You have been provided some background materials for a bioequivalence (BE) study with pharmacokinetic (PK) endpoint. Please review the background materials and answer question #1.

Section 1

Background

A Sponsor conducted a study ABCD2013 entitled "An open-label, three treatment, three-period, six-sequence crossover study comparing the bioavailability of suspension (100 mg/5ml) and enteric-coated tablets of DRUG X (100 mg) to bioavailability of enteric-coated reference listed drug (RLD), DRUG® (100mg) Health Volunteers in fasted condition" This study was conducted at a single clinical site, Best Clinical Research Services. The study was an inpatient, open-label, randomized, 3-period, 3-treatment, 6 sequence crossover study involving 30 healthy volunteers aged 18 to 45 years with 4-day washout period between each treatment. DRUG X has a mean half-life of 60 min and has known instability in matrix. Due to drug shortage, FDA decided to prioritize review of the application.

Due to logistics and enrollment issues, Best Clinical Services conducted the bioequivalence study in two groups. Dosing of the first subject in the study occurred on 11-09-2013 and the last sample collection was on 12-02-2013. All samples were stored in a -20^{o}C freezer till they were shipped to the analytical site. The primary aliquot of all subject samples were shipped to Accurate Analytical Service Ltd. on 01-17-2014.

A total of 1440 plasma samples for ABCD2013 were received by Accurate Analytical Services on 01-18-2014 from Best Clinical Research Services. The samples were stored in a -70°C freezer from 01-18-2014 to 02-20-2014 and then transferred to a -20°C freezer following sample analysis.

Several plasma samples were noted to be hemolyzed during sample inventory at Accurate Analytical Services. Per Method Validation Protocol No. VP-031-00 for analysis of DRUG X, Accurate Analytical Services conducted precision and accuracy and matrix effect experiments on normal subject plasma and not in hemolyzed matrix.

During a previous inspection of this clinical site, FDA investigator noted protocol compliance issues, reporting of protocol deviations, and lack of training of laboratory technicians. The current inspection revealed repeated issues with reporting protocol deviations and inadequate and incomplete source documentation. OSIS reviewer in FDA reviewing the findings found it concerning.

Study Design

This will be an inpatient open-label, randomized, 3-period, 3-treatment, 6-sequence, crossover study involving 48 healthy volunteers. Subjects will be assigned to one of the 6 sequences. After obtaining informed consent, subjects will be screened for eligibility to participate in the study, including medical history, physical examination, height, weight, BMI, clinical chemistry, coagulation markers, hematology, infectious disease serology, urinalysis, urine toxicology screen, vital signs and ECG. On the day after clinic admission, subjects will be administered the study drug in randomized order with a 4-day washout period between doses until all 3 doses have been administered. Blood will be collected for DRUGX PK prior to dosing and approximately 2.5, 5, 10, 15, 20, 30, 45, 60, 120, 180, 240, 300, 360, 480, and 720 minutes after the start of study drug administration. On days of study drug administration, a 12-lead ECG will be performed approximately 60 minutes prior to dosing and 60 and 480 minutes post-dose. Vital signs will be measured pre-dose and approximately 30, 60, 120, and 480 minutes post-dose. On dosing days, the order of assessments will be ECG, vital signs, then PK blood collection when scheduled at the same nominal times. The target time of the PK blood collection is considered the most critical and if the collection is more than ± 1 minute from the scheduled time for the first 60 minutes of collections or more than ± 5 minutes for the scheduled time points thereafter, this will be considered a protocol deviation. ECG and vital signs should be collected within the 10 minute period before the nominal time of blood collections. At screening, admission, discharge, and follow-up, ECG, and vital signs will be checked once per day. Vital signs will also be checked once the day after dosing. Clinical laboratory measurements will be repeated after the last PK blood draw prior to clinic discharge. AEs will be assessed by spontaneous reports by subjects, by measuring vital signs, ECG, and clinical laboratory parameters.

Procedure for Collection, Storage, and Delivery of Plasma Samples for Analysis of DRUGX Levels

Blood Drawing Procedure:

Blood drawn from all subjects should be considered infectious and extreme caution should be used to avoid needle sticks and direct contact with blood or plasma.

Using a 6 mL sodium heparin tube:

(1) draw one tube of blood, filling it as completely as possible;

(2) gently invert 8-10 times;

(3) centrifuge the blood (3,000 x g for 15 min.) immediately in a refrigerated centrifuge to prevent hemolysis;

(4) using a disposable pipet, immediately transfer 1 mL of plasma from the tube to the primary plastic plasma storage vial, and secure the cap tightly; transfer the remaining plasma to the backup tube and secure the cap tightly.

(5) label the vials as described below, and;

(6) freeze sample at -20°C immediately afterwards in an upright position. Keep frozen at ≤ -20°C until shipment.

There should be no more than 60 minutes between collection of the blood sample and placement of the plasma sample in the freezer.

Labeling Procedure:

Fill out a specimen inventory form. Use preprinted labels to label tubes. The label will include:

(1) the protocol number,

(2) subject identification number and alpha code, and

(3) study day and nominal time point.

Sample Storage: Store all plasma samples at ≤ -20°C, until sufficient numbers have been collected to submit to the laboratory for analysis. The primary aliquot will be submitted to the laboratory for analysis. The back-up samples will be held at the clinical site as a

retention sample in case there are problems with the shipment or analysis and may need to be shipped later for analysis. Pack the samples on dry ice in an insulated Styrofoam container and deliver the samples to the laboratory for analysis as soon as possible after packing. Provide a copy of the sample inventory sheet with the samples.

Question #1

Based on what you have learnt so far in the workshop, information provided about the study protocol and the study drug, if you were assigned to conduct an inspection of the study at the clinical site, what sample processing steps would you pay special attention at the clinical site that may have potential impact on **accuracy** of the bioanalytical results and why?


STOP

Section 2

FDA Investigator conducted an inspection of Best Clinical Services. The inspection included a thorough examination of study records, case report forms (CRFs), informed consent process, protocol deviations, institutional review board approvals, sponsor and monitor correspondence, test article accountability and storage records, randomization, dosing, sample collection and adverse events. The FDA investigator observed objectionable conditions and collected the following exhibits to support the findings. Please review the exhibits and answer question #2.

Instructions: Place in -20C freezer within 60 minutes of collection time.

H= Hemolyzed L= Lipemic SS= Short Sample ED = Early Draw LD = Late Draw ND = Not Drawn ETC = Early to Centrifuge

LTC = Late to Centrifuge LTF = Late to Freezer

Date of Visit (Day 1): Nov 9 ~~13~~ 11/9/13 2013

Subject Number	Timepoint	Scheduled Collection Time	Actual Collection Time	Scheduled Centrifuge Time	Time in Centrifuge	Time out of Centrifuge	Scheduled Freezer Time	Time in Freezer	Number of Aliquots	Staff initials	Aliquot check	Comments
3323	predose	8:00	8:00		8:20	8:36	9:00	08:58	2	BC	MN	
3323	2.5min	8:07:30	8:07:30		8:20	8:36	9:07:30	9:07	2	BC	MN	
3323	5min	8:10	8:15		8:45	9:01	9:10	9:15	2	BC	MN	LTF, H, LD
3323	10min	8:15	8:15		8:45	9:01	9:15	9:15	2	BC	MN	
3323	15min	8:20	8:20		8:45	9:01	9:20	9:18	2	BC	MN	
3323	20min	8:25	8:25		8:58	9:13	9:25	9:32	2	BC	MN	H, LTF
3323	30 min	8:35	8:35		9:06	9:21	9:35	9:32	2	BC	MN	H, LTF
3323	45min	8:50	8:52		9:26	9:41	9:50	9:52	2	BC	MN	LD, LTF
3323	60min	9:05	9:07		9:55	10:10	10:05	10:22	2	BC	MN	LD, LTF
3323	120min	10:05	10:07		10:42	10:57	11:05	11:05	2	BC	MN	LD, H
3323	180min	11:05	11:05		11:36	11:52	12:05	12:03	2	BC	MN	
3323	240min	12:05	12:05		12:42	12:58	13:05	13:12	2	BC	MN	LTF
3323	300min	13:05	13:07		13:38	13:53	14:05	14:00	2	BC	MN	LD
3323	360min	14:05	14:05		14:45	15:02	15:05	15:12	2	BC	MN	LTF
3323	480min	16:05	16:05		16:32	16:48	17:05	17:03	2	BC	MN	
3323	720min	20:05	20:07		20:29	20:45	21:05	21:10	2	BC	MN	LD, LTF

Instructions: Place in -20C freezer within 60 minutes of collection time.

H= Hemolyzed L= Lipemic SS= Short Sample ED = Early Draw LD = Late Draw ND = Not Drawn ETC = Early to Centrifuge

LTC = Late to Centrifuge LTF = Late to Freezer

Date of Visit (Day 1): NOV 11, 2013

Subject Number	Timepoint	Scheduled Collection Time	Actual Collection Time	Scheduled Centrifuge Time	Time in Centrifuge	Time out of Centrifuge	Scheduled Freezer Time	Time in Freezer	Number of Aliquots	Staff initials	Aliquot check	Comments
2323	predose	7:55	7:55		08:20	8:35	8:55	08:44	2	ZY	YZ	
2323	2.5min	8:02:30	8:02:30		08:20	8:35	9:02:30	08:44	2	ZY	YZ	
2323	5min	8:05	08:07		08:40	8:55	9:05	09:07	2	ZY	YZ	LD
2323	10min	8:10	08:10		08:40	8:55	9:10	09:07	2	ZY	YZ	
2323	15min	8:15	08:15		08:40	8:55	9:15	09:07	2	ZY	YZ	
2323	20min	8:20	08:23		08:58	9:13	9:20	09:28	2	ZY	YZ	LD
2323	30 min	8:30	8:30		08:58	9:13	9:30	09:45	2	ZY	YZ	
2323	45min	8:45	8:48		09:20	9:35	9:45	09:45	2	ZY	YZ	LD
2323	60min	9:00	9:01		09:20	9:35	10:00	10:00	2	ZY	YZ	LD
2323	120min	10:00	10:00		10:32	10:47	11:00	11:05	2	ZY	YZ	
2323	180min	11:00	11:02		11:47	12:03	12:00	12:20	2	ZY	YZ	LD
2323	240min	12:00	12:05		12:42	12:58	13:00	13:00	2	ZY	YZ	LD
2323	300min	13:00	13:02		13:37	13:52	14:00	13:55	2	ZY	YZ	LD
2323	360min	14:00	14:00		14:30	14:46	15:00	14:58	2	ZY	YZ	
2323	480min	16:00	16:00		16:32	16:48	17:00	17:10	2	ZY	YZ	
2323	720min	20:00	20:00		20:45	21:02	21:00	21:00	2	ZY	YZ	

Instructions: Place in -20C freezer within 60 minutes of collection time.

H= Hemolyzed L= Lipemic SS= Short Sample ED = Early Draw LD = Late Draw ND = Not Drawn ETC = Early to Centrifuge

LTC = Late to Centrifuge LTF = Late to Freezer

Date of Visit (Day 1): Dec 02, 2013

Subject Number	Timepoint	Scheduled Collection Time	Actual Collection Time	Scheduled Centrifuge Time	Time in Centrifuge	Time out of Centrifuge	Scheduled Freezer Time	Time in Freezer	Number of Aliquots	Staff initials	Aliquot check	Comments
5567	predose	8:00	8:00		08:32	08:47	9:00	08:50	2	BC	MN	H
5567	2.5min	8:07:30	8:07:30		08:32	08:47	9:07:30	08:50	2	BC	MN	H
5567	5min	8:10	08:15		08:32	08:47	9:10	09:15	2	BC	MN	LD, LTF
5567	10min	8:15	08:15		08:32	08:47	9:15	09:15	2	BC	MN	
5567	15min	8:20	08:20		09:05	09:22	9:20	09:35	2	BC	MN	LTF, H
5567	20min	8:25	08:27		09:05	09:22	9:25	09:35	2	BC	MN	H, LTF, LD
5567	30 min	8:35	08:35		09:05	09:22	9:35	09:35	2	BC	MN	
5567	45min	8:50	08:50		09:17	09:32	9:50	09:50	2	BC	MN	
5567	60min	9:05	09:07		09:17	09:32	10:05	10:05	2	BC	MN	LD
5567	120min	10:05	10:07		10:45	11:01	11:05	12:06	2	BC	MN	LD, H, LTF
5567	180min	11:05	11:05		11:30	11:45	12:05	12:06	2	BC	MN	LTF
5567	240min	12:05	12:05		12:20	13:05	13:05	13:10	2	BC	MN	LTF
5567	300min	13:05	13:05		13:15	13:30	14:05	14:05	2	BC	MN	
5567	360min	14:05	14:05		14:37	14:47	15:05	15:32	2	BC	MN	LTF
5567	480min	16:05	16:05		16:42	16:57	17:05	18:35	2	BC	MN	LTF
5567	720min	20:05	20:05		20:29	20:44	21:05	21:05	2	BC	MN	

Instructions: Place in -20C freezer within 60 minutes of collection time.

H= Hemolyzed L= Lipemic SS= Short Sample ED = Early Draw LD = Late Draw ND = Not Drawn ETC = Early to Centrifuge

LTC = Late to Centrifuge LTF = Late to Freezer

Date of Visit (Day 1): Dec 2, 2013

Subject Number	Timepoint	Scheduled Collection Time	Actual Collection Time	Scheduled Centrifuge Time	Time in Centrifuge	Time out of Centrifuge	Scheduled Freezer Time	Time in Freezer	Number of Aliquots	Staff initials	Aliquot check	Comments
4456	predose	7:55	7:55		08:20	8:35	8:55	08:44	2	YZ	ZY	
4456	2.5min	8:02:30	08:02:30		08:20	8:35	9:02:30	08:44	2	YZ	ZY	
4456	5min	8:05	08:07		08:40	8:55	9:05	09:07	2	YZ	ZY	LD, H, LTF
4456	10min	8:10	08:10		08:40	8:55	9:10	09:07	2	YZ	ZY	H
4456	15min	8:15	08:15		08:40	8:55	9:15	09:07	2	YZ	ZY	
4456	20min	8:20	08:23		08:58	9:13	9:20	09:28	2	YZ	ZY	LD, H, LTF
4456	30 min	8:30	08:30		08:58	9:13	9:30	09:28	2	YZ	ZY	
4456	45min	8:45	08:48		09:20	9:35	9:45	09:45	2	YZ	ZY	LD, H
4456	60min	9:00	09:01		09:20	9:35	10:00	10:00	2	YZ	ZY	LD
4456	120min	10:00	10:00		10:32	10:47	11:00	11:05	2	YZ	ZY	LTF
4456	180min	11:00	11:06		11:47	12:03	12:00	12:20	2	YZ	ZY	LD, H, LTF
4456	240min	12:00	12:05		12:42	12:58	13:00	13:00	2	YZ	ZY	LD
4456	300min	13:00	13:02		13:37	13:52	14:00	13:55	2	YZ	ZY	LD
4456	360min	14:00	14:00		14:30	14:46	15:00	14:58	2	YZ	ZY	
4456	480min	16:00	16:00		16:32	16:48	17:00	17:10	2	YZ	ZY	LTF
4456	720min	20:00	20:00		20:45	21:02	21:00	21:34	2	YZ	ZY	LTF

Freezer Temperature Log for ABCD2013 Study

Document temperature readings for each day/week/month in C° or F° in accordance with the study protocol.

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	-20C			-16C	-20C			-20C		-20C	-17C	
2	-20C	-20C			-20C		-20C	-20C			-18C	
3	-20C	-14C	-20C	-17C	-20C	-15C	-20C	-20C		-20C		
4	-14C	-16C	-20C				-16C		-17C			
5	-18C	-20C			-20C	-16C	-15C	-18C	-16C	-15C		
6	-19C	-20	-17C			-20C	-15C	-19C	-15C	-14C		
7	-20C	-16C	-18C	-20C	-17C	-20C	-20C	-20C	-20C	-16C		
8		-20C	-20C		-18C	-20C		-10C		-20C		
9	-17C	-7C	-20C		-20C	-17C		-15C	-20C			
10	-20C	-19C	-16C	-22C	-20C	-18C	-17C	-14C		-17C		
11	-21C	-18C	-15C		-20C	-20C	-20C		-14C	-20C		
12	-16C	-20C	-11C		-18C	-21C	-20C	-17C	-17C			
13	-15C	-20C	-20C	-17C	-19C		-16C	-16C	-18C	-20C		
14	-20C	-20C	-20C	-18C	-19.5C			-12C	-20C	-20C		
15	-20C	-18C	-17C		-18C	-22C	-18C	-18C	~~-19~~	-21C		
16	-20C	-15C	-18C	-20C	-20C	-20C	-19C	-20C	-20C	-13C		
17	-20C	-17C	-19C		-20C		-		-20C	-17C		
18	-20C	-20C	-20C	-20C				-20C	-20C			
19	-19C	-17C	-20C	-14C	-20C	-16C		-20C				
20	-20C	-18.5C		-15C	-20C	-15C		-20C	-20C	-20C		
21	-20C	-20C		-15C	-17C	-20C	-17C		-11C			
22	-18C	-20C	-20C	-16C	-18C	-20C		-20C	-12C			
23	-20C	-15C		-18C	-20C		-12C		-20C			
24	-20C	-16C	-20C	-18C	-20C	-20C	-13C		-7C	-20C		
25	-17C	-17C	-18C	-19C	-14C	-14C			-10C	-20C		
26	-20C	-20C	-19C	-20C		-20C			-11C	-18.5C		
27	-16C	-20C	-20C	-20C			-20C			-20C		
28	-15C	-20C	-21C	-20C		-20C			-20C	-19C		
29	-20C	-20C	-20C	-15C			-20C		-20C			
30	-18C	-20C	-20C									
31	-19.5C	-20C		-20C								

Sample Storage Dates 11/9/2013 - 12/2/2013

Date Shipped to Accurate Analytical Services Ltd 01/17/2014 MN 1/17/14

Date Samples Received by Accurate Analytical Services Ltd 01/18/2014 9:33 AM B 1/18/2014

ABCD2013 Study Protocol Deviation Log

Deviation Codes:

ND = Not Drawn **LD = Late Draw** **ED = Early Draw** **LTF = Late to Freezer**

ICF = Consent Deviation **DE = Dosing Error** **OPD = Other Protocol Deviation**

Subject ID	Date of Deviation	Deviation Description and Code
2323	NOV 11, 2013	Code LD 5min PK collection delayed by 2min
2323	NOV 11, 2013	Code LD 45min PK collection delayed by 3min
2323	NOV 11, 2013	Code ICF Subject consented with incorrect version of ICF
3323	NOV 9, 2013	Code OPD Day 1 EKG not done
3323	NOV 9, 2013	Code LD 5min PK collection delayed by 5min
3323	NOV 9, 2013	Code LD 45min PK collection delayed by 2min
3323	NOV 9, 2013	Code OPD Day 1 subject vitals missed
2323	NOV 11, 2013	Code LD 20 min PK collection delayed by 3min
7723	NOV 15, 2013	Code ND 180 min PK collection missed

ABCD2013 Study Protocol Deviation Log

Deviation Codes:

ND = Not Drawn **LD = Late Draw** **ED = Early Draw** **LTF = Late to Freezer**

ICF = Consent Deviation **DE = Dosing Error** **OPD = Other Protocol Deviation**

Subject ID	Date of Deviation	Deviation Description and Code
4456	Dec 2, 2013	Code OPD Day 1 vitals missed – See NTF
4456	Dec 2, 2013	Code LD 60 min PK Collection delayed by 1min
5567	Dec 2, 2013	Code ICF Subject did not sign correct version
5567	Dec 2, 2013	Code LD 20min PK collection delayed by 2min
2323	NOV 11, 2013	Code OPD Survey missing pages 3 and 4 for Day 1 visit
8869	NOV 14, 2013	Code DE Day 1 dosing error
2323	NOV 11, 2013	Code LD 60 min PK collection delayed by 1min
4456	Dec 2, 2013	Code LD 5min PK collection delayed by 2min
4456	Dec 2, 2013	Code LD 45 min PK collection delayed by 3min

Question # 2: FDA investigator observed objectionable conditions during the inspection and issued a form FDA-483 to the management at inspection closeout. Based on the study material and source data you reviewed, what are the likely findings on form FDA-483?


STOP

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

DISTRICT OFFICE ADDRESS AND PHONE NUMBER	DATE(S) OF INSPECTION
MHRA GCP Workshop 2020 Street 12131477200/2020	February 13, 2020
	FEI NUMBER 123456789

NAME AND TITLE OF INDIVIDUAL TO WHOM REPORT IS ISSUED

TO: Mr. Best Himself, Vice President

FIRM NAME	STREET ADDRESS
Best Clinical Research Services	Simply The Best 2020
CITY, STATE AND ZIP CODE	TYPE OF ESTABLISHMENT INSPECTED
Best City, Best State	Contract Research Organization

THIS DOCUMENT LISTS OBSERVATIONS MADE BY THE FDA REPRESENTATIVE(S) DURING THE INSPECTION OF YOUR FACILITY. THEY ARE INSPECTIONAL OBSERVATIONS, AND DO NOT REPRESENT A FINAL AGENCY DETERMINATION REGARDING YOUR COMPLIANCE. IF YOU HAVE AN OBJECTION REGARDING AN OBSERVATION, OR HAVE IMPLEMENTED, OR PLAN TO IMPLEMENT CORRECTIVE ACTION IN RESPONSE TO AN OBSERVATION, YOU MAY DISCUSS THE OBJECTION OR ACTION WITH THE FDA REPRESENTATIVE(S) DURING THE INSPECTION OR SUBMIT THIS INFORMATION TO FDA AT THE ADDRESS ABOVE. IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT FDA AT THE PHONE NUMBER AND ADDRESS ABOVE.

DURING AN INSPECTION OF YOUR FIRM (I) (WE) OBSERVED:

Observation 1

An investigation was not conducted in accordance with the investigational plan.

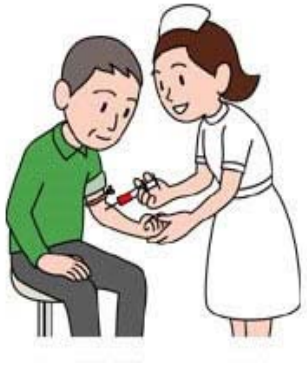
A. Per Section 1.5 of the investigational plan, samples are to be centrifuged immediately in a refrigerated centrifuge to prevent hemolysis. During the inspection, it was discovered that several PK samples were not centrifuged immediately. Samples were centrifuged 30-45 minutes after collection.

B. Per Section 1.5 of the investigational plan, samples are to placed in freezer within 60 minutes of sample collections. Several PK samples were not placed in the freezer within 60 minutes of sample collection.

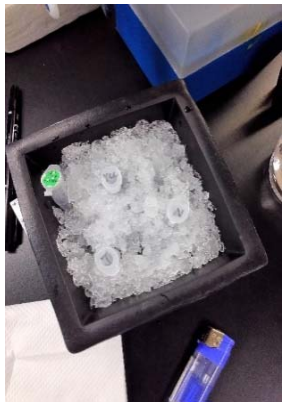
C. Per Section 3.5.2 of the investigational plan, all protocol deviations are to be reported. During the inspection, source records revealed several unreported protocol deviations.

SEE REVERSE OF THIS PAGE	EMPLOYEE(S) SIGNATURE	EMPLOYEE(S) NAME AND TITLE (Print or Type)	DATE ISSUED
	T. Greatest	T. Greatest, Investigator	Feb 13, 2020

Observations at the clinical site



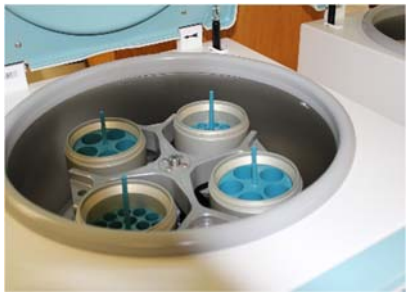
PK Sample collection

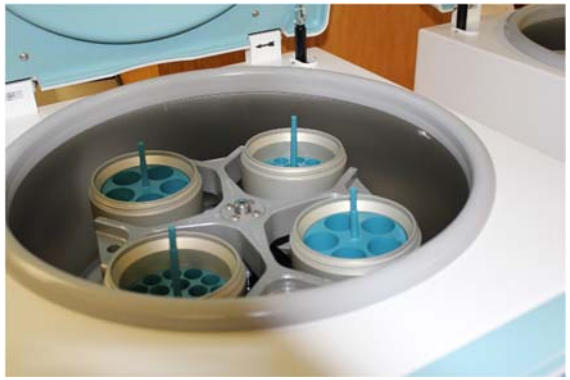


4°C? How long?

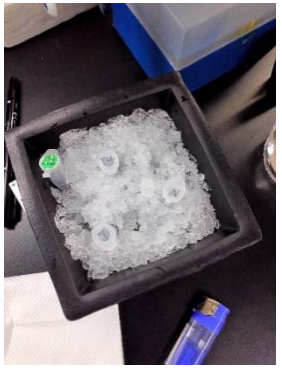
RT? How long?

(Protocol says immediately)





Plasma Sample



4°C? How long?

RT? How long?

(Protocol says 60min to Freezer from sample collection)

Section 3

Accurate Analytical Services Ltd. was inspected by Investigators from FDA. The inspection included a thorough examination of study records, facilities, laboratory equipment, method validation, and sample analysis, and interviews with the firm's management and staff. They confirmed that the source records matched the data submitted to FDA. No additional experiments were conducted as part of validation other than what was presented in the validation summary. The FDA investigators reviewed documents related to sample receipts and noted that study samples were received from Best Clinical services on 01-18-2014 They also noted that several study samples received were recorded to be hemolyzed in the sample inventory documents. The FDA Investigators were concerned with the clinical study conduct due to documentation, reporting and protocol deviations at the clinical site. They discussed with their management potential mitigation strategies which included requesting the analytical site to conduct additional experiments. Please review the exhibits and answer question #3.

BIOANALYTICAL METHOD VALIDATION REPORT FOR
ESTIMATION OF DRUG X IN HUMAN PLASMA

RESULT SUMMARY

A High-performance liquid chromatographic method using tandem mass spectrometry detection for determination of DRUG X in human plasma is described in AMP-031-00. The method was validated in bioanalytical laboratory of Accurate Analytical Services Ltd., according to Analytical Method Procedure No. "AMP-031-00" and Method Validation Protocol No. VP-031-00.

DRUG X acid was extracted from an aliquot of human plasma using Solid Phase Extraction, and then injected into a liquid chromatograph equipped with a tandem mass spectrometry detector; quantitation was done by peak area ratio method. A weighed ($1/x^2$) linear regression was performed to determine the concentration of analyte. All regressions and figures presented in this validation report were generated by Analyst® Software. This method demonstrates acceptable performance and is suitable for the determination of DRUG X in human plasma over the range of 0.0100 to 100ng/mL using Solid Phase Extraction Procedure on Instrument ID.: AAS/BL/001 & AAS/BL/002.

A summary of the validation results are as follows

For DRUGX:

Linearity, Accuracy, Precision and Recovery

Linearity	r 0.9988
Inter Batch Accuracy	93.47% to 102.99%
Inter Batch Precision	1.07% to 5.22%
Intra Batch Accuracy of P & A 1	89.49% to 102.00%
Intra Batch Precision of P & A 1	0.91% to 5.24%
Intra Batch Accuracy of P & A 2	96.30% to 102.66%
Intra Batch Precision of P & A 2	0.43% to 1.50%
Intra Batch Accuracy of P & A 3	96.54% to 101.66%
Intra Batch Precision of P & A 3	0.72% to 2.48%
% recovery of Analyte at LQC, MlQC, M2QC & HQC level	77.78% (LQC), 83.96% (MlQC), 75.51% (M2QC) & 81.74% (HQC)
Mean recovery of Analyte across QC level	79.75%
Mean % CV recovery of Analyte across QC level	4.78%
Recovery of Internal Standard	81.01%
% CV recovery of Internal standard	7.85%

Dilution Integrity

Accuracy	101.15% (1/2nd dilution), 104.52% (1/5th dilution)
Precision	1.42% (1/2nd dilution), 1.32% (1/5th dilution)

Matrix Effect

No significant effect

Ruggedness

Different Analyst	Meets acceptance Criteria
Different Instrument	Meets acceptance Criteria

Stability

Matrix Bench-Top Stability at Room temperature for 6 hrs 06 min	Mean % change at LQC -4.67% & HQC 7.42%
Matrix Freeze/Thaw Stability 5 cycles at -20°C	Mean % change at LQC 4.5% & HQC -5.34%
Long Term Stability at -20°C (32 days)	Mean % change at LQC 4.75% & HQC -4.83
Long Term Stability at -70°C (90 days)	Mean % change at LQC 3.63% & HQC 4.97%
Stock solution stability at Bench-Top (10 hrs)	% Diff -1.10
IS Spiking solution stability at Bench-Top (10 hrs)	% Diff -2.89
IS Spiking solution stability (55 Days at 4°C)	%Diff 1.85
In-injector stability at 10°C for 40 hrs 10 min	Mean % change at LQC 6.86% & HQC 9.46%

Best Clinical Research Services Specimen Tracking Log

Subject	Specimen Timepoint	Total Aliquots Collected	Date Collected	Date Shipped	Date Received by Accurate Analytical Services
2323	Predose 2.5min 5min H 10 min 15 min 20 min H 30 min 45 min 60 min 120min 180 min H 240 min 300 min 360min 480min 720min H	24 4/17/14 32/32	NOV 11, 2013	01/17/14	01/18/2014 BL ✓32
3323	Predose 2.5min 5min H 10 min 15 min 20 min H 30 min H 45 min 60 min 120min 180 min 240 min 300 min 360min 480min 720min	32/32 24 4/17/14	NOV 9, 2013	01/17/14	01/18/2014 BL ✓32
5567	Predose H 2.5min H 5min 10 min 15 min H 20 min H 30 min 45 min 60 min 120min H 180 min 240 min 300 min 360min 480min 720min	32/32 24 4/17/14	Dec 2, 2013	01/17/14	01/18/2014 BL ✓32
4456	Predose 2.5min 5min H 10 min H 15 min 20 min H 30 min 45 min H 60 min 120min 180 min H 240 min 300 min 360min 480min 720min	32/32 42 4/17/14	Dec 2, 2013	01/17/14	01/18/2014 BL ✓32
7793	Predose H 2.5min H 5min H 10 min 15 min H 20 min H 30 min 45 min H 60 min 120min H 180 min 240 min H 300 min 360min 480min 720min H	32/32 24 4/17/14	NOV 21, 2013	01/17/14	01/18/2014 ✓32
1122	Predose 2.5min 5min H 10 min H 15 min H 20 min H 30 min 45 min 60 min 120min 180 min 240 min H 300 min 360min H 480min H 720min H	32/32 24 4/17/14	NOV 21, 2013	01/17/14	01/18/2014 ✓32
3355	Predose 2.5min H 5min H 10 min H 15 min H 20 min H 30 min H 45 min H 60 min 120min H 180 min H 240 min H 300 min 360min 480min 720min H	32/32 24 4/17/14	NOV 27, 2013	01/17/14	01/18/2014 ✓32

*H = Hemolyzed

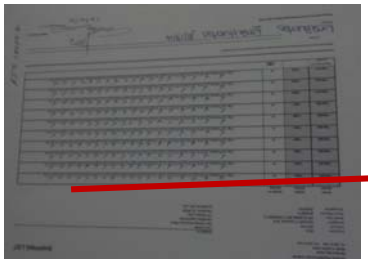
Question #3

Based on the information you have reviewed from the inspection of the clinical site about the clinical study conduct, shipping manifest, method validation summary and information about the analytical site:

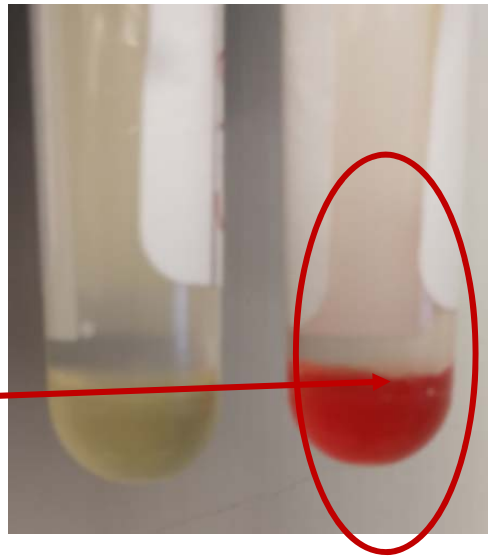
- Can you mitigate the findings at the clinical site? If yes, where? Clinical or analytical site?
- What additional concerns did the FDA Investigators identify at the analytical site?
- What potential mitigation strategies do you think the FDA Investigators came up with?
- What experiments would the FDA Investigators request the analytical site to conduct to address their concerns?


STOP

Observations at the analytical site



Sample inventory



(20% of the study samples received were Hemolyzed.

Validation experiment on effect of Hemolysis on precision and accuracy was not demonstrated during method validation)

Plasma Sample

-20°C Freezer (clinical site)

(Samples stored for 69 days.

Storage at -20°C Freezer validated

for 32 days per validation plan)

-70°C Freezer (analytical site)