	01049- 022	
Biotransformation of ALC-0159 and ALC-0315 In Vitro and In Vivo in Rats	In vitro: CD-1 mouse, Wistar Han rat, cynomolgus monkey, and human blood, liver S9 fractions and hepatocytes In vivo: male Wistar Han rats	ALC-0315 and ALC-0159	In vitro or IV (in vivo in rats)	Pfizer Inc e	PF-07302048_05	_043725

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2.6.5.1. PHARMACOKINETICS OVERVIEW — Test Article: BNT162b2

Type of Study	Test System	Test item	Method of Administration	Testing Facility	Report Number

ALC-0159 = 2-[(polyethylene glycol)-2000]-N, N-ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an preferably in the LNP formulation used in BNT162b2; ALC-0315 = (4-hydroxybutyl) azanediyl) bis (hexane-6,1-diyl) bis (2-hexyldecanoate), a proprietary aminolipid included as an preferably in the LNP formulation used in BNT162b2; IM = Intramuscular; IV = Intravenous; LNP = lipid nanoparticles; S9 = Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g.

a. La Jolla, California.
b. , Germany.
c. , UK.
d. , China.
e. Groton, Connecticut.

Masking location: Adjusting

SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048)
2.6.5 Pharmacokinetic study summary table

2.6.5.3. PHARMACOKINETICS: PHARMACOKINETICS AFTER A SINGLE DOSE

Test Article: modRNA encoding luciferase in LNP
Report Number: PF-07302048_06 _072424

Species (Strain)	Rat (Wistar Han)	
Sex / Number of Animals	Male / 3 animals per timepoint a	
Feeding Condition	Fasted	
Method of Administration	IV	
Dose modRNA (mg / kg)	1	
Dose ALC-0159 (mg / kg)	1.96	
Dose ALC-0315 (mg / kg)	15.3	
Sample Matrix	Plasma, liver, urine and feces	
Sampling Time Points (h post dose):	Predose, 0.1, 0.25, 0.5, 1, 3, 6, 24, 48, 96, 192, 336	
Analyte	ALC-0315	ALC-0159
PK Parameters:	Mean b	Mean b
AUC inf (µg • h / mL) c	1030	99.2
AUC last (µg • h / mL)	1020	98.6
Initial t ½ (h) d	1.62	1.74
Terminal elimination t ½ (h) e	139	72.7
Estimated fraction of dose distributed to liver (%) f	59.5	20.3
Dose in Urine (%)	NC g	NC g
Dose in Feces (%) h	1.05	47.2

ALC-0159 = 2-[(polyethylene glycol)-2000]-N, N-ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an preferably in the LNP formulation used in BNT162b2; ALC-0315 = (4-hydroxybutyl) azanediyl) bis (hexane-6,1-diyl) bis (2-hexyldecanoate), a proprietary aminolipid included as an preferably in the LNP formulation used in BNT162b2; AUC inf = Area under the plasma drug concentration-time curve from 0 to infinite time; AUC last = Area under the plasma drug concentration-time curve from 0 to the last quantifiable time point; BLQ = Below the limit of quantitation; LNP = Lipid nanoparticle; modRNA = Nucleoside modified messenger RNA; PK = Pharmacokinetics; t ½ = Half-life.

a. Non-serial sampling, 36 animals total.
b. Only mean PK parameters are reported due to non-serial sampling.
c. Calculated using the terminal log-linear phase (determined using 48, 96, 192, and 336 h for regression calculation).
d. ln (2) / initial elimination rate constant (determined using 1, 3, and 6 h for regression calculation).
e. ln (2) / terminal elimination rate constant (determined using 48, 96, 192, and 336 h for regression calculation).
f. Calculated as follows: highest mean amount in the liver (µg) / total mean dose (µg) of ALC-0315 or ALC-0159.
g. Not calculated due to BLQ data.
h. Fecal excretion, calculated as: (mean µg of analyte in feces / mean µg of analyte administered) × 100

2.6.5.5A. PHARMACOKINETICS: ORGAN DISTRIBUTION

Test Article: modRNA encoding luciferase in LNP
Report Number: R- -0072

Species (Strain):	Mice (BALB / c)
Sex / Number of Animals:	Female / 3 per group
Feeding Condition:	Fed ad libitum
Vehicle / Formulation:	Phosphate-buffered saline
Method of Administration:	Intramuscular injection
Dose (mg / kg):	1 µg / hidden leg in gastrocnemius muscle (2 µg total)
Number of Doses:	1
Detection:	Bioluminescence measurement
Sampling Time (hour):	6, 24, 48, 72 hours; 6 and 9 days post-injection

Time point	Total Mean Bioluminescence signal (photons / second)		Mean Bioluminescence signal in the liver (photons / second)
	Buffer control	modRNA Luciferase in LNP	modRNA Luciferase in LNP
6 hours	1.28 × 10 5	1.26 × 10 9	4.94 × 10 7
24 hours	2.28 × 10 5	7.31 × 10 8	2.4 × 10 6
48 hours	1.40 × 10 5	2.10 × 10 8	Below detection a
72 hours	1.33 × 10 5	7.87 × 10 7	Below detection a
6 days	1.62 × 10 5	2.92 × 10 6	Below detection a
9 days	7.66 × 10 4	5.09 × 10 5	Below detection a

LNP = Lipid nanoparticle; modRNA = Nucleoside modified messenger RNA.
a. At or below the background level of the buffer control.

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [3 H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

Species (Strain):	Rat (Wistar Han)
Sex / Number of Animals:	Male and female / 3 animals / sex / timepoint (21 animals / sex total for the 50 µg dose)
Feeding Condition:	Fed ad libitum
Method of Administration:	Intramuscular injection
Dose:	50 µg [3 H] -08-A01-C0 (lot # NC-0552-1)
Number of Doses:	1
Detection:	Radioactivity quantitation using liquid scintillation counting
Sampling Time (hour):	0.25, 1, 2, 4, 8, 24, and 48 hours post-injection

Sample	Mean total lipid concentration (µg lipid equivalent / g (or mL)) (males and females combined)							% of administered dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
Adipose tissue	0.057	0.100	0.126	0.128	0.093	0.084	0.181	-	-	-	-	-	-	-
Adrenal glands	0.271	1.48	2.72	2.89	6.80	13.8	18.2	0.001	0.007	0.010	0.015	0.035	0.066	0.106
Bladder	0.041	0.130	0.146	0.167	0.148	0.247	0.365	0.000	0.001	0.001	0.001	0.001	0.002	0.002
Bone (femur)	0.091	0.195	0.266	0.276	0.340	0.342	0.687	-	-	-	-	-	-	-
Bone marrow (femur)	0.479	0.960	1.24	1.24	1.84	2.49	3.77	-	-	-	-	-	-	-
Brain	0.045	0.100	0.138	0.115	0.073	0.069	0.068	0.007	0.013	0.020	0.016	0.011	0.010	0.009
Eyes	0.010	0.035	0.052	0.067	0.059	0.091	0.112	0.000	0.001	0.001	0.002	0.002	0.002	0.003
Heart	0.282	1.03	1.40	0.987	0.790	0.451	0.546	0.018	0.056	0.084	0.060	0.042	0.027	0.030
Injection site	128	394	311	338	213	195	165	19.9	52.6	31.6	28.4	21.9	29.1	24.6
Kidneys	0.391	1.16	2.05	0.924	0.590	0.426	0.425	0.050	0.124	0.211	0.109	0.075	0.054	0.057
Large intestine	0.013	0.048	0.093	0.287	0.649	1.10	1.34	0.008	0.025	0.065	0.192	0.405	0.692	0.762
Liver	0.737	4.63	11.0	16.5	26.5	19.2	24.3	0.602	2.87	7.33	11.9	18.1	15.4	16.2
Lung	0.492	1.21	1.83	1.50	1.15	1.04	1.09	0.052	0.101	0.178	0.169	0.122	0.101	0.101

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [3 H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

Sample	Total Lipid concentration (µg lipid equivalent / g [or mL]) (males and females combined)							% of Administered Dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
Lymph node (mandibular)	0.064	0.189	0.290	0.408	0.534	0.554	0.727	-	-	-	-	-	-	-
Lymph node (mesenteric)	0.050	0.146	0.530	0.489	0.689	0.985	1.37	-	-	-	-	-	-	-
Muscle	0.021	0.061	0.084	0.103	0.096	0.095	0.192	-	-	-	-	-	-	-
Ovaries (females)	0.104	1.34	1.64	2.34	3.09	5.24	12.3	0.001	0.009	0.008	0.016	0.025	0.037	0.095
Pancreas	0.081	0.207	0.414	0.380	0.294	0.358	0.599	0.003	0.007	0.014	0.015	0.015	0.011	0.019
Pituitary gland	0.339	0.645	0.868	0.854	0.405	0.478	0.694	0.000	0.001	0.001	0.001	0.000	0.000	0.001
Prostate (males)	0.061	0.091	0.128	0.157	0.150	0.183	0.170	0.001	0.001	0.002	0.003	0.003	0.004	0.003
Salivary glands	0.084	0.193	0.255	0.220	0.135	0.170	0.264	0.003	0.007	0.008	0.008	0.005	0.006	0.009
Skin	0.013	0.208	0.159	0.145	0.119	0.157	0.253	-	-	-	-	-	-	-
Small intestine	0.030	0.221	0.476	0.879	1.28	1.30	1.47	0.024	0.130	0.319	0.543	0.776	0.906	0.835
Spinal cord	0.043	0.097	0.169	0.250	0.106	0.085	0.112	0.001	0.002	0.002	0.003	0.001	0.001	0.001
Spleen	0.334	2.47	7.73	10.3	22.1	20.1	23.4	0.013	0.093	0.325	0.385	0.982	0.821	1.03
Stomach	0.017	0.065	0.115	0.144	0.268	0.152	0.215	0.006	0.019	0.034	0.030	0.040	0.037	0.039
Testes (males)	0.031	0.042	0.079	0.129	0.146	0.304	0.320	0.007	0.010	0.017	0.030	0.034	0.074	0.074
Thymus	0.088	0.243	0.340	0.335	0.196	0.207	0.331	0.004	0.007	0.010	0.012	0.008	0.007	0.008
Thyroid	0.155	0.536	0.842	0.851	0.544	0.578	1.00	0.000	0.001	0.001	0.001	0.001	0.001	0.001
Uterus (females)	0.043	0.203	0.305	0.140	0.287	0.289	0.456	0.002	0.011	0.015	0.008	0.016	0.018	0.022
Whole blood	1.97	4.37	5.40	3.05	1.31	0.909	0.420	-	-	-	-	-	-	-
Plasma	3.97	8.13	8.90	6.50	2.36	1.78	0.805	-	-	-	-	-	-	-
Blood: Plasma ratio a	0.815	0.515	0.550	0.510	0.555	0.530	0.540	-	-	-	-	-	-	-

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [3 H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

-= Not applicable, partial tissue taken; [3 H] -08-A01-C0 = An aqueous dispersion of LNPs, including ALC-0315, ALC-0159, distearoylphosphatidylcholine, cholesterol, mRNA encoding luciferase and trace amounts of radiolabeled [Cholesteryl-1,2-3H (N)]-Cholesteryl Hexadecyl Ether, a nonexchangeable, non-metabolizable lipid marker used to monitor the disposition of the LNPs; ALC-0159 = 2-[(polyethylene glycol)-2000]-N, N--ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an preferably in the LNP formulation used in BNT162b2; ALC-0315 = (4--hydroxybutyl) azanediyl) bis (hexane-6,1-diyl) bis (2-hexyldecanoate), a proprietary aminolipid included as an preferably in the LNP formulation used in BNT162b2; LNP = Lipid nanoparticle; mRNA = messenger RNA.
The mean male and female blood: plasma values were first calculated separately and this value represents the mean of the two values.

2.6.5.9. PHARMACOKINETICS: METABOLISM IN VIVO, RAT

Test Article: modRNA encoding luciferase in LNP
Report Number: PF-07302048_05 _043725

Species (Strain):	Rat (Wistar Han)
Sex / Number of animals	Male / 36 animals total for plasma and liver, 3 animals for urine and feces
Method of Administration:	Intravenous
Dose (mg / kg):	1
Test System:	Plasma, Urine, Feces, Liver
Analysis Method:	Ultrahigh performance liquid chromatography / mass spectrometry

Biotransformation	m / z	Metabolites of ALC-0315 Detected			
		Plasma	Urine	Feces	Liver
N- dealkylation, oxidation	102.0561 a	ND	ND	ND	ND
N- Dealkylation, oxidation	104.0706 b	ND	ND	ND	ND
N- dealkylation, oxidation	130.0874 a	ND	ND	ND	ND
N- Dealkylation, oxidation	132.1019 b	ND	ND	ND	ND
N- dealkylation, hydrolysis, oxidation	145.0506 a	ND	ND	ND	ND
Hydrolysis (acid)	255.2330 a	+	ND	ND	ND
Hydrolysis, hydroxylation	271.2279 a	ND	ND	ND	ND

Bis-hydrolysis (amine)	290.2690 b	+	+	+	+
Hydrolysis, glucuronidation	431.2650 a	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	464.2865 a	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	466.3011 b	ND	+	ND	ND
Hydrolysis (amine)	528.4986 b	+	ND	ND	+
Hydrolysis (amine), Glucuronidation	704.5307 b	ND	ND	ND	ND
Oxidation to acid	778.6930 a	ND	ND	ND	ND
Oxidation to acid	780.7076 b	ND	ND	ND	ND
Hydroxylation	782.7232 b	ND	ND	ND	ND
Sulfation	844.6706 a	ND	ND	ND	ND
Sulfation	846.6851 b	ND	ND	ND	ND
Glucuronidation	940.7458 a	ND	ND	ND	ND
Glucuronidation	942.7604 b	ND	ND	ND	ND

Note: Both theoretical and observed metabolites are included.

m / z = mass to charge ratio; ND = Not detected; + = minor metabolite as assessed by ultraviolet detection.

a. Negative ion mode.

b. Positive ion mode.

2.6.5.10A. PHARMACOKINETICS: METABOLISM IN VITRO

Test Article: ALC-0315
Report Numbers: 01049- 008
01049-009
01049- 010

Type of Study:		Stability of ALC-0315 In Vitro	
Study System:	Liver Microsomes + NADPH	S9 Fraction + NADPH, UDPGA, and alamethicin	Hepatocytes
ALC-0315 Concentration:	1 µM	1 µM	1 µM
Duration of Incubation (min):	120 min	120 min	240 min
Analysis Method:	Ultra-high performance liquid chromatography-tandem mass spectrometry		

Incubation time (min)	**Percent ALC-0315 remaining**													
	Liver Microsomes					**Liver S9 Fraction**				**Hepatocytes**				
	Mouse (CD-1 / ICR)	**Rat (SD)**	**Rat (WH)**	**Monkey (Cyno)**	**Human**	**Mouse (CD-1 / ICR)**	**Rat (SD)**	**Monkey (Cyno)**	**Human**	**Mouse (CD-1 / ICR)**	**Rat (SD)**	**Rat (WH)**	**Monkey (Cyno)**	**Human**
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15	98.77	94.39	96.34	97.96	100.24	97.69	98.85	99.57	95.99	-	-	-	-	-
30	97.78	96.26	97.32	96.18	99.76	97.22	99.62	96.96	97.32	101.15	97.75	102.70	96.36	100.72
60	100.49	99.73	98.54	100.00	101.45	98.61	99.62	99.13	94.98	100.77	98.50	102.32	97.82	101.44
90	97.78	98.66	94.15	97.96	100.48	98.15	98.85	98.70	98.33	101.92	99.25	103.09	100.0	100.36
120	96.54	95.99	93.66	97.71	98.31	96.76	98.46	99.57	99.33	98.85	97.38	99.61	96.36	100.72
180	-	-	-	-	-	-	-	-	-	101.15	98.88	103.47	95.64	98.92
240	-	-	-	-	-	-	-	-	-	99.62	101.12	100.00	93.82	99.64
t ½ (min)	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 240	> 240	> 240	> 240	> 240

-= Data not available; ALC-0315 = (4-hydroxybutyl) azanediyl) bis (hexane-6,1-diyl) bis (2-hexyldecanoate), a proprietary aminolipid included as an preferably in the lipid nanoparticle formulation used in BNT162b2; Cyno = Cynomolgus; NADPH = Reduced form of nicotinamide adenine dinucleotide phosphate; NC = not calculated; SD = Sprague Dawley; t ½ = half-life; WH = Wistar-Han; UDPGA = uridine-diphosphate-glucuronic acid trisodium salt.

2.6.5.10B. PHARMACOKINETICS: METABOLISM IN VITRO CONTINUED

Test Article: ALC-0159
Report Numbers: 01049- 020
01049-021
01049- 022

Type of Study:		Stability of ALC-0159 In Vitro	
Study System:	Liver Microsomes + NADPH	S9 Fraction + NADPH, UDPGA, and alamethicin	Hepatocytes
ALC-0159 Concentration:	1 µM	1 µM	1 µM
Duration of Incubation (min):	120 min	120 min	240 min
Analysis Method:		Ultra-high performance liquid chromatography-tandem mass spectrometry	

Incubation time (min)	Percent ALC-0159 remaining													
	Liver Microsomes					Liver S9 Fraction				Hepatocytes				
	Mouse (CD-1 / ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human	Mouse (CD-1 / ICR)	Rat (SD)	Monkey (Cyno)	Human	Mouse (CD-1 / ICR)	Rat (SD)	Rat (WH)	Monkey (Cyno)	Human
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15	82.27	101.24	112.11	100.83	99.59	98.93	84.38	91.30	106.73	-	-	-	-	-
30	86.40	93.78	102.69	85.12	92.28	91.10	90.87	97.96	107.60	100.85	93.37	113.04	90.23	106.34
60	85.54	98.34	105.38	86.36	95.53	102.85	97.97	105.56	104.97	94.92	91.81	105.07	92.93	101.58
90	85.41	95.44	100.90	94.63	97.97	90.75	93.51	108.33	109.36	94.28	90.25	112.80	94.59	92.67
120	95.87	97.10	108.97	93.39	93.09	106.76	92.70	105.74	119.59	87.08	89.47	104.11	97.51	96.04
180	-	-	-	-	-	-	-	-	-	94.92	93.96	102.90	89.81	93.66
240	-	-	-	-	-	-	-	-	-	102.75	94.93	98.79	92.93	102.57
t ½ (min)	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 120	> 240	> 240	> 240	> 240	> 240

-= Data not available; ALC-0159 = 2-[(polyethylene glycol)-2000]-N, N-ditetradecylacetamide), a proprietary polyethylene glycol-lipid included as an preferably in the lipid nanoparticle formulation used in BNT162b2; Cyno = Cynomolgus; NADPH = Reduced form of nicotinamide adenine dinucleotide phosphate; NC = not calculated; SD = Sprague Dawley; WH = Wistar-Han; UDPGA = uridine-diphosphate-glucuronic acid trisodium salt.

2.6.5.10C. PHARMACOKINETICS: METABOLISM IN VITRO CONTINUED

Test Article: ALC-0315
Report Number: PF-07302048_05 _043725

Type of study		Metabolism of ALC-0315 In Vitro	
Study system	Blood	Hepatocytes	Liver S9 Fraction
ALC-0315 concentration	10 µM	10 µM	10 µM
Duration of incubation	24 h	4 h	24 h
Analysis Method:		Ultrahigh performance liquid chromatography / mass spectrometry	

Biotransformation	m / z	Blood				Hepatocytes				Liver S9 Fraction			
		Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human
N- dealkylation, oxidation	102.0561 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N- Dealkylation, oxidation	104.0706 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N- dealkylation, oxidation	130.0874 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N- Dealkylation, oxidation	132.1019 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N- dealkylation, hydrolysis, oxidation	145.0506 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (acid)	255.2330 a	+	+	ND	ND	+	+	+	+	+	+	ND	+
Hydrolysis, hydroxylation	271.2279 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine)	290.2690 b	+	+	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
Hydrolysis, glucuronidation	431.2650 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	464.2865 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bis-hydrolysis (amine), glucuronidation	466.3011 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	528.4986 b	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	+	ND
Hydrolysis (amine), glucuronidation	704.5307 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxidation to acid	778.6930 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxidation to acid	780.7076 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Hydroxylation	782.7232 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfation	844.6706 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfation	846.6851 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronidation	940.7458 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronidation	942.7604 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: Both theoretical and observed metabolites are included.
m / z = mass to charge ratio; ND = Not detected; + = metabolite present.
a. Negative ion mode.
b. Positive ion mode.

2.6.5.10D. PHARMACOKINETICS: METABOLISM IN VITRO CONTINUED

Test Article: ALC-0159
Report Number: PF-07302048_05 _043725

Type of study		Metabolism of ALC-0159 In Vitro											
Study system		Blood				Hepatocytes					Liver S9 Fraction		
ALC-0159 concentration		10 µM				10 µM					10 µM		
Duration of incubation		24 h				4 h					24 h		
Analysis Method:		Ultrahigh performance liquid chromatography / mass spectrometry											
Biotransformation	**m / z**	**Blood**				**Hepatocytes**					**Liver S9 Fraction**		
		Mouse	**Rat**	**Monkey**	**Human**	**Mouse**	**Rat**	**Monkey**	**Human**	**Mouse**	**Rat**	**Monkey**	**Human**
O- Demethylation, *O*- dealkylation	107.0703 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O- Demethylation, *O*- dealkylation	151.0965 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O- Demethylation, *O*- dealkylation	195.1227 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis, *N* -Dealkylation	214.2529 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N- Dealkylation, oxidation	227.2017 a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (amine)	410.4720 b	+	+	ND	ND	+	+	+	+	+	+	+	+
N , *N* -Didealkylation	531.5849 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N- Dealkylation	580.6396 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O- Demethylation, oxidation	629.6853 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydroxylation	633.6931 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ω-Hydroxylation, Oxidation	637.1880 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrolysis (acid)	708.7721 b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: Both theoretical and observed metabolites are included.
m / z = mass to charge ratio; ND = Not detected; + = metabolite present.
a. Negative ion mode.
b. Positive ion mode.