

## Enhanced Oral Bioavailability of Alitretinoin by Lipid Drug Delivery System

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### Abstract

Alitretinoin is an endogenous retinoid and acts as a pan-agonist at retinoid receptors, binding with high affinity to both retinoic acid receptors and retinoid X receptors. Oral alitretinoin once daily is approved for use in patients with severe chronic hand eczema unresponsive to treatment with potent topical corticosteroids. The bioavailability of alitretinoin after oral administration is low and subject to large inter-individual variation. The purpose of this study was to evaluate the relationship of solubility and dissolution profiles of alitretinoin *in vitro* and their impact on the oral bioavailability of alitretinoin in rats. The work plan involves using simple and widely used practices under small scale pharmaceutical industry set-ups in India. The solubility of alitretinoin in vehicles of varying Hydrophilic lipophilic balance (HLB) values was determined and selected components were used to formulate different compositions for *in vitro* drug release dissolution studies. The prototypes composition of soybean oil and stearyl macrogol-32 glycerides provided a stable and maximum drug releasing product. The pharmacokinetic study in rats for the optimized formulation was performed and compared against innovator composition. Lipid formulation system of alitretinoin had significantly increased the peak serum concentration ( $C_{max}$ ), area under the curve (AUC) and reduction was observed in time to reach peak serum concentration ( $T_{max}$ ) compared to marketed formulation. The data although reaffirms established correlations between dissolution and bioavailability, it interestingly points towards a complex relationship exist between HLB and thermodynamic activity profile of drug in vehicles in affecting alitretinoin's solubility, dissolution and bioavailability.

**Keywords:** Alitretinoin; Solubility; Dissolution; Lipid formulation; Bioavailability

### Introduction

Alitretinoin (9-*cis*-retinoic acid) is a unique panagonist retinoid, capable of binding to all six known retinoid receptors [1]. With an annual incidence rate of 0.5 percent(%) per year, work-related hand eczema is the highest occupational disease with regard to incidence. This condition takes a huge toll on psychological state and linked to huge economy linked morbidity [2]. Orally administered alitretinoin at 10 and 30 mg once daily, is the only approved treatment for severe chronic hand eczema unresponsive to potent topical corticosteroids [3]. Clinical studies had shown that the drug shows dose dependent response with 27.7-47.7 % patients of chronic hand dermatitis responded favourably to the treatment [4]. Topical treatment with alitretinoin is actively looked for expansion into other areas such as photo-aged skin and cutaneous T-cell lymphoma [5].

Approximately 20% of an oral administered dose of alitretinoin is absorbed with systemic exposure variability reducing from 70 % to 40% when taken with food. Alitretinoin is currently approved in several European Union countries and the first commercial pharmaceutical product was launched under the brand name Tactino® by Basilea pharmaceuticals as 10 mg and 30 mg as soft gelatin capsule [3].

In the current work it is hypothesised that by selection of suitable lipid, surfactant ratios, desired thermodynamic properties can be achieved which can be indirectly measured using suitable *in vitro* drug release and *in vivo* drug absorption studies [6]. The aim of this study was to determine the solubility and dissolution properties of alitretinoin, and to evaluate its effects on the oral bioavailability in rats.

### Materials and Methods

#### Materials

Alitretinoin was provided as gift sample by Sword and Shield

Pharma (India); sunflower oil, castor oil, soybean oil, olive oil were supplied by SNN (India); glycerol monolinoleate, lauroyl macrogol-6 glycerides, caprylic triglyceride, caprylocaproyl macrogol-8 glycerides, propylene glycol dicaprylocaprate, polyglyceryl-3 dioleate, stearyl macrogol-32 glycerides, propylene glycol monocaprylate (type I) were from Gattefosse (France); sorbitan monooleate, polysorbate 80, glycerol mono and distearate and polyoxyethylene-stearate were from Croda (UK); polyoxyl 35-hydrogenated castor oil were from Corel Pharma (India); polyglyceryl-6 octastearate, propylene glycol dicaprate, glyceryl monocaprylate were from Abitec (USA); caprylic / capric triglyceride was from Sasol (Germany); partially hydrogenated soybean oil was Cargill (India); empty hard gelatin capsule were from Capsugel (India) and gelatin bloom 200 from Sterling Gelatin (India). All other chemicals and solvents were of reagent grade and were used without further purification.

#### Solubility study

Solubility studies were conducted by placing an excess amount of alitretinoin (approximately 400 mg) in 10 ml amber colour vial containing 5 gm vehicle. Semi-solid excipients should be fully melted at 20°C above their melting points. Sonicate the mixture for 10 minutes (min) and kept for 24 hours (h) at 25°C to equilibrate,

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followed by centrifugation at 3000g for 15min. All the operations were performed under yellow light and all containers were wrapped with aluminium foil [7].

The supernatant was taken and diluted with acetonitrile: methanol (40:60 v/v) for quantification of alitretinoin by reverse phase-high performance liquid chromatography (RP-HPLC) equipped with a UV-Visible detector and a photodiode array detector, manual injector with 20 ml loop, LC-solution software and Phenomenex C<sub>18</sub> column (250 mm x 4.6 mm, 5 µm particle size). The solvents were filtered through nylon 0.45 µm membrane filter and was eluted at a flow rate of 1 ml/min. Effluents were monitored at 347 nm. The inter- and intra-day variance of this high performance liquid chromatography (HPLC) method were within the acceptable range of less than 2% (correlation coefficient R<sup>2</sup> = 0.9989).

### Compatibility study

Drug samples (0.5 % w/w solution) with the shortlisted vehicles as described in section 2.2, packed in amber type-I glass vials, sealed with a butyl rubber cap and aluminium flip-off seal closures and stored at 40 ± 2°C/75 ± 5% RH. The vials were withdrawn at initial, 15 day and 30 day time intervals, whole content is retrieved and analysed for alitretinoin content.

### Preparation of dosage forms

The innovator product Tactino® is commercially available strengths of 10mg and 30 mg soft gelatin capsule [8]. The test formulations were prepared as 3.57% alitretinoin suspension/solution in different oil and surfactant blends, shortlisted basis the work done in solubility and compatibility section. These were then filled under stirring at 10mg dose strength using an eppendorph micro-pipette in size 0 hard gelatine capsules. In order to prevent leakage, band-seal the filled capsules using sealing solution, gelatine bloom 200 (5%) and polysorbate 80 (0.5%) in water. All the measurements were on weight basis.

### Drug release studies

First phase would involve drug release studies from alitretinoin blends using United States Pharmacopeia (USP) Apparatus II with sinkers in 900ml Borate buffer (pH 8.0) containing 0.5% cetrimide, and 50 mg/l pancreatin at 37 ± 0.5°C with the paddle speed adjusted to 75rpm [9]. Alitretinoin 10mg test formulations and equivalent innovator composition were placed in a dissolution tester (Sotax, Switzerland). At predetermined time Intervals (15, 30,45,60,90,120 min), aliquot (0.5 ml) were collected, filtered and analysed for alitretinoin content. In second phase, the shortlisted formulations blends and control formulation were filled in liquid-in-hard gelatin capsules and tested for release studies for optimising oil to surfactant ratios.

### Differential scanning calorimetric (DSC) studies

The physical state of alitretinoin as plain drug, test and control lipid formulations were characterized by the DSC (Perkin Elmer, USA). The samples (about 4-5 mg) were placed in standard aluminium pans, and dry nitrogen (flow rate 20ml/min) was used as effluent gas. All samples were scanned at a temperature ramp speed of 10 C/min and at the heat flow from 50 - 300°C [10].

### In vivo study

The *in vivo* performance of the test and control formulations of alitretinoin were compared in adult wister male rats(6-8 weeks old;250-300 g) at 1.04 mg/kg [11]. Male Wistar rats (twelve rats were

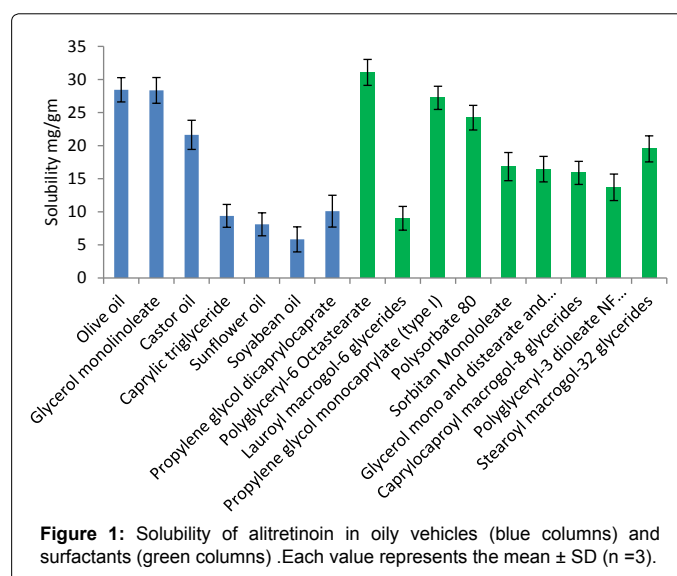
divided into two groups) were fasted for 10 h prior to the experiments but allowed free access to water. All animals care and procedures were conducted according to the guidelines issued by Committee for Purpose of Control and Supervision of Experiments on Animals, Government of India.

Samples were collected as peri-orbital bleeding procedure at 0.5, 1, 2, 3, 6, 12 and 24 hour time intervals after administration from alternate rat [12]. The blood samples were collected into heparinized tubes and 100 µl of plasma was collected by centrifuging blood samples at 3000g for 15 min. Plasma samples were stored with photo protection at -20°C until further analysis. 200 µl plasma was pipetted into a 2 ml polypropylene tube and acetonitrile (1.5ml) was added. The mixture was vortex mixed briefly, and after standing for 30s at room temperature, the mixture was centrifuged at 3500rpm for 10min and the supernatant (20µL) was injected into HPLC system. The calibration curve was constructed over a range of 1.0-250 ng/ml in plasma (R<sup>2</sup> = 0.9947) [13]. Pharmacokinetic parameters C<sub>max</sub>, T<sub>max</sub> and AUC were calculated using PK Solver Software [14]. Student's *t*-tests were performed to evaluate the significant differences between the two formulations. Values are reported as mean ± standard deviation (SD) and the data were considered statistically significant at *P* < 0.05.

## Results and Discussion

### Solubility study

The solubility data of alitretinoin in various oils and surfactant provides a range of values as provided in Figure 1. The drug is plagued from slow and variable dissolution due to its poor aqueous solubility which in turn increases its susceptibility of precipitation in gastrointestinal lumen from the lipid formulation. Therefore the general expectation remains that the shortlisted vehicles should provide highest solvent capacities. On the contrary, excessive solubility in a vehicle may further retard release of drug from the formulations owing to low thermodynamic activity profile. Since a fine balance is required to ensure super-saturation and ideal thermodynamic activity of drug in colloidal system to get desired bioavailability, vehicles representing extreme alitretinoin solubility profiles, were selected for the next stages of development studies. For instance, olive oil (28.44 mg/g ± 1.84) showed highest, soybean oil (5.83mg/g ± 1.89) showed lowest and castor oil (21.63 mg/g ± 2.21) showed intermediate solubility's



**Figure 1:** Solubility of alitretinoin in oily vehicles (blue columns) and surfactants (green columns). Each value represents the mean ± SD (n =3).

for alitretinoin. In similar way, surfactants candidates' polyglyceryl-6 octastearate, stearyl macrogol-32 glycerides, lauroyl macrogol-6 glycerides were shortlisted. As next step, multiple combinations will be prepared with above vehicles basis the criteria defined for lipid formulation classification system (oil: surfactant in ratio 80:20) in the first phase of prototype development studies [15,16].

Compatibility study

DSC is one of the most widely used techniques to understand the drug interactions with the other excipients and capsule shell components in academia. But in absence of these facilities in-house and outsourcing being a costly and time-consuming affair, this practice is not hugely popular in most of the mid-scale pharmaceutical Research and Development (R&D) departments in India. These companies are in the business of generic development where the development goal is come up with a product in similar lines as innovator in record time.

The drug interaction data with oils and surfactants solubility data of alitretinoin in various oils and surfactant provides a range of values (Figure 2). None of the shortlisted ingredients had shown any significant drop in the drug content during the study period. Therefore, these excipients were used for further development work with reasonable amount of confidence that the resulting formulation will show satisfactory stability during the full scale stability studies.

Preparation of dosage forms

For an experienced R&D Industrial pharmacist, while designing optimisation studies, multiple consideration ranging from optimal performance in lab conditions along with the possible scalability issues, had to be factored. The alitretinoin commercial formulation is Type I lipid formulations. Such formulations solely address solvent capacity issues but not the emulsification aspect of final system in gut. Therefore, by selecting surfactants across the HLB spectrum, the current work involves manufacturing of both Type II and type III lipid formulations (Table 1) to the study the impact of these lipid systems on release rates of alitretinoin [16].

One important aspect generally overlooked is the impact of un-intended exposure of these drugs the on the production personnel during the manufacturing operations. Considering the potential side effects of these potent retinols (like teratogenicity), it is preferable to develop drug-in-solutions or suspensions system to minimise both drug exposure levels and stages to this un-intended healthy population. This work would also address this concern by wetting the drug in the first manufacturing step itself, thus eliminating the risk of respiratory exposure of powdered drug during next manufacturing & packaging stages.

Dissolution studies

Following oral administration, risk of drug precipitation in gut always exists. In type I lipid formulations, fate of drug is determined following digestion of triglycerides and subsequent emulsification with bile salts. Similarly for type II and type III lipid formulations, formation of viscous crystalline gel and migration of hydrophilic compounds in aqueous phase respectively, could impact the precipitation potential. Unfortunately, there is absence of any practical method which can preciously predict the fate of the drug precipitation [16]. The only available and most widely practiced tool is the dissolution studies using a bio-relevant media involving enzymatic systems.

The drug release data of alitretinoin in various lipid formulations provides a range of values as shown in Figure 3. The lipid formulation

containing soyabean oil and stearyl macrogol-32 glycerides provided highest alitretinoin release among the tested combinations. The other two surfactants with low HLB values failed to provide the release superior to innovator formulation. These results are interesting, since in light of solubility studies, these results points towards a complex relationship between drug, its solubility and the thermodynamic activity on the overall performance of the formulation system. The results suggest that type III lipid formulation is the way forward for alitretinoin lipid formulations for further optimization.

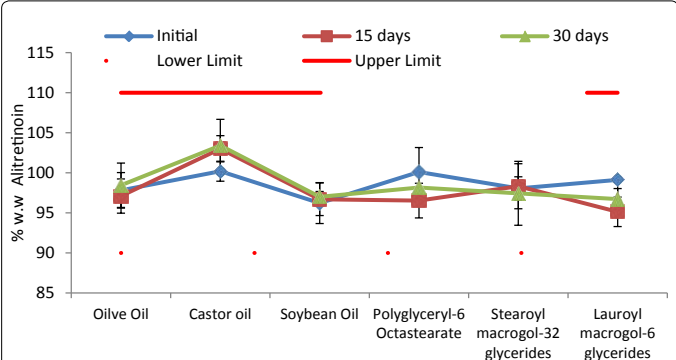


Figure 2: Compatibility studies of Alitretinoin in oils and surfactants. Each value represents the mean ± RSD (n =3).

| Ingredients                    | Batch        |     |     |     |     |     |     |     |     |
|--------------------------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|
|                                | mg/unit dose |     |     |     |     |     |     |     |     |
|                                | A            | B   | C   | D   | E   | F   | G   | H   | I   |
| AIN                            | 10           | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  |
| Olive oil                      | 216          | 216 | 216 | -   | -   | -   | -   | -   | -   |
| Castor Oil                     | -            | -   | -   | 216 | 216 | 216 | -   | -   | -   |
| Soybean Oil                    | -            | -   | -   | -   | -   | -   | 216 | 216 | 216 |
| Polyglyceryl-6 octastearate    | 54           | -   | -   | 54  | -   | -   | 54  | -   | -   |
| Stearyl macrogol-32 glycerides | -            | 54  | -   | -   | 54  | -   | -   | 54  | -   |
| Lauroyl macrogol-6 glycerides  | -            | -   | 54  | -   | -   | 54  | -   | -   | 54  |
| Total                          | 280          | 280 | 280 | 280 | 280 | 280 | 280 | 280 | 280 |

Table 1: Details of experiments performed for selection of oil and surfactant for alitretinoin lipid formulation.

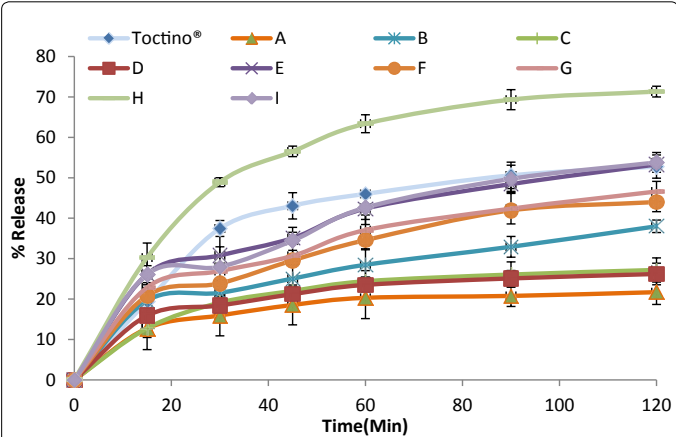


Figure 3: Drug release studies of Alitretinoin in various lipid formulations. Each value represents the mean ± RSD (n =3).

Hence, type III formulations containing hydrophilic surfactant in range of 20 - 40% were prepared and finished formulations (in capsule format) containing varying level of stearyl macrogol-32 glycerides were evaluated for alitretinoin release (Table 2). The data (Figure 4) seems to indicate some level of plateau effect at 30% levels beyond which no meaningful improvement in dissolution profile has been observed. There seems to be an initial lag in release for 1<sup>st</sup> 15 min which can be attributed to opening time for hard gelatin capsule in dissolution media. Interestingly inter-compositions variability does not seem to be very different including the innovator composition.

### DSC studies

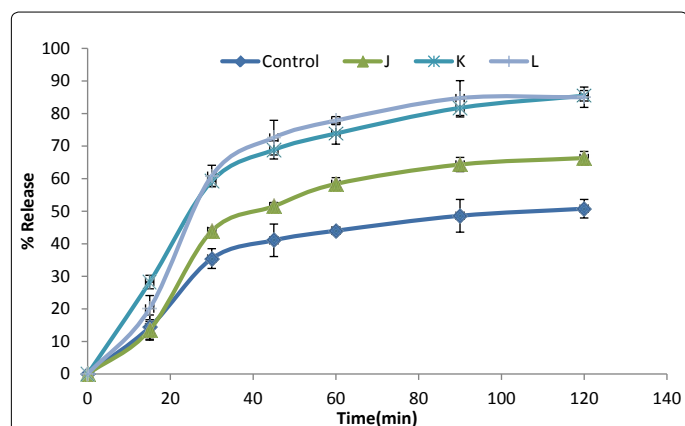
DSC thermograms of alitretinoin plain drug, optimized test formulation and innovator composition were presented in the Figure 5. The DSC curve of pure alitretinoin showed a single sharp endothermic peak at 197°C corresponding to the melting point of alitretinoin. The DSC curves for test and control formulations showed slight shift in endotherms peaks with reduced peak intensities at 189.53°C and 194.12°C, indicating presence of crystalline form, although in lower levels in test formulations as compared to control product. These finding points towards some drug-lipid formulation interaction and molecular distribution of drug in lipid formulation matrix. This may also provide the possible reasoning for the increase in the dissolution rate of test composition as compared to control formulations.

### In vivo absorption studies

Being a Class IV BCS drug, the improvement in dissolution cannot be the sole guarantor of superior *in vivo* performance. Therefore, *in vivo* pharmacokinetic release (as shown in Figure 6) and evaluation of the optimized alitretinoin type III lipid (formulation K in Table 2) and marketed product was performed and AUC, T<sub>max</sub> and C<sub>max</sub> were calculated (Table 3).

| Ingredients                    | Batch<br>mg/unit dose |     |     |
|--------------------------------|-----------------------|-----|-----|
|                                | J                     | K   | L   |
| AIN                            | 10                    | 10  | 10  |
| Soybean oil                    | 216                   | 189 | 162 |
| Stearyl macrogol-32 glycerides | 54                    | 81  | 108 |
| Total                          | 280                   | 280 | 280 |

**Table 2:** Details of experiments performed for optimisation of alitretinoin lipid formulations.

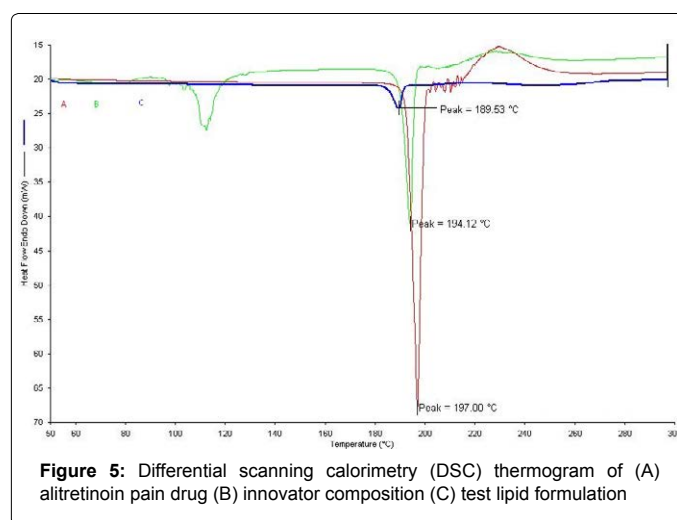


**Figure 4:** Drug release from alitretinoin lipid formulation during optimisation studies. Each value represents the mean  $\pm$  RSD (n = 6).

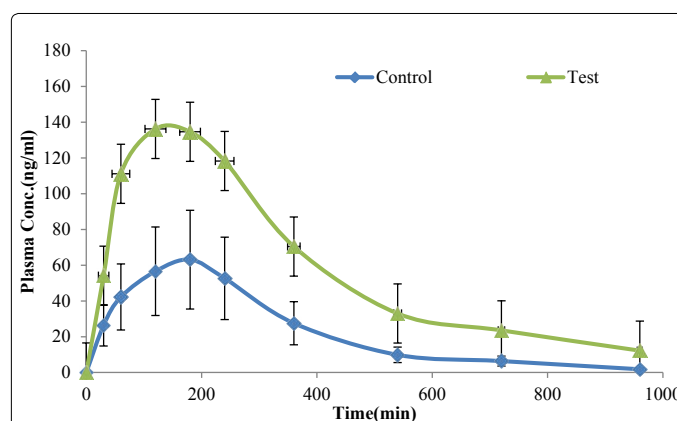
The pharmacokinetic values indicated a statistically significant improvement ( $P < 0.05$ ) in bioavailability for test alitretinoin lipid formulation. Typically type III lipid formulation undergo shorter gastric transit and will be in colloidal state earlier than type I lipid formulation leading to more rapid absorption and higher peak concentration of drug [16]. The test alitretinoin capsules significantly increased alitretinoin plasma levels and 2-fold jump was observed in the relative oral bioavailability compared to innovator composition. The T<sub>max</sub> values of 180 min for innovator composition was quite close to the reported values for alitretinoin in humans (18-240 min) [17]. In fact, time to C<sub>max</sub> was reduced by 30 min in test formulation when compared against the control product. The improved oral bioavailability of alitretinoin indicates that the poor bioavailability of alitretinoin can be increased by improving its dissolution properties.

### Conclusion

Alitretinoin was formulated as type III lipid formulation in an



**Figure 5:** Differential scanning calorimetry (DSC) thermogram of (A) alitretinoin plain drug (B) innovator composition (C) test lipid formulation



**Figure 6:** Plasma profiles of alitretinoin test lipid formulations and innovator composition in rats. Each value represents the mean  $\pm$  SD (n = 6).

| Parameter                | Control Alitretinoin Capsule | Test Alitretinoin Capsule |
|--------------------------|------------------------------|---------------------------|
| T <sub>max</sub> (min)   | 180.0 $\pm$ 0.0              | 120.0 $\pm$ 0.0           |
| C <sub>max</sub> (ng/ml) | 63.12 $\pm$ 27.62            | 136.26 $\pm$ 18.14        |
| AUC (ng min/ml)          | 22084.714 $\pm$ 9663.150     | 56444.400 $\pm$ 9108.150  |

**Table 3:** Pharmacokinetic profiles of alitretinoin lipid formulations and innovator composition in rats. Each value represents the mean  $\pm$  SD (n = 6).



attempt to increase its dissolution and bioavailability. An optimized formulation containing alitertinoin was developed by estimating its solubility, indicative compatibility and drug release studies. Following oral administration in rats, lipid formulation provided significant increase in the bioavailability. Any possible reduction in dosage amount without compromising the efficacy aspect will provide a commercial edge while marketing such product to prospective registered medical practitioners. The current development strategy is also expected prevent exposure to unintended healthy production personnel during manufacturing operation.

Overall, the current work has indicated that it is indeed possible to develop an effective and safely scalable formulation via a simple one-step process using basic laboratory equipment's and procedures. Still, considering the limited sample size, the results are at best indicative and further clinical work can be undertaken to develop and commercially viable suprabioavailable format of alitertinoin liquid-in-hard gelatin capsules for human use.

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