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RESEARCH ARTICLE
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In-Vivo Study of Dipyridamole Liquisolid Compacts

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ABSTRACT

The purpose of this study was to employ a 32-factorial design to develop and assess dipyridamole liquisolid compacts. An in vivo study was conducted in albino Wistar rats to determine the pharmacokinetic parameters for the optimized formulation. Dipyridamole plasma concentration-time data pharmacokinetic analysis yielded the following pharmacokinetic parameters: Cmax values ranging from 511.77 ± 10.18 ng/mL to 621.16 ±14.32 ng/mL; Tmax values ranging from 1.25 ± 0.03 to 0.75 ± 0.02 hours; and AUC values ranging from 2332.90 ± 112.10 h.ng/mL to 2059.80 ± 124.42 h.ng/mL. Results obtained for the formulated product prepared with liquid-solid compacts technology show rapid release in comparison to the conventional formulation showing variability in pharmacokinetic parameters.

Keywords: *Dipyridamole, Liquisolid, Factorial, AUC, Cmax, Tmax*

INTRODUCTION

Solubility or dissolution enhancement strategies continue to be the most active areas of formulation science research. This is one of the most often debated but unresolved questions. Dissolution and solubility are basic principles in physical and chemical sciences, as well as biopharmaceutical and pharmacokinetic challenges. A drug's solubility/dissolution behaviour determines its oral bioavailability, with the gastrointestinal tract serving as the rate-limiting stage in its absorption. More than 40% of novel drug candidates fail to reach drug development pipelines due to suboptimal biopharmaceutical properties. [1].

Over time, the dissolution profile and, as a result, the absorption efficiency and bioavailability of water-insoluble drugs and/or liquid lipophilic medications have improved [2].

Several studies have shown that liquisolids are the most promising method for promoting drug dissolution [3-5]. Liquid lipophilic medications can be turned into liquisolids without further modification. When creating a solid water-insoluble drug, the choice of non-volatile solvent is critical. It should first be dissolved or suspended in the non-volatile solvent system that will yield the desired concentration of drug solution or drug suspension. It is advised that inert, water-mixable organic solvents with a high boiling point be used for liquid vehicles. Propylene glycol, liquid polyethylene glycols, polysorbates, fixed oils, and glycerine are a few examples [5]. Dipyridamole is a PDE3 inhibitor and a nucleoside transport inhibitor that prevents blood clot formation [6]. When given in high doses over a short period of time, it produces blood vessel dilatation when given chronically.

Dipyridamole inhibits the phosphodiesterase enzymes that typically break down cAMP and/or cGMP (raising cellular cAMP levels and inhibiting platelet aggregation in response to ADP). Dipyridamole inhibits adenosine cellular absorption into platelets, red blood cells, and endothelial cells, resulting in higher extracellular adenosine concentrations. [7]. As a result, the goal of this study was to evaluate the pharmacokinetics of an optimized liquisolid formulation of dipyridamole reported in literature. [8]

METHOD

Experimental Design

Design Expert trial version 13.00 (StatEase Inc., Minneapolis, MN, USA) was used to optimize the formulations. To determine the optimum values of the most influencing factors, 32 factorial design was applied, and a response surface equation was derived in order to investigate the interaction between the factors. In this design 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations as shown in Table 1. The two independent variables were selected as X1 and X2. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the response. [8]

TABLE 1: Formulation of Liquisolid Tablets of Dipyridamole: [8]

Ingredients (mg)	DPY1	DPY2	DPY3	DPY4	DPY5	DPY6	DPY7	DPY8	DPY9
Dipyridamole	50	50	50	50	50	50	50	50	50
Peceol	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Avicel 112	100	130	70	70	70	100	130	130	100
Aerosil 200	3.5	9.5	9.5	6.5	3.5	9.5	6.5	3.5	6.5
Sodium starch glycolate	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
PVP K- 30	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Dicalcium phosphate	66.44	30.44	90.44	93.44	96.5	60.44	33.44	36.44	63.44
Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Talc	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total weight	250	250	250	250	250	250	250	250	250

The optimum formulation was found to exhibit angle of repose of 25.12°, hardness of 3.1 kg/cm2 and drug release of 99.22 % at 10 mins. The observed values were found to be similar to the predicted values. Based on these observations, DPY–O can be considered to be the optimum formulation. [8]

Animal Ethics

All animal experiments approved and performed in Jeeva Life Sciences accordance with the guidelines of Institutional Animal Ethics Committee (CPCSEA Registration No: CPCSEA/IAEC/JLS/18/07/22/019).

Animal Husbandry and Maintenance

Healthy adult male Wistar strain albino rats (weighing 200-300 g, 4-5 weeks of age) were obtained from Animal house; Standard laboratory diet, water and libitum were provided to the caged animals. Animals should be individually housed. The temperature of the experimental animal room should be 20°-26°C

for rats. Although the relative humidity should be at least 30% and preferably not exceed 70%, other than during room cleaning, the aim should be 50-60%. The lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unrestricted supply of drinking water. Only healthy animals were assigned for these studies according to OECD Guidelines 404 [9]. Approval to carry out these studies was obtained from the Institutional Animal Ethics Committee and an experiment was performed in compliance with the Principles of Laboratory Animal Care (NIH Publication 85-23, revised 1985) [10]. All of the animal experimental protocols were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals, Ministry of Forest and Environment, Government of India.

Liquid Chromatography Conditions

Liquid chromatography. The chromatography was performed on an Acquity Ultra Performance LCTM system (Waters Corp.) with cooling auto sampler. Separation was achieved on an Agilent Poroshell 120 EC-*C18*, *3* x *50 mm*, 2.7 *µm* column at ambient temperature with an isocratic mobile phase consisting of methanol–ammonium acetate (5 mM; 75 : 25, v/v) at a flow rate of 0.3 mL/min. The autosampler temperature was kept at 5°C and 15 µL of sample solution was injected in full loop mode. After each injection, the sample manager underwent a needle wash process, including strong wash (methanol: water = 90: 10, v/v) and weak wash (methanol: water = 10: 90, v/v). [11]

Mass Spectrometry

A triple-quadrupole tandem mass spectrometric detection was carried out on a Micromass® Quattro micro TM API mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) interface set in positive ionization mode. Quantification was performed using multiple-reaction monitoring (MRM) of the transitions of m/z 505.4 → 429.3 for dipyridamole and m/z 285.0 → 193.0 for diazepam, with a scan time of 0.20 s per transition. The optimal MS parameters were as follows: capillary voltage 3 kV, cone voltage 60 kV for dipyridamole and 30 kV for diazepam, source temperature 110°C and desolvation temperature 350°C. Nitrogen was used as the desolvation and cone gas with a fl ow rate of 400 and 30 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 0.265 psi. The optimized collision energy for dipyridamole was 40 eV, and for diazepam 30 eV. All data collected in the centroid mode were acquired and processed using MassLynxTM NT 4.1 software with QuanLynxTM program (Waters Corp.).[11]

Preparation of calibration standards and quality control samples

Stock standard solutions of dipyridamole and diazepam were both prepared by dissolving the accurately weighed standard compounds in methanol with final concentrations of 8000 ng/mL, respectively. Working solutions were obtained from the stock solution by a series dilution with methanol. For the preparation of three levels of quality control samples (low, LQC, medium, MQC, and high-quality controls, HQC), separately prepared stock solution were further diluted. All the stock and working solutions were stored at 4°C and brought to room temperature before use. Calibration standards were prepared in the range of 20, 40, 80, 400, 800, 4000 and 8000 ng/mL. The quality control samples were prepared with blank plasma sample at the concentrations of 20, 400 & 8000 ng/mL for LQC, MQC, HQC and aliquots were stored at −20°C after preparation. One set of standards and quality controls was analyzed on each analysis day with the same procedure for plasma samples as described below. [11]

Sample preparation

An aliquot of 100 µL diazepam was transferred into a 1.5 mL polypropylene micro-centrifuge tube, and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was mixed with 200 µL of plasma sample and vortexed vigorously for 30 s. Then 400 µL methanol was added to precipitate the protein, followed by vortex for 60 s and centrifugation for 15 min at 14,000 rpm. The supernatant was transferred into an autosampler vial, and an

aliquot of 20 µL was injected into the HPLC-MS/MS system for the analysis. [11]

Application of the method

The LC–MS-MS procedure was developed to determine dipyridamole concentrations in rat plasma 0–24 h. After an initial period of acclimatisation for one week to laboratory conditions, the rats were randomly divided into 2 groups of 3 subjects each. All the rats were fasted for twelve hours with impromptu access to water before the experiment. Dose of drug was administered according to Animal Equivalent Dose Calculations.

Maximum Dose Per Day- 400mg/Day/60kg-6.666mg/kg

AED = 6.666/0.162 = 4.111 mg

Group 1: Administered with Pure Drug

Group 2: Administered with Optimized Formulation (DPY-O)

These rats were administered with conventional formulation and Optimized Liquisolid Tablets (DPY-O) dissolving in normal saline.

The conventional formulation and DPY-O were administered at the rear of the throat using a stomachic canulation tube (made of silicone rubber) and immediately 5 ml of water was administered through the tube to facilitate swallowing. Animals had access to food 4 h after dose administration. Concerning 0.2 ml of blood sample was withdrawn from tail vein into heparinized Eppendorf tubes at time intervals of 0 (pre-dose), 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 4, 6, 12 & 24 hours post administration. [12]

RESULTS

Chromatograms of Pharmacokinetic Study

The chromatograms and retention times of Blank plasma, Dipyridamole, Internal Standard (Diazepam), plasma spiked with Dipyridamole and Diazepam are shown in figures 1-4 and Table 2. From the retention times of all the plasma samples, it has been observed that blank plasma has no interference from endogenous substance at the retention times of IS and analyte. The retention time minutes for IS and minutes for Dipyridamole showing good resolution between IS and analyte. The retention time for the plasma samples collected after 2 hours from the subject administered via oral administration are found to be similar indication no interference between the analyte and plasma.

TABLE 2: Retention time of Chromatograms

S.No	Name of the Sample	Retention Time
1	Blank Plasma	0.0
2	Analyte (Dipyridamole)	4.96
3	IS (Diazepam)	2.03
4	Blank + Dipyridamole + Diazepam	0.0 + 4.93 + 1.99

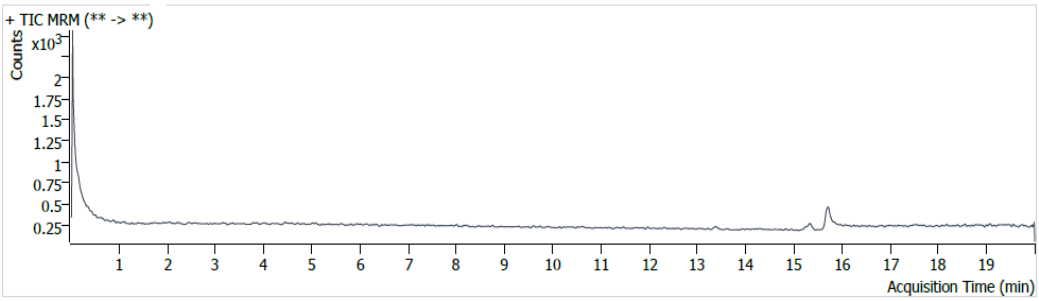

FIGURE 1: Chromatogram of Blank plasma

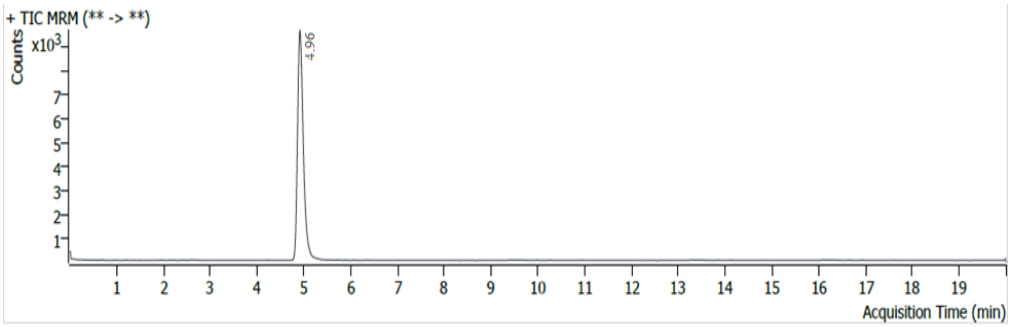

FIGURE 2: Chromatogram of Dipyridamole

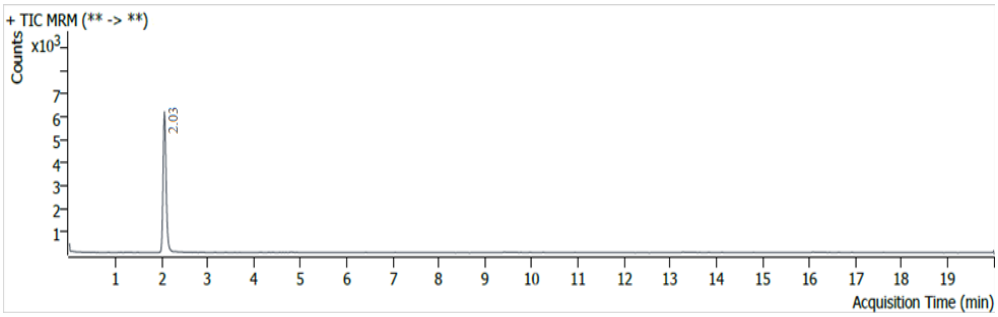

FIGURE 3: Chromatogram of IS Diazepam

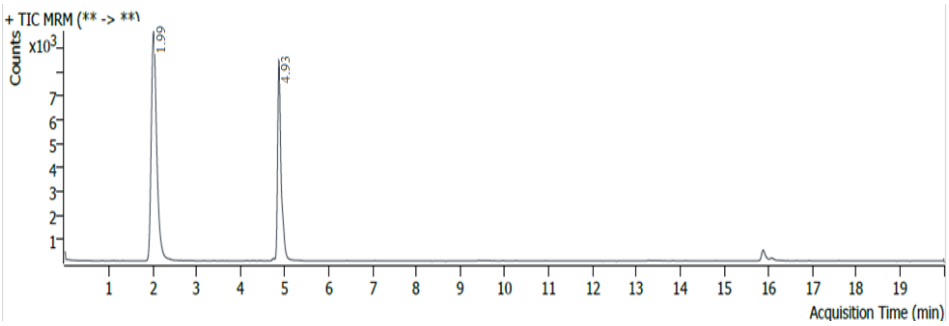

FIGURE 4: Chromatogram of plasma spiked with Analyte & Internal Standard

Standard Linearity Curve of Dipyridamole

The calibration curve results observed over the concentration range of 20 to 8000 ng/ml were satisfactory. The regression equation was found to be Y = 15.71*X-202.2 with a regression coefficient of 0.9998. The linearity of results was depicted in Table and Figure.

TABLE 3: Calibration curve of Dipyridamole

S.No	Concentration ng/ml	Peak Area
1	20	276
2	40	543
3	80	1125
4	400	6124
5	800	11322
6	4000	63731
7	8000	125021

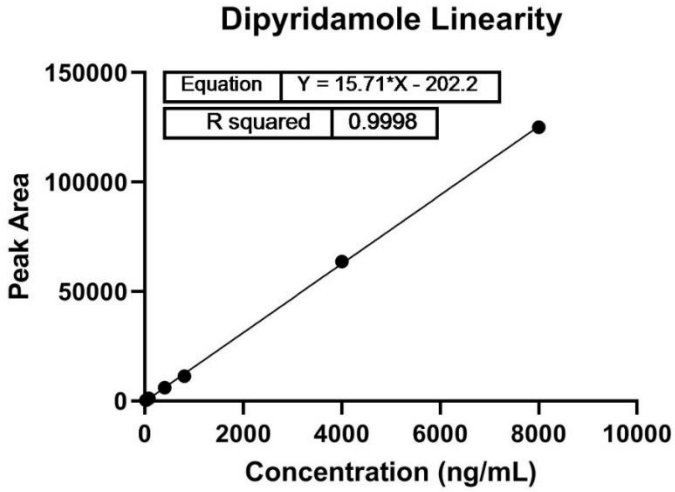

FIGURE 5: Linearity of Dipyridamole

TABLE 4: QC Samples for Dipyridamole

Amount Added (ng/mL)			
Amount Found	20	400	8000
	20.26 ± 0.04	400.11 ± 0.34	7999.23 ± 16.8
	19.58 ± 0.02	399.31 ± 0.10	8001.74 ± 22.6
	20.12 ± 0.01	401.01 ± 0.18	8000.15 ± 31.3
	19.78 ± 0.03	400.88 ± 0.31	8002.43 ± 44.2
	20.66 ± 0.02	398.26 ± 0.28	7999.68 ± 66.9
	21.00 ± 0.01	400.51 ± 0.10	8000.39 ± 27.7
Mean	20.23 ± 0.02	400.01 ± 0.17	8000.60 ± 6.6
Standard Deviation	0.532	1.055	1.234

TABLE 5: Experimental Mean Plasma Concentration Values of Pure Drug

First group of Rats Administered with Pure Drug (Dipyridamole)					
Concentration ng/mL					
Time in Hours	Subject				
	1	2	3	Mean	Standard Deviation
0	0	0	0	0	0
0.25	120.36	121.74	121.22	121.11	0.70
0.5	249.1	247.49	248.17	248.25	0.81
0.75	368.23	369.18	368.77	368.73	0.48
1	428.39	427.56	428.18	428.04	0.43
1.25	512.2	511.34	511.78	511.77	0.43
1.5	473.16	472.92	473.31	473.13	0.20
2	357.29	357.14	356.88	357.10	0.21
4	218.88	220.19	220.01	219.69	0.71
6	149.12	149.27	150.14	149.51	0.55
12	83.76	84.46	83.98	84.07	0.36
24	29.18	30.32	29.11	29.54	0.68

TABLE 6: Experimental Mean Plasma Concentration Values of Optimized Liquisolid Formulation DPY-O

Experimental Mean Plasma Concentration Values of Optimized Liquisolid Formulation DPY-O					
Concentration ng/mL					
Time in Hours	Subject				
	1	2	3	Mean	Standard
0	0	0	0	0	0
0.25	182.58	181.29	182.66	182.18	0.77
0.5	397.12	398.22	397.57	397.64	0.55
0.75	621.91	620.14	621.43	621.16	0.92
1	571.29	570.43	571.88	571.20	0.73
1.25	52.11	522.67	521.07	365.28	271.22
1.5	448.41	448.09	449.65	448.72	0.82
2	318.87	317.14	317.28	317.76	0.96
4	177.09	178.44	177.62	177.72	0.68
6	108.62	107.35	108.1	108.02	0.64
12	57.13	58.1	57.22	57.48	0.54
24	20.64	21.01	21.31	20.99	0.34

TABLE 7: Pharmacokinetic parameters of Pure Drug and Optimized Liquisolid Formulation DPY-O

Parameters	Pure Drug	Optimized Liquisolid Formulation DPY-O
Cmax (ng/mL)	511.77 ± 10.18	621.16 ± 14.32
Tmax (Hours)	1.25 ± 0.03	0.75 ± 0.02
AUC(0-24) (h.ng/mL)	2332.90 ± 112.10	2059.80 ± 124.42
T1/2 (Hours)	10 ± 0.5	9 ± 0.5
Ke (hr-1)	0.0693 ± 0.00115	0.0770 ± 0.00112

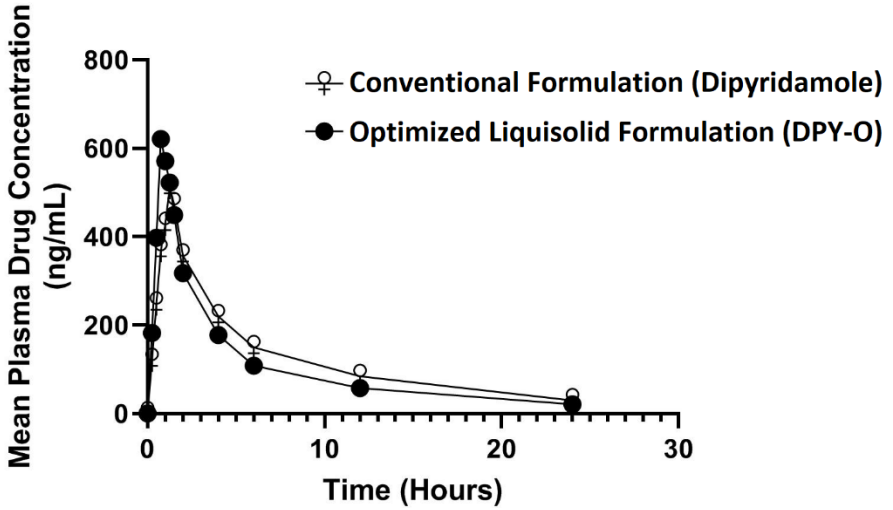

FIGURE 6: Mean Plasma Concentration Time Profile in Wistar Rats obtained after single dose oral administration of Dipyridamole Conventional Formulation and Optimized Liquisolid Formulation (DPY-O)

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The mean ± SD plasma Dipyridamole concentration time curve after oral administration of pure drug and Optimized Liquisolid Formulation DPY-O were administered to 2 groups of 3 healthy subjects each is illustrated in Figure. Pharmacokinetic analysis of Dipyridamole plasma concentration–time data provided the following pharmacokinetic parameters like Cmax values ranging (511.77 ± 10.18 ng/mL to 621.16 ± 14.32 ng/mL), Tmax values ranging (1.25 ± 0.03 to 0.75 ± 0.02 Hours), AUC values ranging (2332.90 ± 112.10 h.ng/mL to 2059.80 ± 124.42 h.ng/mL) and other pharmacokinetic parameters are depicted in Table 6.

It is evident from the data obtained in Table.6 demonstrates the variability in pharmacokinetic parameters like Tmax, Cmax, T1/2 (Hours), AUC & Ke. It has been observed that decreased Tmax value (0.75 hours), Half Life (9 ± 0.5 Hours) and AUC Value with increased Cmax value (621.16 ± 14.32 ng/mL) and Ke (0.0770 ± 0.00112 hr-1) values obtained for the formulated product prepared with liquisolid compacts technology showed immediate release in comparison to the Conventional Formulation.

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