RESEARCH METHODOLOGY
LEC. 2

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❑Ex-vivo permeation study :

1. Fresh tissue(nasal mucosa or intestinal part or piece of skin) was carefully taken from sheep(provided by the local butcher) or rats(from the college animal house).

2. Rinsed in phosphate buffer at pH 6.4 or 6.8 or 7.4, then mounted on the Franz diffusion cell with a permeation area of 1.76 cm2 between the donor and receptor compartments as shown in the Figure below.

3. Eleven milliliters of PBS with a pH of 7.4 were poured into the receptor chamber.

4. After a 20-minute pre-incubation period, separate additions, for example of 0.25 g of prepared dosage form and aqueous drug suspension (8% Drug suspended in DD) were made. The contents of the receptor chamber were maintained at 37°C and continually stirred using the magnetic bar.

5. Over the course of three hours, 0.5 ml samples were taken at regular intervals and analyzed using a Shimadzu UV/VIS spectrophotometer to determine the drug concentration.

6. The results were expressed as the amount that reached the receptor chamber.

7. The steady-state flux (Jss) was established by calculating the slope of the line that resulted from graphing the drug's permeation rate (mg/cm^2) against various time intervals (min). By dividing the resulting slope value (Jss) over the initial LAS concentration (C_0 **or** Cd) in the donor compartment, we can determine the permeability coefficient (P) as shown in the equations listed below:

$$Jss = dM / (S.\ dt) \text{ ----------Eq. (6)}$$

$$P = Jss / Cd \text{----------Eq. (7)}$$

Where **M** is the amount of material that transfers through a unit section **S** of a barrier in unit time **t**.

Cd initial drug concentration in donar compartment,
J ss Flux value at steady state

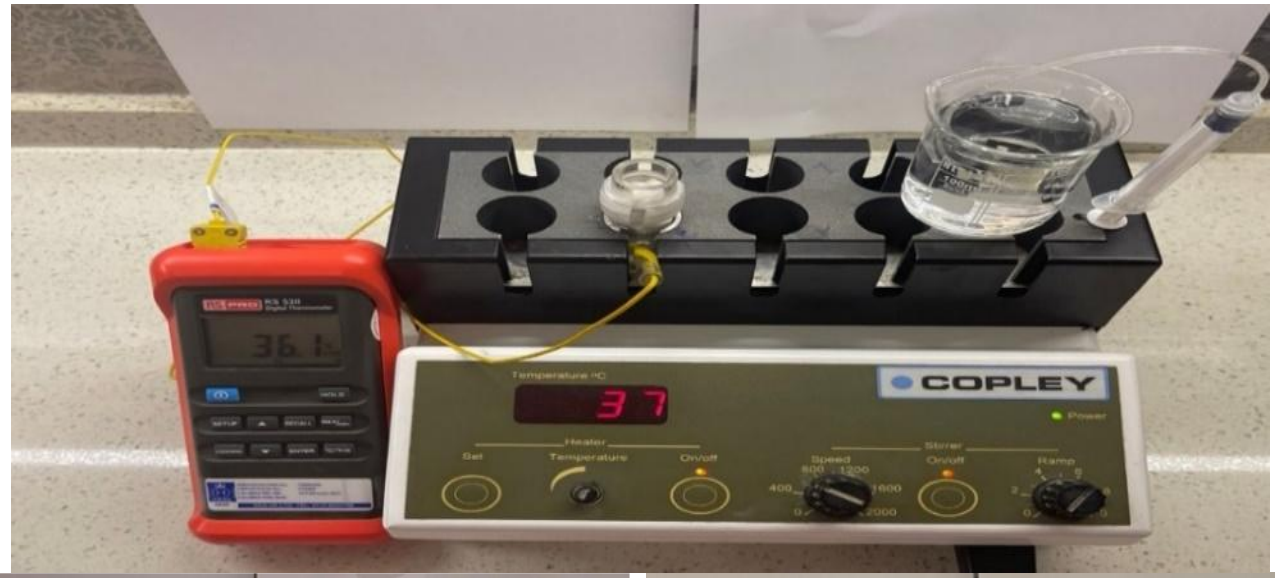
Calculation:

1. calibration curve of the drug should be performed in PBS pH7.4.

2. The saturated solubility of the drug should be made in PBS pH7.4 to check the experiment; is it **within sink condition** or not otherwise the dose should be reduced or replacement of the receptor media is required for each withdrawal.

3. Absorbance substituted in the calibration curve equation to obtain the corresponding concentration, then multiplied by the DF.

4. Obtain the amount permeated (M) by multiplying the calculated concentration by the receptor cell volume (11ml). → M/S
5. Obtain Q value (amount of drug permeated per certain surface area).
6. Plotted **d M/S**(amount of drug permeated per certain surface area at each time) versus **time**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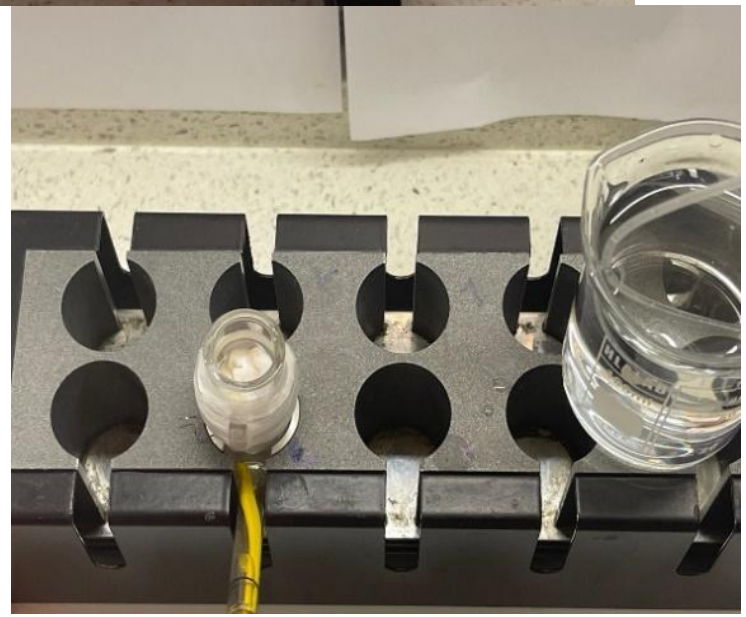
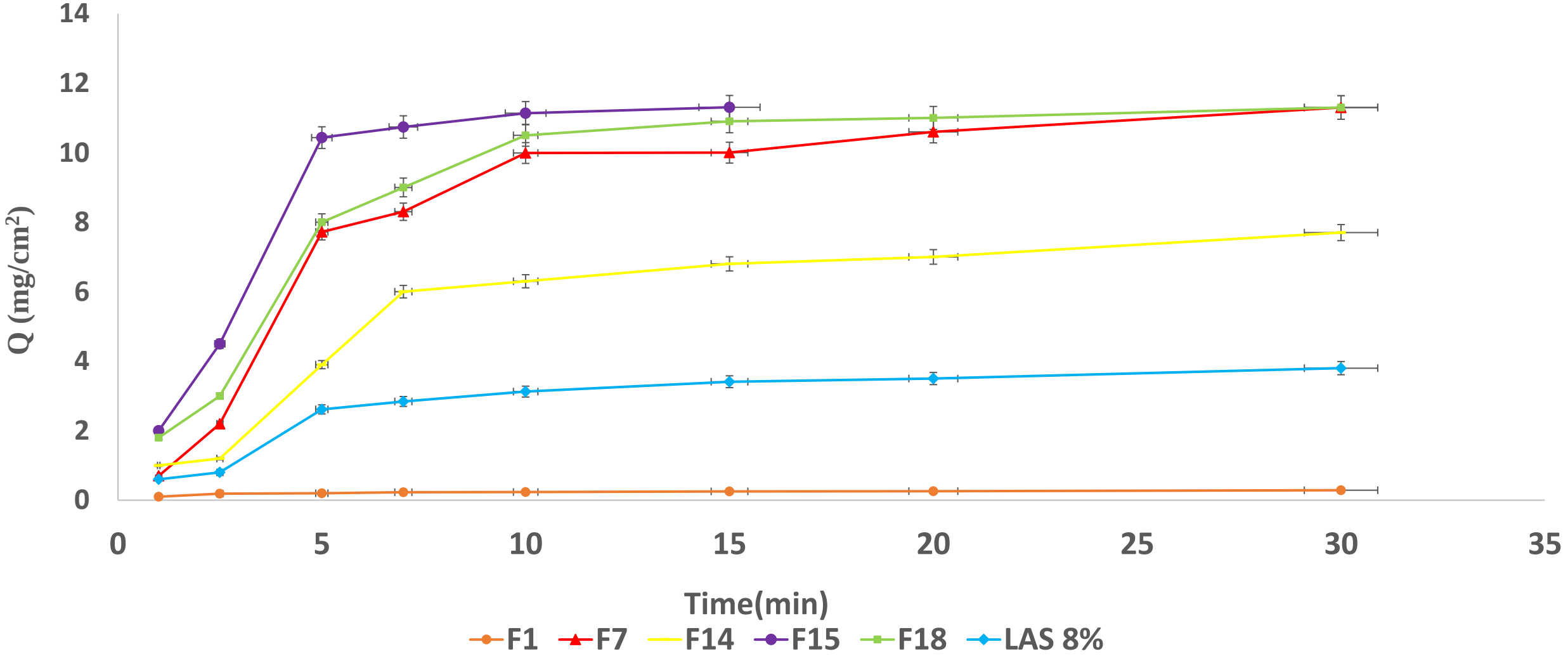




Ex-vivo permeation of the optimized NEs (F1, F7, F14, F15, F18) formulas with the pure drug (LAS): Figure (a) represents the experimental duration of only 30 min.

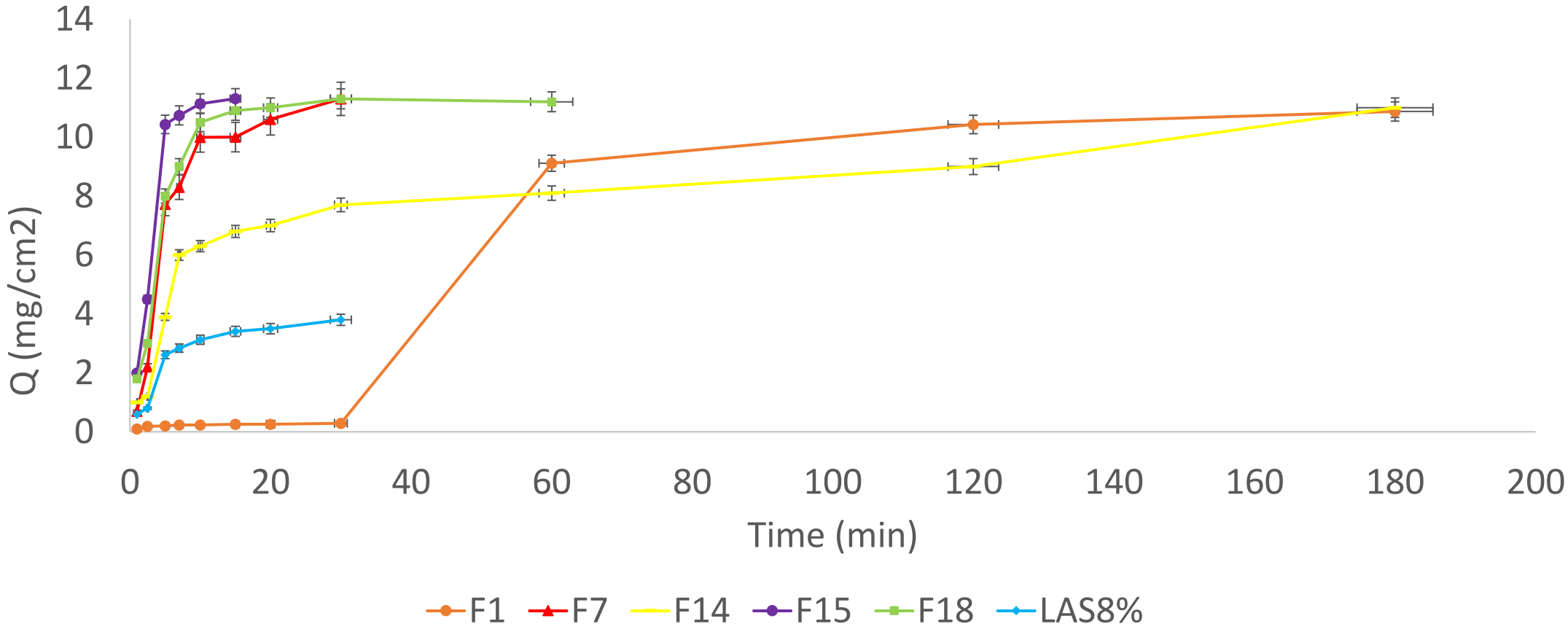


figure (b) represents the study duration for 3 h (180 min)

Table. *Ex-Vivo* Permeation Parameters

Formula code	Lag time (min)	Flux (J)- ($mg/cm^2.min$)	$P=J/Cd *10^{-2}$ (cm/min)
F1	30	0.294	0.368
F7	1.6	2.21	2.76
F14	1.5	1.05	1.31
F15(NE)	0.5	2.37	2.97
F18	1.1	2	2.5
Drug-LAS	2	0.724	0.91

In-Vivo Pharmacokinetics Study

Research Plan

1. Ethics Committee of the College of Pharmacy at the University of Baghdad with the approval name REACUBCP2542022A.

2. **The BSA normalization method** and the human equivalent dose (HED) of pharmaceuticals, including the species (**Km**) factor (**body weight in kg divided by BSA in m2**), were used to calculate the animal nasal.

HED (mg) = (Animal Km/ Human Km) x Animal Dose (mg)----Eq. 8[1]

OR:

Animal Dose (mg) = (Animal Km/ Human Km)/ HED (mg) ----Eq. 9

Drug X intravascular single daily dose is advised to be 0.28 mg/kg for adults, and the values of Km were **37** and **12** for adult humans and rabbits, respectively. Hence, the dose for rabbits based on the BSA normalization method was **1.72 mg** and reached **17.26 mg** after multiplying by 10 (safety factor) [2].

1. Maraie NK, Almajidi YQ, Alshadher A. Absolute and relative bioavailability study for the newly developed nasal nanoemulsion in situ gel of ondansetron HCl in comparison to conventionally prepared in situ gel and intravenous dosage forms. *Int J Appl Pharm*. 2018;10(5):105-109.

2. Shin JW, Seol IC, Son CG. Interpretation of animal dose and human equivalent dose for drug development. *J Korean Med Sci* 2010; 31(3): 1-7.

3. Eighteen albino male rabbits weighing between 1.8 and 2.0 kg were employed in the *in vivo* study. They were divided into three groups, each with six rabbits. Before the experiment began, the rabbits fasted for 24 hours.

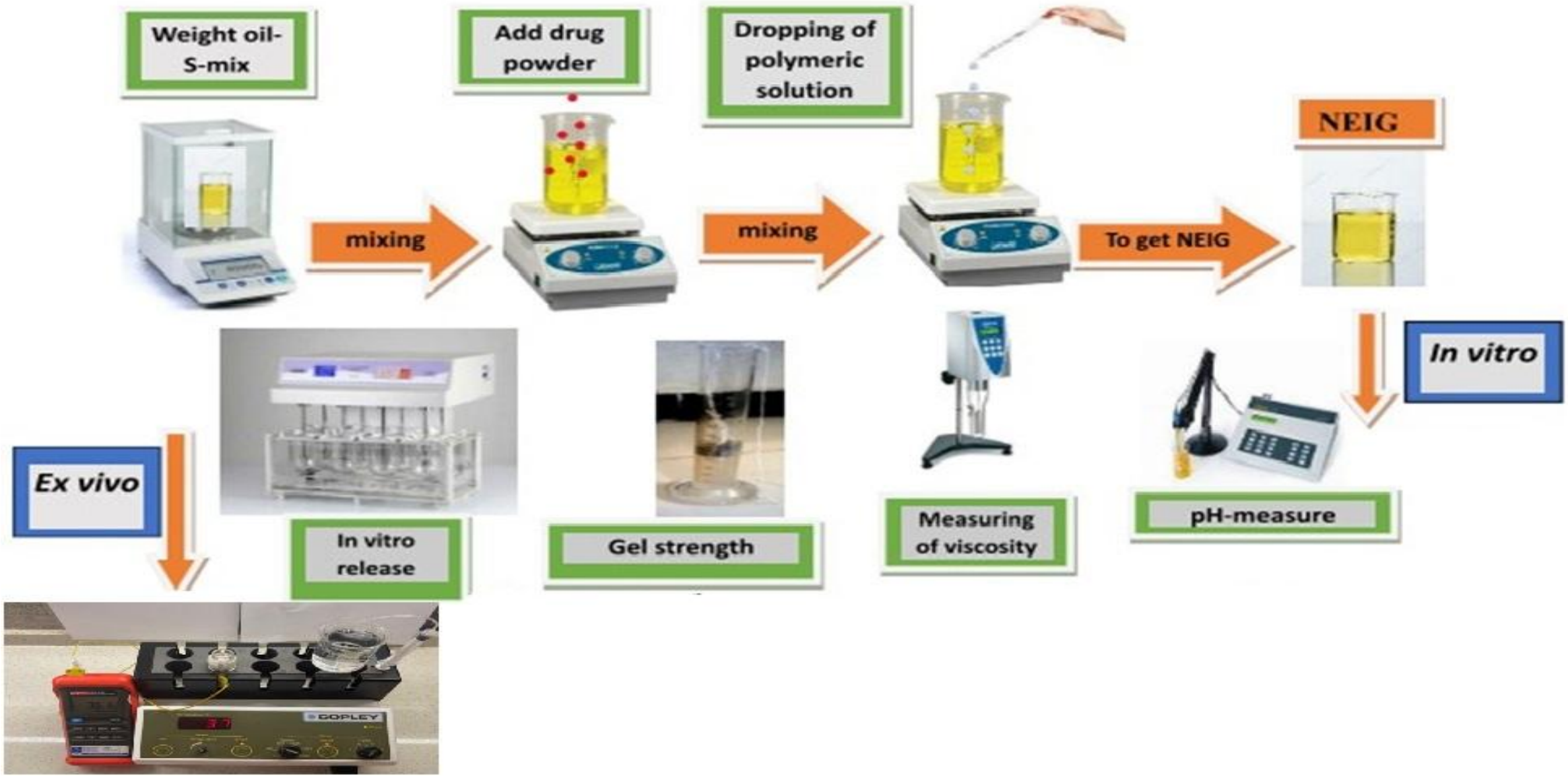
4. At a dosage of 0.863 mg/kg (calculated as predicted in the previous section), **Group I** received drug X as AQS (reference). **Group II** received **the first selected formula** intranasally at a dosage of 0.863 mg/kg as well as **Group III** received **the second selected formula** intranasally at the same dose.

5. 2ml blood samples were collected in EDTA tubes before the medication was administered (control sample), and then again at 0.25, 0.5,0.75, 1.0, 2.0, 3.0, 5.0, 7.0, 12, and 19 h after the drug was administered, centrifuged as shown in the Figure listed below.

6. Samples(supernatant) were kept at -20C° and then subjected to analysis utilizing the HPLC technique.

NOTE: Based on the results of the *in vitro* and *ex vivo* studies, the selected formulas were passed into *in vivo* examination.

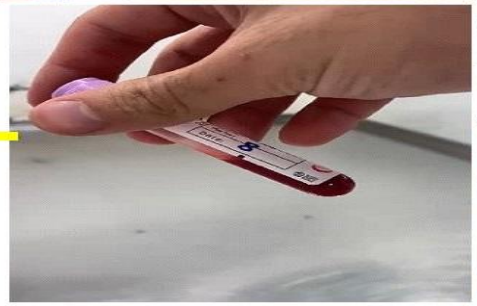
GRAPHICAL ABSTRACT



In vivo

Collection of blood samples

Centrifuge

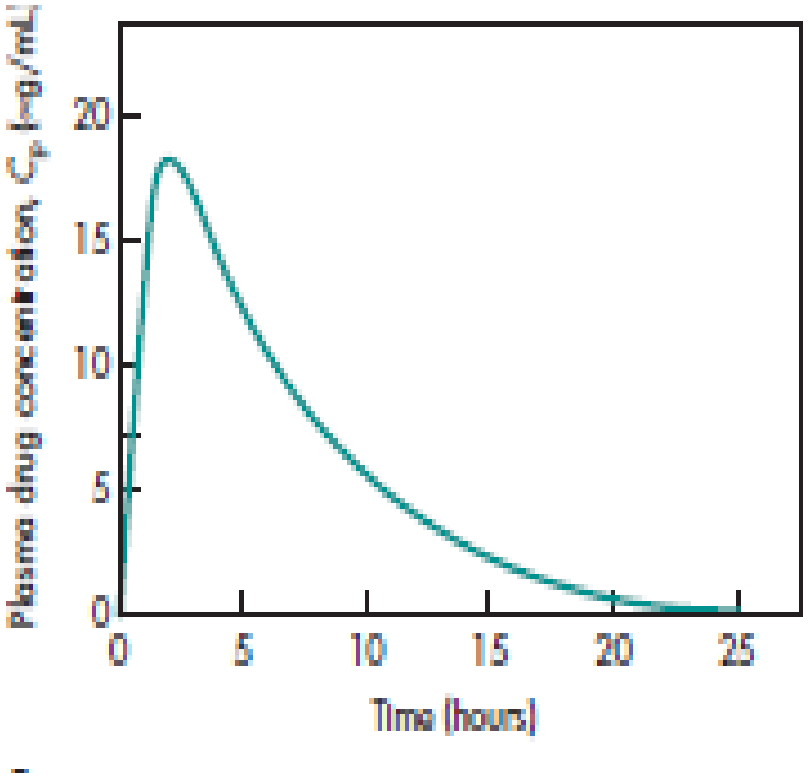


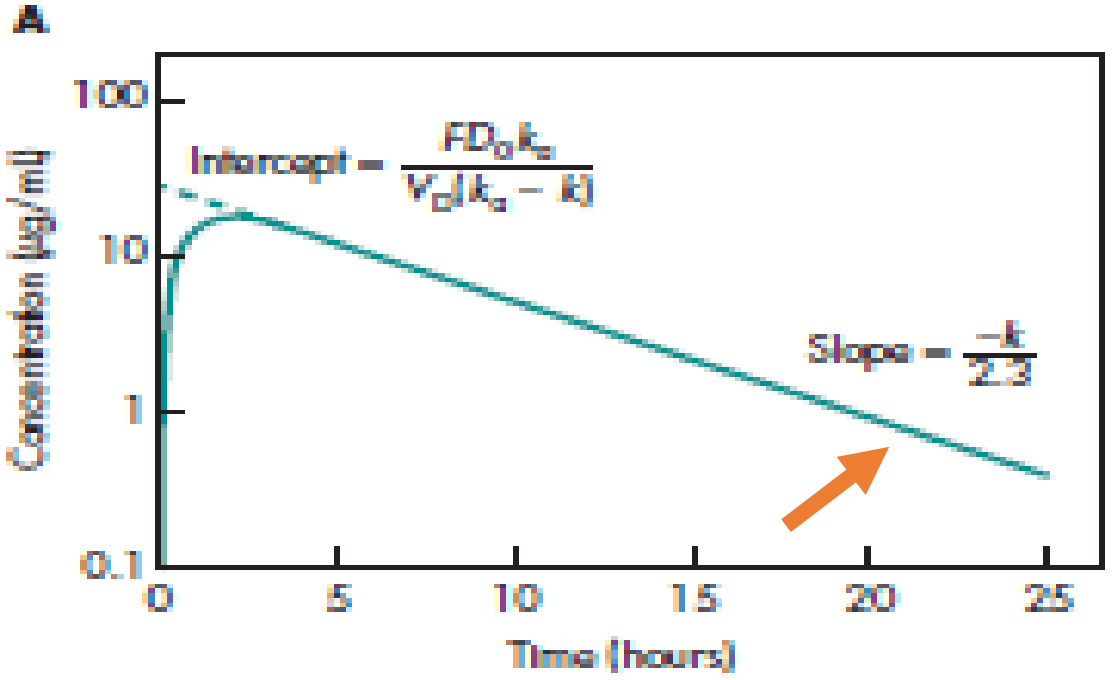
Analysis By HPLC

- **Analysis of data**:
- By using the plasma concentration-time curve (Cp versus T curve),
- C max and T max following oral and intranasal delivery can be calculated.
- T0.5 is calculated by dividing the value of Ln 2 over Ke.
- Ke is predicted from the slope of the straight section of the terminal phase (where the slope is equal to - Ke/2.303).
- Calculate the AUC for each period using the trapezoidal method.
- This was done for each rabbit by calculating the area under the Cp-time curve from time 0-19 h (AUC t 0-19).
- Where Cp 19 is the concentration of drug after 19 h.

Calculation of elimination rate constant using plasma data

- The first-order elimination rate constant may be determined from the elimination phase of the plasma level–time curve at later time intervals when drug absorption has been completed.





Calculate AUC for each time interval as follow: (using trapezoidal method by plotting Cp versus time on ordinary paper or using the data in the table only)

- AUC $_1$=(CP_1/2)t_1
- AUC 2 =[CP_2+CP_1/2](t_2-t_1)
- AUC 3=[CP_3+CP_2/2](t_3-t_2)
- AUC 4=[CP_4+CP_3/2](t_4-t_3)
- AUC 5=[CP_5+CP_4/2](t_5-t_4)

- AUC 19=[CP_{19}+ CP_{18}/2](t_{19}-t_{18})
- AUC last= Cp last/k

Or AUC last =CP 19/K

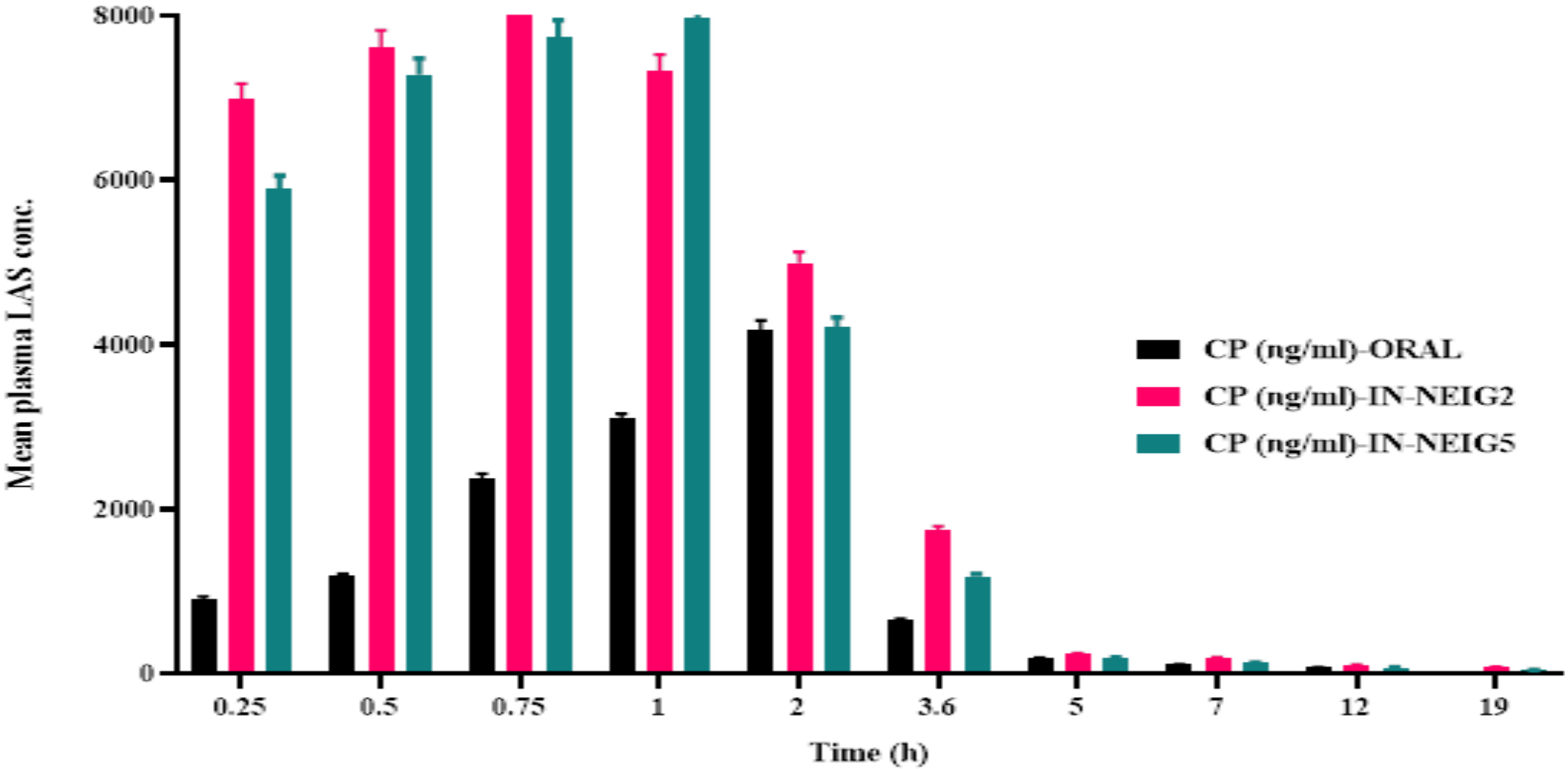
- AUC total =AUC1 +AUC 2+AUC 3+AUC 4+ AUC 5+-----+ AUC last

- The last measured area (AUC last), calculated by dividing Cp last over Ke.
- Calculate cumulative AUC value for each time [AUC]0t

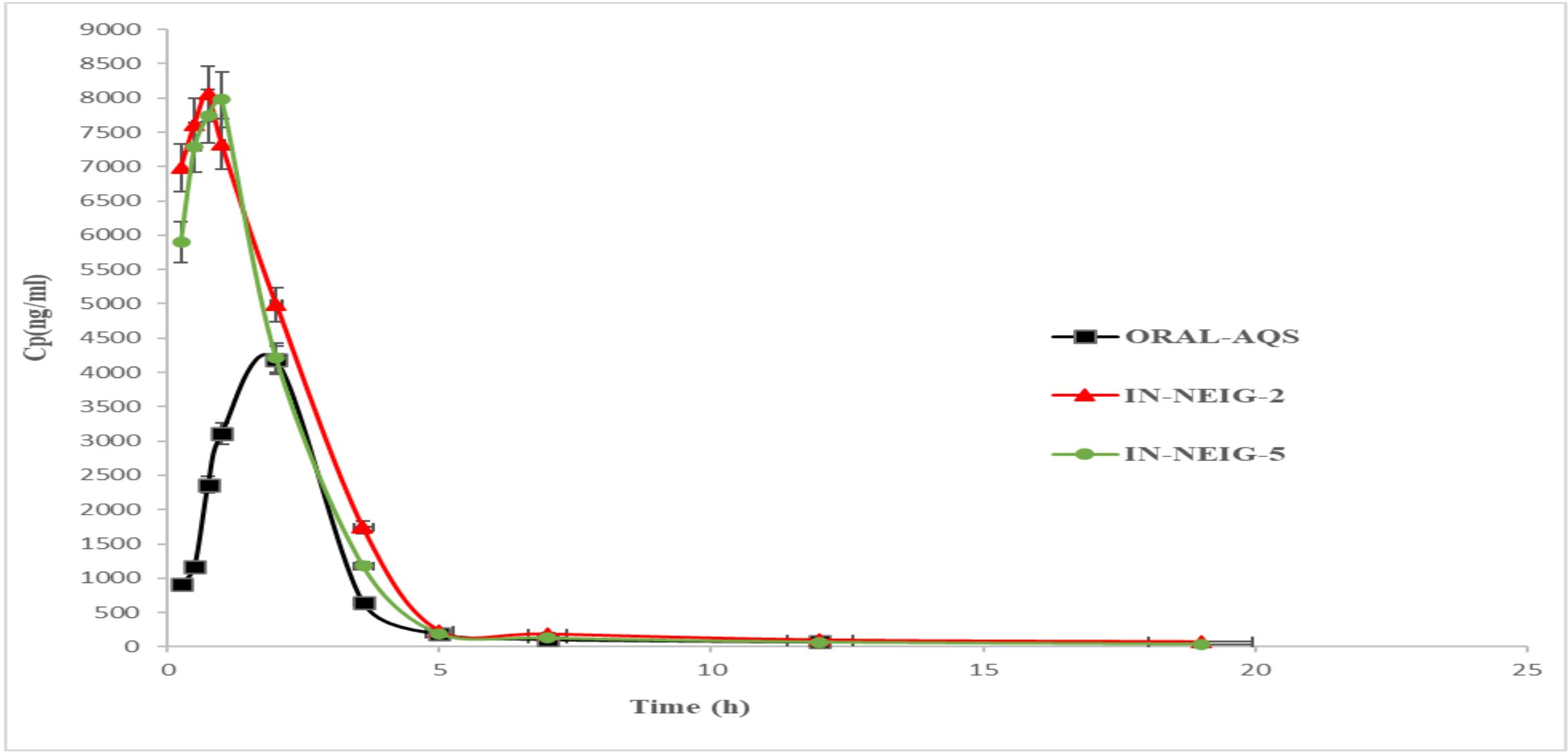
$$\mathbf{AUC_{0-\alpha} = AUC_{0-19} + [\ Cp_{\ last} / Ke]}$$

- The below equation was used to determine F relative :

$$\mathbf{F_{\ relative} = (AUC_{\ SF} / AUC_{\ oral} \times Dose_{\ oral} / Dose_{\ NEIG}) \times 100}$$



Arithmetic means and standard deviation values of plasma drug concentration (ng/ml) of LAS (Mean Cp) at the selected time intervals



The profile of Mean Cp versus time during the pharmacokinetic experiment applied on 18 rabbits each 6 were given a single dose of drug as SF1**, the other 6 were given** SF2 **(both given intranasally), and the last group received aqueous drug suspension AQS (given orally).**

Parameters of A single Intranasal Dose of LAS as Optimized SF1, SF2, and Single Oral AQS (8%LAS).

Kinetic parameters	Oral-LAS (control)	Intranasal LAS (SF1)	Intranasal LAS (SF2)
C max(ng/ml)	4181.09±125	8066±242	7975.67±239
T max(h)	2±0.2	0.75±0.05	1±0.05
AUC0---19 (ng. h/ml)	8852.67±266	19616.86±589	17912.36±537

- Calculation of each AUC:

IN -AUC las	AUC of hydroch.	AUC ratio	CP(Mg/ml)	AUC NO.	AUC 0---T(Mg.h/ml)	time (h)
133254.08	1980.49	67.28338946	6986.647919	AUC1	873.33	0.25
145256.08	1979.85	73.36721469	7618.405471	AUC2	1825.58125	0.5
154120.12	1984.08	77.6783799	8066.086179	AUC3	1960.5	0.75
140114.98	1984.79	70.59436011	7330.466263	AUC4	1924.5	1
95542.98	1988.05	48.05864038	4990.30845	AUC5	6160	2
33256.05	1981.49	16.78335495	1742.61526	AUC6	2019.6	2.6
4521.08	1983.65	2.279172233	236.4695984	AUC7	2374.2	5
3655.12	1986.58	1.839905768	190.8552199	AUC8	427.35	7
2017.25	1985.89	1.015791408	105.2774048	AUC9	740.325	12
1490.22	1983.69	0.751236332	77.8054343	AUC10	640.78	19
				AUC terminal	670.6896552	
				AUC total 0---∞	19616.85591	

AUC NO.	n1	n2	n3	n4	n5	n6	Mean value	STDEVA
AUC1	873.33	873.33	873.33	873.33	916.9965	829.6635	873.33	27.6
AUC2	1825.58125	1825.58	1825.58	1825.58125	1916.860313	1734.302188	1825.58125	57.7
AUC3	1960.5	1960.50	1960.50	1960.5	2058.525	1862.475	1960.5	62.0
AUC4	1924.5	1924.50	1924.50	1924.5	2020.725	1828.275	1924.5	60.9
AUC5	6160	6160.00	6160.00	6160	6468	5852	6160	194.8
AUC6	2019.6	2019.60	2019.60	2019.6	2120.58	1918.62	2019.6	63.9
AUC7	2374.2	2374.20	2374.20	2374.2	2492.91	2255.49	2374.2	75.1
AUC8	427.35	427.35	427.35	427.35	448.7175	405.9825	427.35	13.5
AUC9	740.325	740.33	740.33	740.325	777.34125	703.30875	740.325	23.4
AUC10	640.78	640.78	640.78	640.78	672.819	608.741	640.78	20.3
AUC terminal	670.6896552	670.69	670.69	670.6896552	704.2241379	637.1551724	670.6896552	21.2
AUC total 0---∞	19616.85591	19616.86	19616.86	19616.85591	20597.6987	18636.01311	19616.85591	620.3

ORAL

AUC NO.	n1	n2	n3	n4	n5	n6	Mean value	STDEVA
AUC1	113.64375	113.64375	113.64375	113.64375	119.3259375	107.9615625	113.64375	3.59
AUC2	259.85625	259.85625	259.85625	259.85625	272.8490625	246.8634375	259.85625	8.22
AUC3	441.71	441.71	441.71	441.71	463.7955	419.6245	441.71	13.97
AUC4	684.8475	684.8475	684.8475	684.8475	719.089875	650.605125	684.8475	21.66
AUC5	3647.945	3647.945	3647.945	3647.945	3830.34225	3465.54775	3647.945	115.36
AUC6	1448.796	1448.796	1448.796	1448.796	1521.2358	1376.3562	1448.796	45.81
AUC7	584.899	584.899	584.899	584.899	614.14395	555.65405	584.899	18.50
AUC8	295.84	295.84	295.84	295.84	310.632	281.048	295.84	9.36
AUC9	296.25	296.25	296.25	296.25	311.0625	281.4375	296.25	9.37
AUC terminal	578.88	578.88	578.88	578.88	607.824	549.936	578.88	18.31
AUC total 0---∞	8352.6675	8352.6675	8352.6675	8352.6675	8770.300875	7935.034125	8352.6675	264.13

IN 2

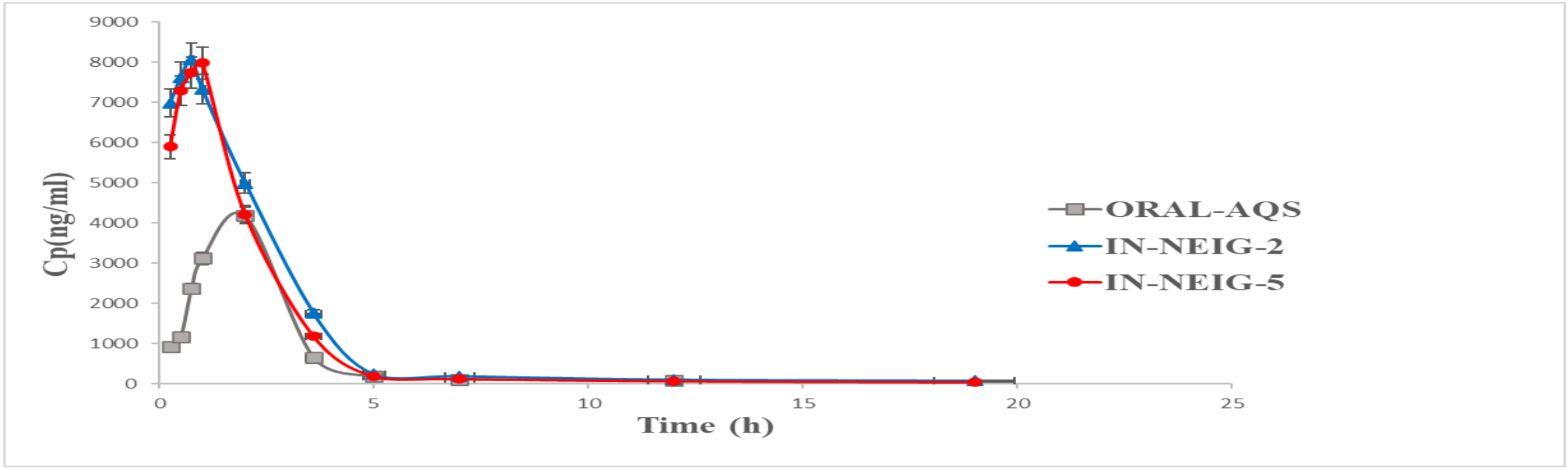
AUC NO.	n1	n2	n3	n4	n5	n6	Mean value	STDEVA
AUC1	736.903208	736.903208	736.90321	736.903208	736.903208	700.0580476	736.903208	15.04
AUC2	1647.390808	1647.390808	1647.3908	1647.390808	1647.390808	1565.021267	1647.390808	33.63
AUC3	1877.70747	1877.70747	1877.7075	1877.70747	1877.70747	1783.822096	1877.70747	38.33
AUC4	1964.178067	1964.178067	1964.1781	1964.178067	1964.178067	1865.969163	1964.178067	40.09
AUC5	6095.533701	6095.533701	6095.5337	6095.533701	6095.533701	5790.757016	6095.533701	124.42
AUC6	2696.235441	2696.235441	2696.2354	2696.235441	2696.235441	2561.423669	2696.235441	55.04
AUC7	1365.790104	1365.790104	1365.7901	1365.790104	1365.790104	1297.500599	1365.790104	27.88
AUC8	315.244312	315.244312	315.24431	315.244312	315.244312	299.4820964	315.244312	6.43
AUC9	489.4074166	489.4074166	489.40742	489.4074166	489.4074166	464.9370458	489.4074166	9.99
AUC10	378.8383048	378.8383048	378.8383	378.8383048	378.8383048	359.8963895	378.8383048	7.73
AUC terminal	345.1310695	345.1310695	345.13107	345.1310695	345.1310695	327.874516	345.1310695	7.04
AUC total 0---∞	17912.3599	17912.3599	17912.36	17912.3599	17912.3599	17016.74191	17912.3599	365.63

time (h)	CP (ng/ml)-ORAL-n1	CP (ng/ml)-ORAL-n2	CP (ng/ml)-ORAL-n3	CP (ng/ml)-ORAL-n4	CP (ng/ml)-ORAL-n5	CP (ng/ml)-ORAL-n6	Mean value-ORAL
0.25	909.15	863.75	954.55	863.8	954.4	909.2	**909.14**
0.5	1169.7	1228.19	1111.21	1228.1	1111.2	1169.7	**1169.68**
0.75	2363.98	2482.18	2248.78	2412.16	2245.71	2363.97	**2352.80**
1	3114.8	3270.55	2959.06	3270.1	2959.1	3114.8	**3114.74**
2	4181.09	4390.145	3972.035	4390.1	3972.01	4181.09	**4181.08**
3.6	648.23	680.63	615.83	680.1	615.5	648.2	**648.08**
5	187.34	196.71	177.97	196.6	177.8	187.3	**187.29**
7	108.5	113.9	103.07	114	102.9	108	**108.40**
12	72.36	75.98	68.7	76	68.68	72.3	**72.34**
19							

time (h)	CP (ng/ml)-IN-NEIG2-n1	CP (ng/ml)-IN-NEIG2-n2	CP (ng/ml)-IN-NEIG2-n3	CP (ng/ml)-IN-NEIG2-n4	CP (ng/ml)-IN-NEIG2-n5	CP (ng/ml)-IN-NEIG2-n6	Mean value -IN-NEIG2
0.25	6986.65	7335.98	6637.32	7336	6636.7	6986.4	**5822.15**
0.5	7618	7998.9	7237.1	7999.1	7236.7	7617.8	**6348.38**
0.75	8066	8469.3	7662.7	8468.7	7663.2	8065.8	**6721.78**
1	7330	7696.5	6963.5	7697	6962.9	7329.8	**6108.48**
2	4990	5239.5	4740.5	5238.9	4739.8	4989.9	**4158.45**
3.6	1742	1829	1654.9	1828.9	1655	1741.8	**1452.23**
5	236.5	248.33	224.7	249	223.9	237	**197.91**
7	190.85	200.4	181.3	199.99	180.9	190.9	**160.07**
12	105.28	110.54	100.02	110.51	99.89	105.3	**89.71**
19	77.8	81.7	73.9	81.67	74	77.6	**68.01**

time (h)	CP (ng/ml)-IN-NEIG5-n1	CP (ng/ml)-IN-NEIG5-n2	CP (ng/ml)-IN-NEIG5-n3	CP (ng/ml)-IN-NEIG5-n4	CP (ng/ml)-IN-NEIG5-n5	CP (ng/ml)-IN-NEIG5-n6	Mean value -IN-NEIG5
0.25	5895.2	6189.96	5600.44	6190	5599.89	5894.89	**5895.06**
0.5	7283.9	7648.09	6919.7	7648.3	6920	7284	**7284.00**
0.75	7737.75	8124.6	7350.97	8123.9	7351	7737.58	**7737.63**
1	7975.67	8374.45	7576.72	8375	7576.8	7976	**7975.77**
2	4215.4	4426.17	4004	4426.2	4005	4214.98	**4215.29**
3.6	1177.07	1235.92	1118.22	1236	1117.98	1176.98	**1177.03**
5	188.72	198.16	179.28	198.2	178.9	189	**188.71**
7	126.52	132.846	120.19	132.9	119.98	126.48	**126.49**
12	69.24	72.74	65.74	72.7	65.69	69.18	**69.22**
19	38.99	41	37.04	40.9	36.98	38.87	**38.96**

time (h)	CP (ng/ml)-ORAL	CP(ng/ml)- IN	Cp(ng/ml)-IN2
0.25	909.15	6986.65	5895.2
0.5	1169.7	7618	7283.9
0.75	2363.98	8066	7737.75
1	3114.8	7330	7975.67
2	4181.09	4990	4215.4
3.6	648.23	1742	1177.07
5	187.34	236.5	188.72
7	108.5	190.85	126.52
12	72.36	105.28	69.24
19		77.8	38.99



PHARMACOKINETICS PARAMETER						
C-MAX OF ORAL	4181.09	4390.145	3972.035	4390.1	3972.01	4181.09
C-MAX OF IN	8066	8469.3	7662.7	8468.7	7663.2	8065.8
C-MAX OF IN2	7975.67	8374.45	7576.72	8375	7576.8	7976

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
C-MAX OF	6	25086.47	4181.078	34961.52
C-MAX OF	6	48395.7	8065.95	129943.4
C-MAX OF	6	47854.64	7975.773	127349.7

ANOVA

Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	59000140	2	29500070	302.8189	7.49E-13	3.68232
Within Gro	1461273	15	97418.19			
Total	60461413	17				

Anova: Single Factor

AUC Total for ORAL	8352.668	8770.300	7935.060	8770.300	7935.060	8352.668
AUC Total for IN	19616.856	20597.660	18636.060	18636.060	20597.660	8352.668
AUC Total for IN2	17912.360	18807.980	17016.700	18807.200	17017.000	8352.668

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AUC Total	6	50116.06	8352.676	139525.2
AUC Total	6	106437	17739.49	21916577
AUC Total	6	97913.91	16318.98	15872280

Anova: Single Factor

SUMMARY

Groups	*Count*
T-MAX OF	6
T-MAX OF	6
T-MAX OF	6

ANOVA

Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	3.07E+08	2	1.54E+08	12.1486	0.000729	3.68232
Within Gro	1.9E+08	15	12642794			
Total	4.97E+08	17				

RATE OF DRUG ABSORPTION							
T-MAX OF ORAL	2	2.1	1.88	1.9	2.09	2	
T-MAX OF IN	0.75	0.71	0.78	0.77	0.7	0.75	
T-MAX OF IN2	1	1.05	0.8	0.88	1	1.1	
Anova: Single Factor							
SUMMARY							

Groups	*Count*	*Sum*	*Average*	*Variance*
T-MAX OF ORAL	6	11.97	1.995	0.00847
T-MAX OF IN	6	4.46	0.7433333	0.0010267
T-MAX OF IN2	6	5.83	0.9716667	0.0124167

Anova: Single Factor

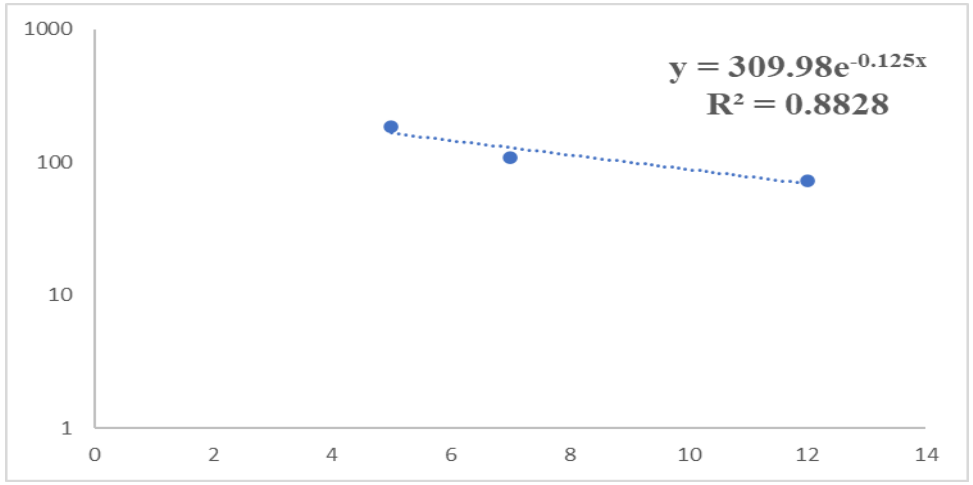
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
T-MAX OF	6	11.97	1.995	0.00847
T-MAX OF	6	4.46	0.743333	0.001027

ANOVA

Source of Varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between G	4.700008	1	4.700008	989.8227	2.47E-11	4.964603
Within Grc	0.047483	10	0.004748			
Total	4.747492	11				

5	187.34	ke= 0.125 h-
7	108.5	t0.5= 5.5h
12	72.36	



5	236.5	ke=0.116h-
7	190.85	t0.5 =5.9 h
12	105.28