International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

RUNNING TITLE: Comparative Pharmacokinetic Study of Voglibose
A Novel Bio-analytical Validated Method Development and Bioequivalence Study
(Comparative Pharmacokinetic Study) of Alpha-Glucosidase Inhibitor Drug Voglibose by LC-ESI-MS/MS in Indian Healthy Male Volunteers Plasma.

Prof. (Dr.) Tapan Kumar Pal,
Pallab Mandal1,3, Balaram Ghosh2, Soumya Chakraborty1, Rakesh Bera 1, Anirbandeep Bose1, Chiranjit Saha1, Sanmoy Karmakar3, Tapan Kumar Pal1*

Ex. Emeritus Medical Scientist (ICMR), Bioequivalence Study Centre,Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata – 700 032, India.Technical Advisor, TAAB Biostudy Services, Kolkata 700032, India

1. *TAAB Biostudy Services, Jadavpur, Kolkata 700032, India*
2. *Calcutta School of Tropical Medicine, Kolkata 700073, India.*
3. *Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.*

Pallab Mandal, Bio-analytical Research Scientist and Laboratory In-charge, IDMA Awardee (2018),TAAB Biostudy Services, Kolkata 700032, India. Kolkata, India.
Dr. Balaram Ghosh, MBBS, MD (Pharmacology), DM, DGO, PhD. Calcutta School of Tropical Medicine, Kolkata 700073, India.
Soumya Chakraborty, Research Scientist, TAAB Biostudy Services, Kolkata 700032, India.
Rakesh Bera, Research Assistant, TAAB Biostudy Services, Kolkata 700032, India.
Dr. Anirbandeep Bose, Head Quality Assurance, TAAB Bio study Services, Kolkata 700032, India.

Chiranjit Saha, Head Documentation, TAAB Biostudy Services, Kolkata, India.
Prof. (Dr.) SanmoyKarmakar, Professor, Department of Pharmaceutical Technology, Jadavpur University, Kolkata,India.
Prof. (Dr.) Tapan Kumar Pal, Ex. Emeritus Medical Scientist (ICMR), Bioequivalence Study Centre, Dept. of Pharmaceutical Technology, Jadavpur University, and Technical Advisor, TAAB Biostudy Services, Kolkata 700032, India.

Submitted: 01-01-2022 Accepted: 10-01-2022

HIGHLIGHTS:

1. Voglibose is a very poor absorbable drug, so any pharmacokinetic parameters were not reported till now except this paper. For this reason it is a novel bio-analytical method which was successfully applied to comparative pharmacokinetic study of voglibose and has been reported a clear pharmacokinetic parameters.
2. In this paper full statistical analysis of this study has been reported.
3. In this paper total fragmentation of voglibose during ionization has been reported.
4. This bio-analytical method is highly selective, sensitive, specific and reproducible.
5. Ionization suppression of voglibose was very low by applying this bio-analytical method.
6. Recovery of voglibose from human plasma was high by applying this bio-analytical method.
7. This paper will be help to all researchers and clinicians for therapeutic dose monitoring.

ABSTRACT: Voglibose is an alpha-glucosidase inhibitor but a very poorly absorbed drug after oral administration, so pharmacokinetic parameters of

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

this drug were not reported in previous literature. The main aim of this study was to develop a highly sensitive bioanalytical method in LC-MS/MS API-4000 by human plasma for quantification of voglibose from human plasma after oral administration.

Voglibose was highly ionized in positive mode. The precursor's ion of voglibose was m/z 268.1/92.3 and propranolol was used as an internal standard which precursors ion was 260.2/116.3 in positive ionization mode. The voglibose was extracted from human plasma by the liquid-liquid extraction method. The plasma calibration concentrations were 0.000160 to 0.020000 ng/ml. The quality control concentrated samples LLOQ, LQC, MQC and HQC were 0.000160, 0.000470, 0.007500, and 0.015000 ng/ml respectively

This bio-analytical method was validated as per the US-FDA guideline and all linearity and stability reports of validation parameters were accurate and précised and within the acceptable limit as per the bioanalytical method validation guideline of US-FDA. The maximum plasma concentration of 0.0007±0.0002 ng/ml or 700±200 fg/ml (C_{max}) at the time 1.60±0.33hr (T_{max}) for reference drug and 0.0006±0.0001ng/ml or 600±100 fg/ml (C_{max}) at the time of 1.84±0.34hr (T_{max}). The renal excretion is negligible that is the plasma elimination constant (K_{el}) were 0.105±0.036hr-1 for reference preparation and 0.103±0.040hr^{-1} for test preparation.

This LC-MS/MS method is highly selective, sensitive, reproducible, low ion suppression, and high recovery, stable method and successfully applied to comparative pharmacokinetic study.

KEY WORDS: Voglibose, Alpha-glucosidase inhibitor, Novel LC-MS/MS method, Bioequivalence study.

I. INTRODUCTION:

One of the valiolamine derivatives and alpha-glucosidase inhibitors voglibose is an oral anti-diabetic drug use to treatment in postprandial (PP) hyperglycemia in diabetes mellitus type-2 by delaying the absorption of absorbable carbohydrates like monosaccharide from the digestion of complex dietary carbohydrates by inhibiting an alpha-glucosidase enzyme.[1] An Exo-type carbohydrate-hydrolase enzyme is the alpha-glucosidase that is a glucosidase that acts on the alpha bond and is located in the brush border of the enterocytes of the jejunum in the small intestine.[5] After the swallowing of complex dietary carbohydrates, it hydrolyses pancreatic alpha-amylase, and this alpha-glucosidase enzyme cleavage the glycosidic bonds in complex dietary carbohydrate into absorbable monosaccharide that is starch and disaccharide to glucose. So voglibose, an alpha-glucosidase inhibitor, are saccharides and act as competitive inhibitors of alpha-glucosidase enzymes, inhibit this membrane-bound enzyme and delay the digestion of dietary carbohydrate and reduce the glucose absorption and decrease the blood glucose level and also reduce the HbA1C level for long term use. [6]

Voglibose is slowly and poorly absorbed through the small intestine. So pharmacokinetic parameters of this drug were not reported till now. The main importance of this study is to develop a validated LC-MS/MS method with plasma extraction procedure which is successfully used to analysis of voglibose in human plasma and calculate the pharmacokinetic parameters of voglibose from human plasma. [7]

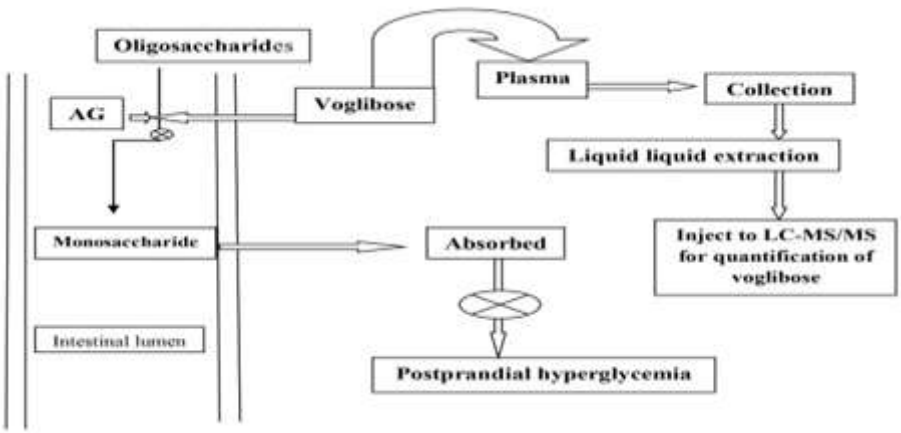

Figure-1 Mechanism of voglibose with analysis in MS

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

II. MATERIALS AND METHODS:

II.I. CHEMICAL AND REAGENTS:

The usable chemicals for this study are methanol, acetonitrile, n-hexane, TBME. Methanol was purchased from J.T.Baker and acetonitrile from Merck. TBME, n-hexane, ammonia solution, ammonium formate, formic acid were used and analytical grade. The human plasma with EDTA was collected in the CPU of TAAB Biostudy services, Kolkata which was stored at -20ºc after collection.

II.II. ETHICAL GUIDELINE: The bioequivalence study of voglibose related documents are approved by the HURIP Independent Bio-ethics committee, Kolkata, India [Central Drugs Standard Control Organization (CDSCO) registration: ECR/103/Indt/WB/2013/RR-19 which is valid up to 21-Nov.-2024] and the ethical clearance was obtained prior to initiation of the study. [2]

II.III METHOD DEVELOPMENT OF VOGLIBOSE BY LC-MS/MS (API-4000) FOR ANALYSIS:

Voglibose (CAS number 83480-29-9) is a valiolamine derivative which chemical name is (1S, 2S, 3R, 4S, 5S)-5-(1, 3-dihydroxypropan-2-ylamino)-1(hydroxymethyl) cyclohexane-1, 2, 3, 4-tetrol. Voglibose Pka values are 12.46 and 7.46, so the total compound is neutral in character. Due to the difference in Pka value 0.2% ammonia Solution in Milli Q water with 10mM ammonium formate was used as an aqueous solvent and 0.1% ammonium hydroxide in methanol and 0.1% formic acid in acetonitrile mixed with a 1:1 ratio was used as an organic solvent for better ionization of voglibose from human plasma in the liquid chromatography-mass spectrometer at 0.5ml/min flow rate. Depending on the chemical characteristic, voglibose was ionized in positive mode. The monoisotopic molecular weight of voglibose is 267.13. The parent ion (Q1) of voglibose in positive mode was m/z 268.1[M+H]+ at declustering potential 51volt and the fragmentation product of parent ion was m/z 92.3 [M- {4OH+CH3OH+(OH-CH2-CH2-CH2-OH)}+ H]+ at collision energy 31volt for aniline (for releasing four hydroxyl group, one methyl alcohol, and one propane-1,3-diol component).

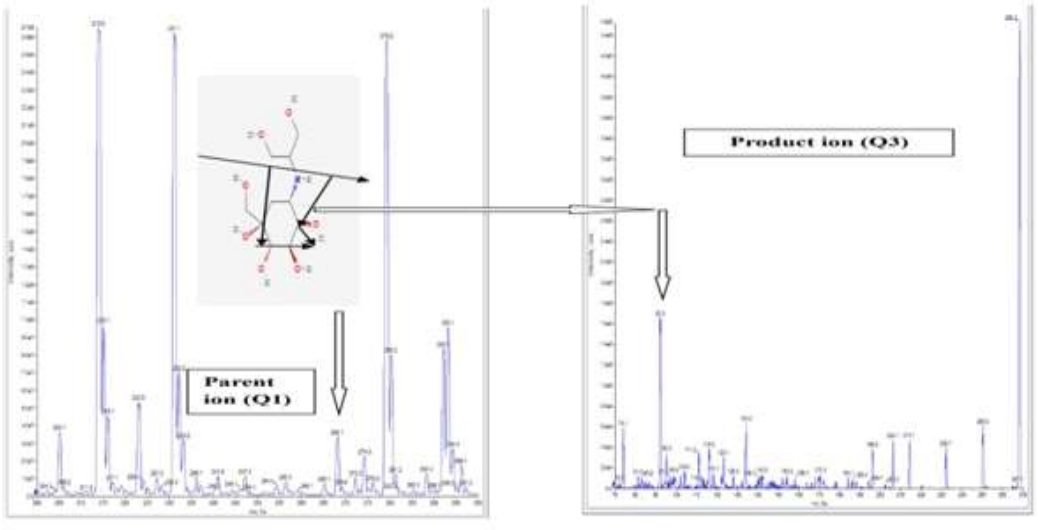

Figure: 2 Parent ion (Q1) and product ion (Q3) scan of voglibose

Figure 2 Parent ion and product ion of voglibose

For quantification of voglibose in LC-MS/MS propranolol was used as an internal standard. The precursor ion and its fragmentation were m/z 260.2/116.3 at 40volt declustering potential and 25volt collision energy. The mass spectrometry parameters for optimization were illustrated in table-1.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

Parameter(s)	Value
Ionization mode	MRM (+ve)
Source temperature (°C)	400
Dwell time per transition (msec)	100
Curtain gas (psi)	30
CAD gas (psi)	7
Ion spray voltage (V)	5500.00
Ion source gas 1 (psi)	40
Ion source gas 2 (psi)	40
Focussing potential (V)	400
Declustering potential (V)	51 (voglibose) and 40 (IS)
Entrance potential (V)	11
Collision energy (V)	31(voglibose) and 25(IS)
Collision cell exit potential (V)	15 (voglibose and IS)
Transition pair of Voglibose (analyte)	268.1/92.3
Transition pair of Propranolol (IS)	260.2/116.3

Table-1: Optimized mass spectrometry parameters for Voglibose and IS

After extraction from plasma voglibose eluate from LC-MS/MS column (Phenomenex Kinetex 5µ C18 100A 50*3mm) by using gradient method where 20% mixture of the organic solution was run from 0.01min to 2.00min and from 2.00min to 4.00min concentration of this solution was raised to 80% the decreased this concentration again 20% from 4.00min to 7.00min for washing purpose.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

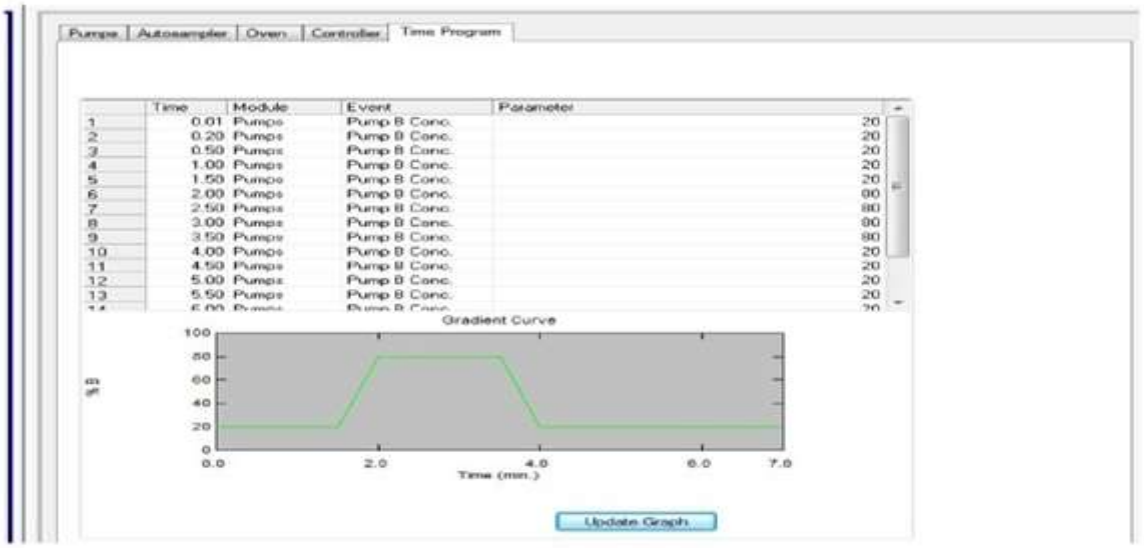

Figure 3 Gradient curve of voglibose method

II.IV. PLASMA EXTRACTION PROCEDURE OF VOGLIBOSE:

The liquid-liquid extraction technique was used for the extraction of voglibose from Indian human plasma. 400µl volume of plasma sample was transferred to a 15ml plastic tarson tube, and then 100µl of internal standard propranolol was spiked to attain a concentration of 0.5µg/ml. After vortexing for 2 min, 700µl n-hexane was added to the sample tubes and again vortexed for 2min. Then 800µl of TBME was added and the sample was vortex -mixed for 2min then centrifuged at 5000 r.p.m for 10min. The 1.5 ml supernatant organic layer was transferred to a 15ml test tube and under a stream of nitrogen; it was evaporated at 4°c. Then the dried extract was reconstituted in 200µl of diluents acetonitrile: Milli Q water (50:50) and vortex for 2 min then was taken into autosampler vial and 10µl aliquot was injected into chromatographic system.

II.V. STOCK SOLUTION, CALIBRATION STANDARDS AND QUALITY CONTROL SAMPLES PREPARATION:

For quantification of voglibose from human plasma, a range of plasma calibration standard concentrations were selected and prepared from a 1mg/ml stock solution. The plasma calibration concentrations were 0.000160, 0.000310, 0.000630, 0.001250, 0.002500, 0.005000, 0.010000, 0.020000 ng/ml. The stock solution was diluted by acetonitrile and methanol mixture at a 1:1 ratio. Then intermediate concentrations were prepared and from these concentrations, plasma calibration concentrations were prepared by spiking 500µl related concentration in 500µl plasma then remaining concentrations were prepared by serial dilution. The quality control concentrated samples LLOQ, LQC, MQC and HQC were 0.000160, 0.000470, 0.007500, and 0.015000 ng/ml respectively which were prepared from separately prepared intermediate solution by spiking into human plasma.

II.VI METHOD VALIDATION:

This bio-analytical method was validated according to the US-FDA guidelines. Three days linearity were done by using freshly prepared calibration concentrations and calculated by formula Y= 1.51X + -7.65e-005(Y= mX +c) and R(regression) square value were calculated from slope m and intercept c. Between run and within run precision and accuracy values were prepared from values of quality control samples. The sample of voglibose was stabilized in different temperatures and was confirmed by quantification of freeze-thaw, short-term autosampler, and bench top stability samples. Plasma samples stabilization days till the completion of the whole analysis were confirmed by long-term stability quantification and for high selectivity, specificity, sensitivity, and suppression of ionization were confirmed by analysis of recovery and matrix effect quality control samples. [3, 4]

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

II.VII COMPARATIVE PHARMACOKINETIC STUDY:

This study was randomized**[Table-2]** open-label, two treatment, two periods, single-dose, cross over, comparative, oral bioequivalence study of test preparation of film-coated tablet containing voglibose IP 0.3mg with reference preparation voglibose IP 0.3mg in 24 healthy adult human, male subjects under fasting conditions.

Subject No.	**PERIOD I**	**PERIOD II**
1	A2	A1
2	A1	A2
3	A1	A2
4	A2	A1
5	A2	A1
6	A1	A2
7	A2	A1
8	A1	A2
9	A1	A2
10	A2	A1
11	A2	A1
12	A1	A2
13	A2	A1
14	A1	A2
15	A1	A2
16	A2	A1
17	A2	A1
18	A1	A2
19	A2	A1
20	A1	A2
21	A1	A2
22	A2	A1
23	A2	A1
24	A1	A2

A1 – Reference Preparation and A2 – Test Preparation

Table-2: Randomization schedule

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

All subjects were adult, human, healthy male volunteers with a mean age of 33.42±4.99 years and mean weight 60.17±6.37kg **.**

Vol. No.	Sex	Age	Height (cm)	Weight (kg)	BMI(kg/m^2)
1	M	32	169	64	22.41
2	M	36	158	51	20.43
3	M	34	167	54	19.36
4	M	39	167	55	19.72
5	M	34	168	60	21.26
6	M	29	166	60	21.77
7	M	31	153	52	22.21
8	M	38	163	62	23.34
9	M	36	162	65	24.77
10	M	26	162	63	24.01
11	M	27	177	62	19.79
12	M	31	176	73	23.57
13	M	40	162	57	21.72
14	M	36	164	64	23.80
15	M	35	169	60	21.01
16	M	29	163	61	22.96
17	M	40	170	70	24.22
18	M	29	171	70	23.94
19	M	39	169	55	19.26
20	M	26	163	52	19.57
21	M	41	170	65	22.49
22	M	25	154	50	21.08
23	M	38	162	54	20.58
24	M	31	167	65	23.31
Mean		**33.42**	**165.50**	**60.17**	**21.94**
S.D.		**4.99**	**5.82**	**6.37**	**1.72**

Table-3: BMI of each volunteer

A total of fifteen blood samples of each volunteer were collected at 0hr. (before drug administration) and 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 2.75, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0hrs. (after drug administration) in the test tubes with EDTA at each time point and washout periods at least seven days between the two dosing sessions. The collected blood samples were centrifuged (3500r.p.m at 4ºc) immediately and plasma was separated and stored frozen at -20ºc with appropriate labeling of volunteer code number with study date and collection time. All plasma samples were analyzed by LC-MS/MS after liquid liquid extraction of the drug voglibose from plasma and injecting it on the LC-MS/MS column for chromatographic analysis. Plasma levels of voglibose for every volunteer at each time point were plotted to obtain time-plasma concentration curves for the study preparation. The mean parameters of bioavailability for this single-dose study were C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, K_{el}

III. RESULTS AND DISCUSSION:

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

III.I. METHOD VALIDATION:

The calibration standards of voglibose were 0.000160 to 0.020000ng/ml and LLOQ 0.000160ng/ml where LOD value was quantified 0.000010ng/ml. The ranges of nominal percentage were 99.38 to 106.61% after the mean calculation of three linearities **.**

Linearity	Concentration (ng/ml)							
	0.000160	**0.000310**	**0.000630**	**0.001250**	**0.002500**	**0.005000**	**0.010000**	**0.020000**
LIN 1	0.000159	0.000315	0.000640	0.001163	0.002524	0.005654	0.009789	0.018514
LIN 2	0.000152	0.000344	0.000632	0.001219	0.002460	0.004760	0.010763	0.019050
LIN 3	0.000166	0.000288	0.000615	0.001250	0.002527	0.005578	0.010355	0.017897
Average	0.000159	0.000316	0.000629	0.001211	0.002504	0.005331	0.010302	0.018487
S.D	0.000007	0.000028	0.000013	0.000044	0.000038	0.000496	0.000489	0.000577
% C.V.	4.40	8.87	2.03	3.64	1.51	9.30	4.75	3.12
Nominal %	99.38	101.83	99.84	96.85	100.15	106.61	103.02	92.44

Table-4a :Pre-study linearity of detector response (n=3)

The plasma calibration curve was present in figure **[Figure-4]**.

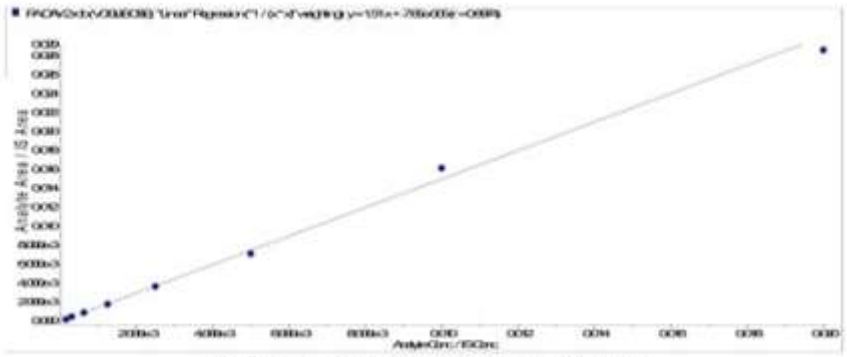

Figure: 4 Plasma calibration curve of voglibose

Figure 4 Plasma calibration curve of voglibose

The regression value was 0.9971, standard deviation 0.0004 and %CV 0.04**[Table-4b]**.

LINEARITY	**STATISTICS**		
LINEARITY CODE	**SLOPE (m)**	**INTERCEPT (c)**	**R square**
LIN 1	1.59000	0.00000263	0.9971
LIN 2	1.51000	-0.0000765	0.9974
LIN 3	1.56000	-0.000169	0.9967
MEAN	**1.55333**		**0.9971**
S.D.	**0.0404**		**0.0004**
C.V.%	**2.60**		**0.04**

Table-4b :Pre-study linearity of detector response statistics (n=3)

Within run and between run precision and accuracy were %CV 3.55 to 7.32 and percentage of absolute percent bias 98.33 to 108.55% and %CV 5.56 to 8.53 and percentage of absolute percent bias100.69 to 101.66% **.**

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

	Between run			Within run		
	Mean ± SD	**C.V.%**	**Absolute bias (%)**	**Mean ± SD**	**C.V.%**	**Absolute bias (%)**
LLOQ (0.000160 ng/ml)	0.000161±0.000014	8.53	100.92	0.000173±0.000013	7.32	107.88
LQC (0.000470 ng/ml)	0.000478±0.000029	6.17	101.66	0.000510±0.000018	3.55	108.55
MQC (0.007500ng/ml)	0.007564±0.000420	5.56	100.85	0.007375±0.000431	5.84	98.33
HQC (0.015000ng/ml)	0.015104±0.000899	5.95	100.69	0.015837±0.000950	6.00	105.58

Table-5: Precision and accuracy (n = 5)

The result of freeze-thaw stability (after three freeze-thaw cycles), short term stability (after 24hr.), bench top stability (after 24hr at sample bench room temperature), autosampler stability (at 15ºc in autosampler machine), long term stability (at -20ºc) were 102.50 to104.86%, 97.44 to 100.84%, 100.47 to 103.25%, 98.82 to 102.64% and 91.80 to 101.94% respectively **.**

		Inj No.	**LQC (0.000470 ng/ml)**	**MQC (0.007500 ng/ml)**	**HQC (0.015000 ng/ml)**
	Freshly Thawed	1	0.000444	0.007607	0.015738
		2	0.000473	0.007510	0.013086
		3	0.000452	0.007377	0.013260
		4	0.000471	0.008091	0.015210
		5	0.000508	0.007426	0.014512
		Mean	**0.000470**	**0.007602**	**0.014361**
Freeze thaw stability	After 3 cycle	1	0.000494	0.008111	0.015604
		2	0.000499	0.007650	0.013549
		3	0.000502	0.007907	0.013753
		4	0.000463	0.007494	0.015695
		5	0.000504	0.008036	0.014997
		Mean	**0.000492**	**0.007840**	**0.014720**
	% Stability		**104.86**	**103.12**	**102.50**
Short term stability	After 24 hours.	1	0.000458	0.007854	0.015757
		2	0.000473	0.007815	0.013537
		3	0.000431	0.007633	0.013583
		4	0.000441	0.007206	0.015233
		5	0.000485	0.007821	0.014240
		Mean	**0.000458**	**0.007666**	**0.014470**
	% Stability		**97.44**	**100.84**	**100.76**
Auto sampler stability	After 24 hours in auto sampler (15ºc)	1	0.000485	0.008112	0.015515
		2	0.000506	0.007679	0.013566
		3	0.000493	0.007075	0.013373
		4	0.000491	0.007117	0.014799
		5	0.000435	0.007579	0.014030
		Mean	**0.000482**	**0.007512**	**0.014257**
	% Stability		**102.64**	**98.82**	**99.27**

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

Bench top stability	After 24 hours in laboratory room temperature	1	0.000432	0.008443	0.015513
		2	0.000507	0.008164	0.013637
		3	0.000511	0.007978	0.013422
		4	0.000430	0.007185	0.015725
		5	0.000509	0.007477	0.013850
		Mean	**0.000478**	**0.007849**	**0.014429**
	% Stability		**101.75**	**103.25**	**100.47**
Long term stability		1	0.000479	0.008413	0.015869
		2	0.000478	0.007333	0.014690
		3	0.000460	0.007997	0.014402
		4	0.000498	0.007894	0.014896
		5	0.000509	0.007680	0.014180
		Mean	**0.000485**	**0.007863**	**0.014807**
	% Stability		**100.83**	**101.94**	**91.80**

Table -6: Stability Study (Freeze thaw, Short term, Auto sampler, Bench top stability, Long term stability

The result of the recovery of voglibose was 90.50 to 95.99% and 89.87 to 97.41% for internal standard **[Table-7(a, b)]**.

INJ No.	Diluent Sample			In Plasma		
	LQC 0.000470 ng/ml	**MQC 0.007500 ng/ml**	**HQC 0.015000 ng/ml**	**LQC 0.000470 ng/ml**	**MQC 0.007500 ng/ml**	**HQC 0.015000 ng/ml**
1	1416.48	26969.87	41917.09	1212.24	19560.61	41866.93
2	1361.75	20638.43	38706.06	1282.95	21588.19	36653.41
3	1342.85	19730.38	39083.89	1248.97	19953.07	37131.93
4	1343.23	19811.38	46111.98	1155.36	19716.58	42102.24
5	1323.90	23426.16	40152.96	1243.75	19720.13	39947.80
Mean	**1357.64**	**22115.24**	**41194.40**	**1228.65**	**20107.72**	**39540.46**
% Recovery				**90.50**	**90.92**	**95.99**

Table-7a: Recovery of Voglibose

INJ No.	Diluent Sample			In Plasma		
	LQC 0.000470 ng/ml	**MQC 0.007500 ng/ml**	**HQC 0.015000 ng/ml**	**LQC 0.000470 ng/ml**	**MQC 0.007500 ng/ml**	**HQC 0.015000 ng/ml**
1	1578715.49	1773311.17	1464786.58	1439761.87	1600473.37	1463118.87
2	1645148.45	1601933.81	1551031.23	1493173.93	1437446.01	1494632.01
3	1550144.61	1580885.49	1527439.46	1391793.74	1456905.92	1489599.85
4	1574401.79	1630642.02	1546518.18	1421689.23	1493449.17	1502847.14
5	1662698.95	1585222.46	1479745.65	1452851.43	1476816.88	1423116.26
Mean	**1602221.86**	**1634398.99**	**1513904.22**	**1439854.04**	**1493018.27**	**1474662.83**
% Recovery				**89.87**	**91.35**	**97.41**

Table-7b: Recovery of IS

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

The result of the matrix factor was 0.93 to 0.98 for voglibose and 0.93 to 0.98 for internal standard **[Table-8]**.

	Matrix effect IS		Matrix effect Voglibose	
	% of ME	**Matrix factor**	**% of ME**	**Matrix factor**
LQC (0.000470/ ml)	97.66±2.25	0.98±0.02	98.12±4.68	0.98±0.05
MQC (0.007500ng/ml)	94.56±2.37	0.95±0.02	93.36±2.27	0.93±0.02
HQC (0.015000ng/ml)	92.62±1.15	0.93±0.01	93.18±2.00	0.93±0.02

Table-8: Matrix effect (area) (N = 5)

Multi reaction monitoring chromatograms of voglibose are represented in the figure. **[Figure-5(a, b, c, d, e, f, g)]**

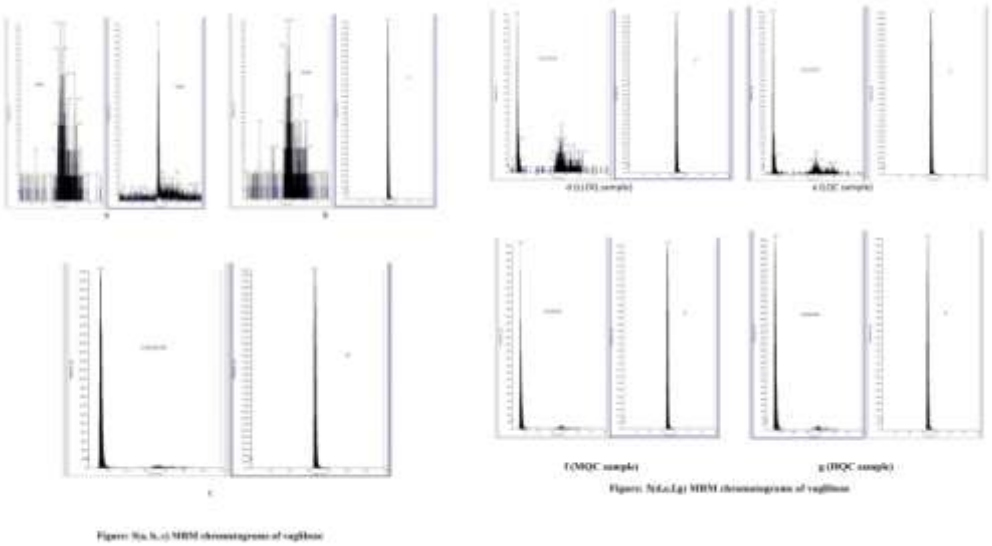

Figure-5(a, b, c, d, e, f, g): MRM chromatograms of voglibose

III.II. RESULT OF COMPARATIVE PHARMACOKINETIC STUDY OF VOGLIBOSE IN HUMAN VOLUNTEERS:

From this analytical study of voglibose, it was observed that after administration of the reference preparation containing voglibose 0.3mg tablet, as a single dose in the fasting state produced the maximum plasma concentration of 0.0007±0.0002 ng/ml (C_{max}) at the time 1.60±0.33hr (T_{max}), whereas the test preparation of one film-coated tablet containing voglibose IP 0.3mg as a single dose in the fasting state produced the maximum plasma concentration 0.0006±0.0001ng/ml at the time of 1.84±0.34hr (T_{max}).

From the pharmacokinetic parameter calculation, it was observed that area under plasma concentration-time curve AUC $_{0-t}$ of reference preparation was 0.0039±0.0010ng.hr./ml, whereas administration of the test preparation produced the area under plasma concentration-time curve AUC $_{0-t}$ was 0.0038±0.0011ng.hr/ml.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

When as a single dose, in the fasting state, the reference preparation has produced the area under plasma concentration-time curve up to infinity (AUC $_{0-\infty}$) 0.0047±0.0013ng.hr./ml, whereas administration of the test preparation produced the area under plasma concentration-time curve up to infinity (AUC $_{0-\infty}$) 0.0047±0.0015ng.hr./ml

After administration of the reference preparation, produced the plasma elimination half-life ($T_{1/2}$) 7.24±2.01hr. Whereas administration of the test preparation was produced plasma elimination half-life ($T_{1/2}$) 7.54±2.38hr.

The plasma elimination constant (K_{el}) were 0.105±0.036hr-1 for reference preparation and 0.103±0.040hr^{-1} for test preparation.

On the basis of comparison of the area under plasma concentration-time curve AUC $_{0-t}$ for voglibose after single-dose administration, the log-transformed relative bioavailability of voglibose test preparation was 100.68%.

Pharmacokinetic parameters		**VOGLIBOSE**	
		Reference Preparation (A1)	Test Preparation (A2)
C_{max} (ng./ml.)	**Mean**	0.0007	0.0006
	± S.D.	0.0002	0.0001
T_{max} (hr.)	**Mean**	1.60	1.84
	± S.D.	0.33	0.34
AUC 0-t (ng. hr./ml.)	**Mean**	0.0039	0.0038
	± S.D.	0.0010	0.0011
AUC 0-inf (ng. hr./ml.)	**Mean**	0.0047	0.0047
	± S.D.	0.0013	0.0015
k_{el} ($hr.^{-1}$)	**Mean**	0.105	0.103
	± S.D.	0.036	0.040
$T_{1/2}$ (hr.)	**Mean**	7.24	7.54
	± S.D.	2.01	2.38
Relative Bioavailability (LN RB %)		100 %	100.68%

Table-9: Pharmacokinetic parameters in 24 volunteers of voglibose

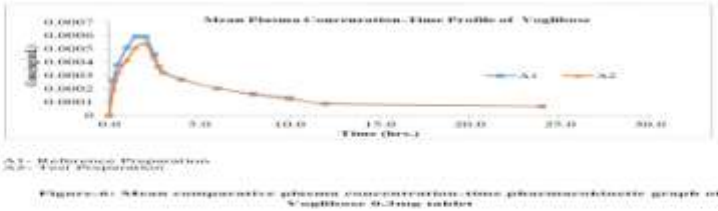

Figure-6 Mean comparative plasma concentration-time graph

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

III.III RESULT OF STATISTICAL ANALYSIS COMPARATIVE PHARMACOKINETIC STUDY:

C_{max}: The results of ANOVAs for untransformed and log-transformed data of C_{max} observed that the parameters like subject, sequence, period, and treatment were not statistically significant at 5% level in both untransformed and log-transformed data.

The 90% confidence interval for the ratio (test/reference) of geometric means, based on the log-transformed data for C_{max} was found to be 96.40 % to 100.07% relative to test preparation with reference preparation.

The 95% confidence interval for the ratio (test/reference) of geometric means, based on the log-transformed data for C_{max} was found to be 96.02 % to 100.45% relative to test preparation with reference preparation.

AUC $_{0-t}$: The results of ANOVAs for untransformed and log-transformed data of AUC $_{0-t}$ observed that the parameters like subject, sequence, and treatment were not statistically significant at 5% level in both untransformed and log-transformed data, the only period was statistically significant at 5% level in log-transformed data i.e. P<0.05 but not in untransformed data.

The 90% confidence interval for the ratio (test/reference) of geometric means, based on the log-transformed data for AUC $_{0-t}$ was found to be 97.76% to 101.21% relative to test Preparation with reference Preparation.

The 95% confidence interval for the ratio (test/reference) of geometric means, based on the log-transformed data for AUC $_{0-t}$ was found to be 97.40% to 101.57% relative to test preparation with reference preparation.

AUC $_{0-\infty}$: The results of ANOVA for untransformed and log-transformed data of AUC $_{0-\infty}$ observed that the parameters like sequence and treatment were not statistically significant at 5% level in both untransformed and log-transformed data but the period was statistically significant at 5% level in both untransformed and log-transformed data i.e. P<0.05. Next, the Subject was statistically significant at a 5% level in log-transformed data i.e. P<0.05 but not in untransformed data.

The 90% confidence interval for the ratio (test/reference) of geometric means, based on the log-transformed data for AUC $_{0-\infty}$ was found to be 97.77 % to 101.70% relative to test preparation with reference preparation.

The 95% confidence interval for the ratio (test/reference) of geometric means, based on the log-transformed data for AUC $_{0-\infty}$ was found to be 97.36 % to 102.11% relative to test preparation with reference preparation.

T_{max}: The results of ANOVA of T_{max} data for the parameters like sequence, period, and treatment were not statistically significant at 5% level but the subject was statistically significant at 5% level i.e P<0.05.

K_{el}: The results of ANOVA of K_{el} data for the parameters like subject, sequence, period, and treatment were not statistically significant at the 5% level.

$T_{1/2}$: The results of ANOVA of $T_{½}$ data for the parameters like sequence and treatment were not statistically significant at 5% level but the period was statistically significant at 5% level i.e P<0.05 Geometric mean has been calculated as the antilog (or exponential) of the least-square means of the log-transformed data.

Application of paired t-test that the C_{max} (Untransformed data) for test preparation and reference preparation was not statistically significant at 5% level and P-value = 0.076. Both the preparations were similar effects on the body.

The relative bioavailability of test preparation was found to be 96.29 % as compared to the reference preparation based on the untransformed data for AUC $_{0-t}$. The relative bioavailability of test preparation was found to be 99.40 % as compared to the reference preparation based on the log-transformed data for LN AUC $_{0-t}$.

IV. DISCUSSION:

The single-dose bioequivalence study of film-coated tablets containing voglibose IP 0.3mg, was conducted in24 adult healthy, Indian humans. male volunteers. Values of Cmax. T_{max}, and AUC $_{0-t}$, were comparable for the reference and the test preparation in the fasting state. Voglibose was detected in plasma from 0.25 hr.to24.0 hrs. for both the preparations. Peak plasma levels of voglibose were achieved for both the preparations between 1.0 to 2.75hrs. The mean peak plasma levels of voglibose with reference preparation, on the study day, ranged between 0.0004-0.00lng/ml. while the test preparation ranged between 0.0004-0.0009ng/ml. On the basis of comparison of the AUC $_{0-t}$, for voglibose after single-dose administration, the log-transformed relative bioavailability of the test preparation was 100.68% in comparison with the reference preparation.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 86-99 www.ijprajournal.com ISSN: 2249-7781

V. OVERALL CONCLUSION:

Voglibose is a very poorly absorbed drug after oral administration and metabolism in the liver is negligible. The plasma concentration of voglibose after oral administration was undetectable but now in this study plasma concentration of voglibose has been detected by liquid-liquid extraction method although it is very minute in the femtogram unit. The maximum plasma concentration of 0.0007±0.0002 ng/ml or 700±200fg/ml (C_{max}) at the time 1.60±0.33hr (T_{max}) for reference drug and 0.0006±0.0001ng/ml or 600±100fg/ml (C_{max}) at the time of 1.84±0.34hr (T_{max}). The renal excretion is negligible that is the plasma elimination constant (K_{el}) were 0.105±0.036hr^{-1} for reference preparation and 0.103±0.040hr^{-1} for test preparation. On the basis of the pharmacokinetic study, it can be concluded that the test preparation is bioequivalent with the reference preparation by this selective, sensitive, specific, high recovery, and less ionic suppression US-FDA guided bio-analytical novel LC-MS/MS method.

SAFETY ASSESSMENT:

This is the CDSCO approved drug and during the study, two volunteers were reported mild headaches and dizziness.

CONFLICT OF INTEREST:

None

ACKNOWLEDGEMENT:

The author is thankful to M/S, TAAB Biostudy Services, Kolkata-700032, India for providing human plasma and for providing the necessary instrumental facilities. The authors are acknowledging to Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India, and Calcutta School of Tropical Medicine, Kolkata 700073, India for supporting this analysis.

REFERENCES:

[1]. Apostolidis E, Kwon YI, Ghaedian R, Shetty K (2007) Fermentation of milk and soymilk by Lactobacillus bulgaricus and Lactobacillus acidophilus enhances functionality for potential dietary management of hyperglycemia and hypertension. Food Biotechnol 21:217–236.

[2]. Dan Subhasis, Bose Anirbandeep, Ghosh Balaram, Mandal Pallab, Pal Tapan Kumar. "New Drugs and Clinical Trials Rules-2018" (GSR-104E) by CDSCO: It's Impact on BA/BE Studies In India. Dan et al.,Pharamaceutical Regulatory Affairs 2018,7:2(DOI:10.4172/2167-7689,1000208)

[3]. Guidance for Industry: Bioavailability and Bioequivalence Studies for orally Administered Drug Products. United States Food and Drug Administration. 2000. Available at: www.fda.gov/downloads/Drugs/.../Guidances/ucm070124.pdf.

[4]. Guideline for Good Clinical Practice. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. E6 (R1).1996.Availableat:http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf.

[5]. Jaiswal N, Srivastava SP, Bhatia V, Mishra A, Sonkar AK (2012) Inhibition of alpha-glucosidase by acacia nilotica prevents hyperglycemia along with improvement of diabetic complications via aldose reductase inhibition. J Diabetes Metab 6:1–7

[6]. Slama G, Elgrably F, Mbemba J, Larger E (2006) Postprandial glycaemia:a plea for the frequent use of delta postprandial glycaemia in the treatment of diabetic patients. Diabetes Metab32:187–192

[7]. Yao Y, Sang W, Zhou M, Ren G (2010) Antioxidant and alphaglucosidase inhibitory activity of colored grains in China. J Agric Food Chem 58:770–774.