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RESEARCH ARTICLE

Selection of oral bioavailability enhancing formulations during drug discovery

Weijia Zheng[1], Akash Jain[2], Dimitris Papoutsakis[2], Rose-Marie Dannenfelser[3], Riccardo Panicucci[2], and Sudhakar Garad[2]

[1]Astrazeneca Pharmaceuticals, Waltham, USA, [2]Novartis Institute for Biomedical Research, Cambridge, USA, and [3]Novartis Pharmaceutical Corporation, One Health Plaza, East Hanover, USA

Abstract

The objective of this paper was to identify oral bioavailability enhancing approaches for a poorly water-soluble research compound during drug discovery stages using minimal amounts of material.

LCQ789 is a pBCS (preclinical BCS) Class II compound with extremely low aqueous solubility (<1 μg/mL) and high permeability, therefore, resulting in very low oral bioavailability in preclinical species (rats and dogs). A number of solubility and/or dissolution enhancing approaches including particle size reduction, solid dispersions, lipid-based formulations and co-crystals, were considered in order to improve the compound's oral bioavailability. High-Throughput Screening (HTS) and *in silico* modeling (GastroPlus™) were utilized to minimize the compound consumption in early discovery stages. *In vivo* evaluation of selected physical form and formulation strategies was performed in rats and dogs. Amongst the formulation strategies, optimized solid dispersion and lipid-based formulation provided significant improvement in drug dissolution rate and hence, oral bioavailability. In addition, a significant impact of physical form on oral bioavailability of LCQ789 was observed. In conclusion, a thorough understanding of not only the formulation technique but also the physical form of research compounds is critical to ensure physical stability, successful pharmacokinetic (PK) profiling and early developability risk assessment.

Keywords: Physical form, high-throughput screening, co-crystal, solid dispersion, lipid-based delivery, bioavailability

Introduction

Most compounds selected from today's drug discovery phase exhibit low aqueous solubility although more and more emphasis has been placed on optimization of drug-like properties[1]. Poor aqueous solubility poses significant development risks for orally administered drug candidates, especially, poor oral absorption/bioavailability and lack of inadequate exposure multiples for toxicology and clinical studies. In order to identify and proactively mitigate such risks, a comprehensive developability assessment is usually performed on selected lead candidates during drug discovery stages. Such developability assessment activities include evaluation of physicochemical and biopharmaceutical properties, selection of physical form and formulation principles that provide adequate exposure multiples in preclinical studies and decision-making[2].

In most instances, the physical form of a compound in the early discovery stage is often not well characterized. The various physical forms of a compound can exhibit a variety of different physicochemical properties (melting point, hygroscopicity, dissolution rate, stability and habit), mechanical properties (hardness, tensile strength, compactability and flow properties) and biopharmaceutical properties (absorption rate and bioavailability[3,4]). Generally speaking, the most thermodynamically stable (TDS) form is preferred for development due to desired stability. However, the TDS form also exhibits the lowest solubility, which often leads to lower bioavailability. In most cases, the TDS form is not available during early drug discovery; therefore, there is a high likelihood that the discovery of more stable forms in later stages of development could significantly impact

Address for Correspondence: Dr. Sudhakar Garad, Novartis Institute for Biomedical Research, 250 Mass Ave, Cambridge, 02139 United States. Tel: 617–871–3162. Fax: 617–871–4079. E-mail: sudhakar.garad@novartis.com

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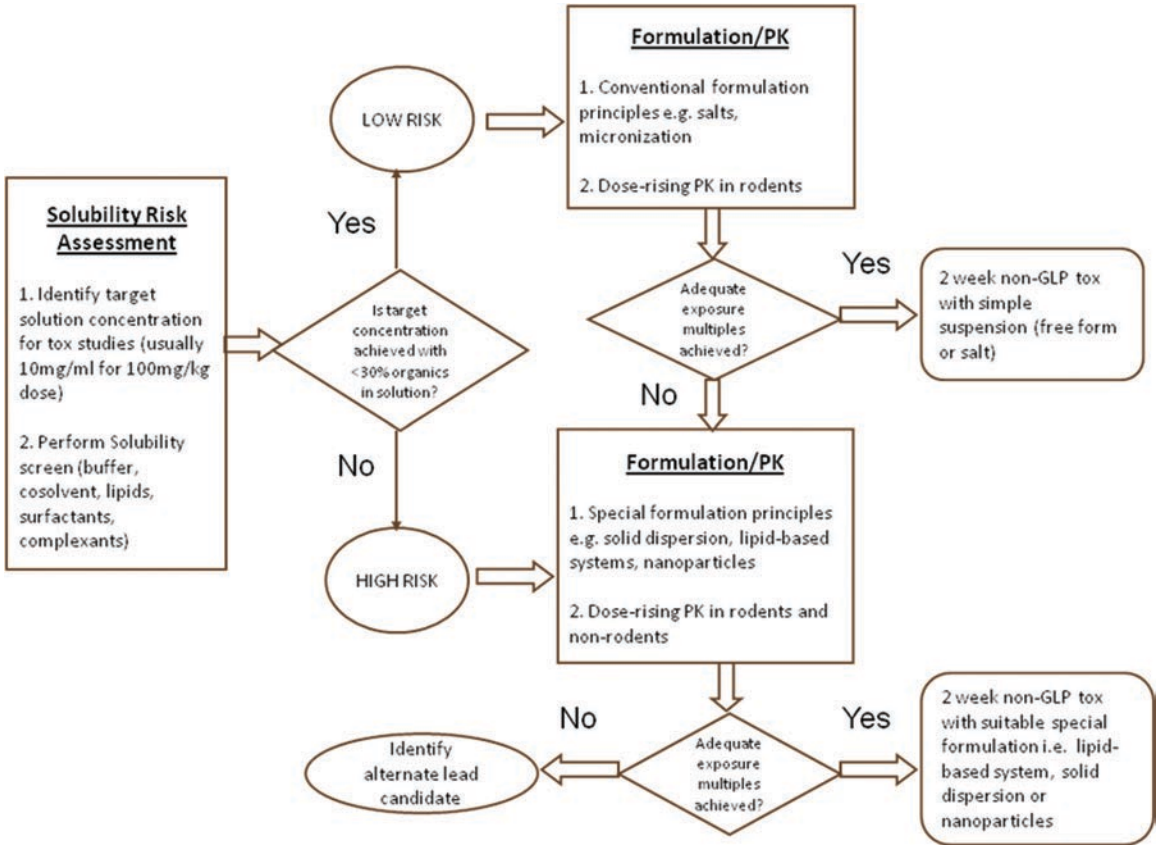

Figure 1. Decision-making approach for selecting optimal formulation strategy for preclinical studies.

the physicochemical properties and bioavailability of a compound.

Another major activity in the developability assessment of poorly soluble research compounds is the development of suitable formulations for early PK (pharmacokinetics) studies, PD (pharmacodynamics) studies, efficacy studies and toxicology studies. Selection of adequate formulation principles is critical for obtaining reliable information during efficacy and toxicity screening, especially for poorly soluble compounds with potential for low and variable exposure[2]. Inadequate exposure in efficacy and toxicity studies due to poor physicochemical properties can lead to poor efficacy and misleading safety evaluation that can jeopardize further development and cause significant delays in achieving first-in-human read-out.

A decision-making approach (Figure 1) based on *in vitro* solubility screening and oral exposure in preclinical species can be used to identify a suitable formulation strategy to conduct high dose toxicology studies and potentially clinical studies, even before candidate selection. As shown in Figure 1, discovered candidates can be classified into "low" and "high" solubility risk categories based on the ability to achieve target dosing concentrations expected for toxicology studies in a low organic (<30%) solution formulation. "low" solubility risk candidates can be formulated using conventional strategies like salt formation or particle size reduction, however, the "high" risk candidates mostly require the use of special formulation principles like solid dispersions, nanoparticles or lipid-based systems. While "low" risk candidates can be advanced into 2-week toxicology studies based on rodent PK evaluation, it is advised that "high" risk candidates should be screened, using most promising formulation principles, in both rodent and non-rodent species before advancing into 2-week toxicology studies. In cases of "high" risk candidates where adequate exposures cannot be achieved with special formulation principles, the feedback to research teams should be to identify alternate lead candidates with a more favorable solubility and formulability profile.

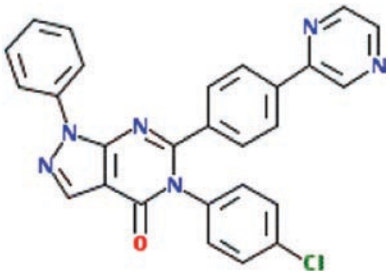

Figure 2. Chemical structure of LCQ789.

LCQ789 is a crystalline, neutral compound with a molecular weight of 476.93, and a ClogP of 5.4 (Figure 2). The compound was selected as a lead candidate based on its promising efficacy profile. Adequate plasma exposure was observed with oral suspensions prepared with

early research batches consisting of metastable forms. However, once the TDS form was discovered, the oral bioavailability significantly decreased due to extremely low solubility (<1 μg/mL). Being a neutral compound, salt formation was not feasible. Because of the extremely low aqueous solubility and poor oral bioavailability, LCQ789 was identified as a "high" risk candidate (Figure 1). A solution formulation with commonly used solubilizing agents was not feasible to achieve higher concentrations for toxicology studies, thus special formulation approaches were investigated to identify a suitable path forward for LCQ789.

Formulation approaches based on particle size reduction, such as nanoparticles, have been used to deliver poorly water-soluble compounds orally, intravenously and ocularly[5–14]. For oral administration, nanoparticles increase the dissolution rate by maximizing the surface area, and therefore enhancing systemic exposure. Physical form modification approaches, such as pharmaceutical co-crystals, have been recently explored as promising approaches to modify the compound properties such as stability, dissolution rate and bioavailability[15–19]. In addition to nanoparticles and salt/co-crystals, lipid-based formulations have been shown to increase the oral bioavailability of lipophilic compounds by improved drug solubility, increased membrane permeability and lymphatic transport[20–27]. Solid dispersions in water-soluble polymers have attracted considerable interest as a means of improving bioavailability of poorly water-soluble compounds. The bioavailability enhancement from a solid dispersion could be attributed to increased dissolution rate and kinetic solubility of a compound by generation of metastable amorphous form and higher supersaturation in presence of a stabilizing polymeric matrix[28–38].

One of the major challenges in formulation selection and optimization during early development is the limited compound availability. As described in the sections below, a combination of *in silico* modeling and high-throughput technology was extensively utilized to overcome the limited compound availability. Once a few promising solubility/dissolution enhancing formulation approaches were identified, they were screened in preclinical animal models (rats and dogs) to determine their potential to improve the oral bioavailability of LCQ789.

Materials and methods

Materials

Polymers used in this study (Eudragit E PO, Eudragit L100, HPC-LF (Klucel), HPMC (Pharmacoat 606), PVP K30) were obtained from BASF (NJ), Evonik Degussa (Kirschenallee, Darmstadt), Dow Chemical company (MI), Hercules (DE) and Optimize (CA). Cremophor EL and Solutol HS-15 were provided by BASF (Ludwigshafen, Germany); VitE TPGS was purchased from EASTMAN (Anglesey, UK); Tween-80 and Sodium Lauryl Sulfate (SLS) were purchased from Fisher Scientific (IL, NJ); Labrasol, Transcutol HP, Lauroglycol FCC, Labrafil M-1944 CS, Labrafil M-2125 CS, Peceol and Gelucire® 44/14 were samples provided by Gattefossé (Saint-Friest Cedex, France); PEG4000 and PEG8000 were purchased from Optimize (CA). Methylcellulose (grade: medium viscosity 400–800), Olive oil and Soybean oil were purchased from Sigma. Arlasolve® was provided by Uniqema (DE); Brij98, Triacetin and ethanol (absolute 200 proof) were obtained from Acros Organics (Morris Plains, NJ); Sesame oil was purchased from MP Biomedicals (Solon, OH); Miglyol sample was requested from Sasol (Sasol Germany GmBH, Witten, Germany); Capmul MCM was obtained from Abitec Corp.(Columbus, OH); Methylene chloride was purchased from Fisher Chemicals (Pittsburgh, PA); Corn oil was obtained from Novartis (Stein, Switzerland).

Methanesulfonic acid, toluenesulfonic acid, ethanesulfonic acid, phosphoric acid, fumaric acid, succinic acid, acetic acid, nitric acid, benzenesulfonic acid, formic acid, sulfuric acid (H_2SO_4), hydrobromic acid (HBr), hydrochloric acid (HCl), malic acid, lactic acid, propionic acid, malonic acid, pyruvic acid, adipic acid, citric acid, mesoconic acid, benzoic acid, bromo sucinic acid, calcium Chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), zinc Chloride ($ZnCl_2$) and magnesium acetate were purchased from Sigma. All other solvents and reagents were HPLC grade and used as received.

In-silico modeling (GastroPlus™)

GastroPlus™ modeling was performed to determine the sensitivity of LCQ789 oral bioavailability to parameters like particle size and solubility. The physicochemical parameters used for modeling include molecular weight (476.93), Clog P (5.4), water solubility (0.0005 mg/mL) and Caco-2 permeability (P_m (A–B) = 1.32 nm/s, P_m (B-A) = 0.36 nm/s). Pharmacokinetic parameters incorporated into the modeling included half-life and clearance obtained from intravenous dosing in rats and AUC, C_{max} and t_{max} values obtained from oral suspension PK in rats.

Physical form characterization

Physical forms of the compound and solid dispersion formulations were evaluated by X-ray Powder Diffraction (XRPD, D8 Discover, Bruker AXS, Madison, WI) with a scan range of 4–40° 2θ (40 KV, 40 mA) and differential scanning calorimetry (DSC, Q2000, TA instrument, New Castle, DE). A ramp rate of 10°C/min was used in conventional DSC runs. Modulation at 2°C/min of ± 1.27°C every 60 s was used as the ramp rate for modulated DSC.

Co-crystal screening

High-throughput co-crystal screening was performed with four solvents and 27 co-crystal formers. Solvents included in the screening were methanol, ethanol, acetonitrile and acetone. Co-crystal formers including methanesulfonic acid, toluenesulfonic acid, ethanesulfonic acid, phosphoric acid, fumaric acid, succinic acid, acetic acid, nitric acid, besylate acid, formic acid, H_2SO_4, HBr, HCl, malic acid, lactic acid, propionic acid, malonic

acid, pyruvic acid, adipic acid, citric acid, mesoconic acid, benzoic acid, bromo sucinic acid, and $CaCl_2$, $MgCl_2$, $ZnCl_2$ and Mg acetate, were screened. Crystalline hits were manually scaled up for further characterization including XRPD, thermal analysis and elemental analysis.

Solid dispersion screening

A high-throughput method, similar to method described by Bak et al.[19], was utilized for solid dispersion screening by solvent evaporation to maximize the success rate and minimize compound consumption. However, the choices of excipients, polymer-surfactant ratios, solvent system to prepare drug and excipient solutions, liquid handling system and solubility media for screening solid dispersions used in our studies varied significantly from those reported in the literature[39].

A total of 64 variants containing seven polymers (row A-G) and seven surfactants (column 2–8) were evaluated with a 10% drug load using a 96 well block. Eudragit E PO, Eudragit L100, HPC-LF (Klucel), HPMC (Pharmacoat 606), PEG4000, PEG8000 and PVP K 30 were selected as polymeric carriers in the screening. Cremophor EL, Gelucire 44/14, Labrasol, SLS, Solutol HS-15, Tween-80 and VitE TPGS were included as surfactants. No polymers were included in Row H, and no surfactants were included in column 1. H-1 served as the control and contained only LCQ789 without any polymers and surfactants. Row H and column 1 reflected the solubility enhancement from only either surfactants or polymers. All other variants reflected the solubilization effect from the combinations of polymers and surfactants. All components were dissolved in a 50:50 mixture of methylene chloride and ethanol, and the components were dispensed by a liquid handling system (Zinsser Analytic, Northridge, CA). After all components were dispensed, the block was gently shaken for 1 h, and then the solvents were removed in a vacuum oven. Kinetic solubility of the solid dispersions in water and buffers was evaluated at 15 and 60 min by HPLC. Water was used as the medium for all water-soluble neutral polymers, whereas 50 mM pH 1 HCl-KCl buffer and pH 6.8 phosphate buffer were used for the cationic and anionic polymers, respectively. This was done to ensure that the compound solubility was not limited by pH-dependent solubility of the polymers.

Based on the kinetic solubility results at 15 and 60 min, lead variants were selected and scaled up using the same solvent system. During scale up, rotary evaporation (BÜCHI R-200, Switzerland) was used to remove the organic solvents. The solid dispersions were further dried in a vacuum oven at 40°C for 24 h, and then sieved through a 35-mesh screen. The resulting solid dispersions were characterized based on physical properties and dissolution behavior.

Dissolution

Due to limited compound availability, dissolution of the various physical forms and solid dispersions of LCQ789 was carried out in µDiss™ a micro-disso device (*p*ION Inc, Woburn, MA) originally invented by collaboration between Novartis scientists and *p*ION engineers. An aliquot of 20 mL of media and a 300 rpm stirring rate were used for all the dissolution studies ($n=2$). The active concentrations were determined by either fiber optic probe or HPLC. These dissolution studies were used to compare the dissolution rate between different physical forms and solid dispersions. Significant amount of compound was saved in comparison to conventional USP dissolution methods. µDiss™ could serve a valuable tool to rank order dissolution rates of different polymorphs, salts, solid dispersions and other formulation principles during early development.

Lipid-based formulation delivery and characterization

Solubility of LCQ789 in 25 GRAS listed and/or compendial status of excipients including solvents, surfactants, oils and lipids were measured by HPLC. Several microemulsion preconcentrate (MEPC) systems were developed based on the solubility in single component systems (except where the said component is dissolved in ethanol), physical stability after dilution and excipient tolerability. MEPCs were diluted 10× with water and simulated gastric fluids and analyzed for their physical appearance and particle size distribution (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). The final lipid-based formulation was selected based on compound solubility, chemical stability in MEPCs, particle size distribution and physical stability of the resulting microemulsions after dilution. The stability of LCQ789 in the MEPCs was evaluated at 4°C, room temperature (RT) and 50°C. Physical stability was monitored by visual observation of any precipitation during storage and chemical stability was determined by a HPLC method.

HPLC Method

The HPLC analysis of LCQ789 was performed on an Agilent 1100 series system using reversed-phase C18 column (Agilent Zorbax, 1.8 µm, 4.6 × 50 mm) with a mobile phase consisting of 0.1% formic acid in water (A)/0.1% formic acid in acetonitrile (B), gradient of A (%) = 90, 10, 10, 90 at 0, 2.5, 5 and 5.1 min respectively and total run time of 7 min. The flow rate was maintained at 1 mL/min and samples were analyzed using an online diode array detector (Agilent 1100) at 296 nm.

In vivo evaluation

Basic pharmacokinetic parameters of LCQ789 such as half-life and clearance were obtained following an intravenous dose (1 mg/kg) in Sprague Dawley (SD) rats. Absolute oral bioavailability of LCQ789 was measured using plasma exposure following an oral solution dose (10 mg/kg) in SD rats. The solution formulation used for both intravenous and oral dosing consisted of 10% N-methyl pyrrolidone (NMP), 30% PEG400, 10% cremophor EL, 5% Vit ETPGS and 45% dextrose 5% for injection. Oral bioavailability from suspensions of three different physical forms of LCQ789 (amorphous, solvate and TDS

forms; all 10 mg/kg) in 0.5% methylcellulose vehicle was also measured in SD rats.

For evaluation of various bioavailability enhancing formulation approaches (co-crystals, solid dispersion and MEPC), additional pharmacokinetic studies on LCQ789 (10 mg/kg) were performed in SD rats and beagle dogs. The neutral form of LCQ789 was administered as a crystalline suspension in 0.5% methylcellulose in both species, whereas the co-crystal form was dosed as a suspension in 0.5% methylcellulose in rats and as powder-filled capsules in dogs. Solid dispersion formulation was administered as an aqueous solution/suspension in rats and as powder-filled capsules in dogs. Plasma samples were taken at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h following the dosing. Plasma exposures obtained from oral dosing of all four formulation approaches were compared and absolute bioavailability from each formulation was calculated using plasma exposure from intravenous dosing.

Results and discussion

Influence of physical form on dissolution rate and bioavailability

The exposure following intravenous and oral administration of different physical forms of LCQ789 are illustrated in Table 1. As seen in the table, a significant difference in oral bioavailability was observed between solution and suspension of TDS form indicating dissolution limited absorption profile for LCQ789. It was also observed that the metastable physical forms (amorphous and solvate forms) provided higher oral exposure than the TDS form. These *in vivo* findings are in correlation with results from *in vitro* dissolution testing in 0.1N HCl (Figure 3) where the TDS form showed significantly lower dissolution as compared to the metastable form. Based on these observations, it was clear that improving the solubility and dissolution rate of LCQ789 is essential to achieve the desired oral bioavailability enhancement and dose proportionality.

Table 1. Exposure of LCQ789 in rat following intravenous (IV) and oral administration using different physical forms.

Formulation	Physical form	AUC0-24 h/dose (μg·h/mL/mg/kg)
IV	Amorphous	1074 ± 121.0
Oral solution	Amorphous	729.2 ± 16.5
Oral suspension	Amorphous	600.9 ± 23.4
	Solvate	458.7
	Thermodynamic stable form	128.3 ± 78.93

IV and oral solutions: PEG400/NMP/cremophor EL/VitE TPGS/D5W (30:10:10:5:45).
Oral suspension: 0.5% w/v methylcellulose.

Selection of bioavailability enhancing formulations of LCQ789

Parameter sensitivity analysis by GastroPlus™

Particle size reduction is one of the most commonly used approaches to increase surface area resulting in enhanced dissolution rate. Because of limited material availability, parameter sensitivity analysis of GastroPlus™ was used as a tool to identify parameter(s) that could potentially enhance the bioavailability of LCQ789. Results from GastroPlus™ (Figure 4) suggested that, improving solubility can significantly increase the bioavailability of LCQ789, whereas, particle size reduction has minimal effect on oral bioavailability of this compound. With 200 nm particles, the bioavailability was predicted to be approximately 2%. In addition, particle size reduction on a previous research compound from the same scaffold as LCQ789 had minimal impact on its oral bioavailability, as confirmed by GastroPlus™ and *in vivo* rat study. Therefore, the nanoparticle approach was not pursued for LCQ789.

Co-crystal

Salt formation is commonly employed in early drug development to increase solubility, dissolution rate and stability of compounds with acidic or basic functional

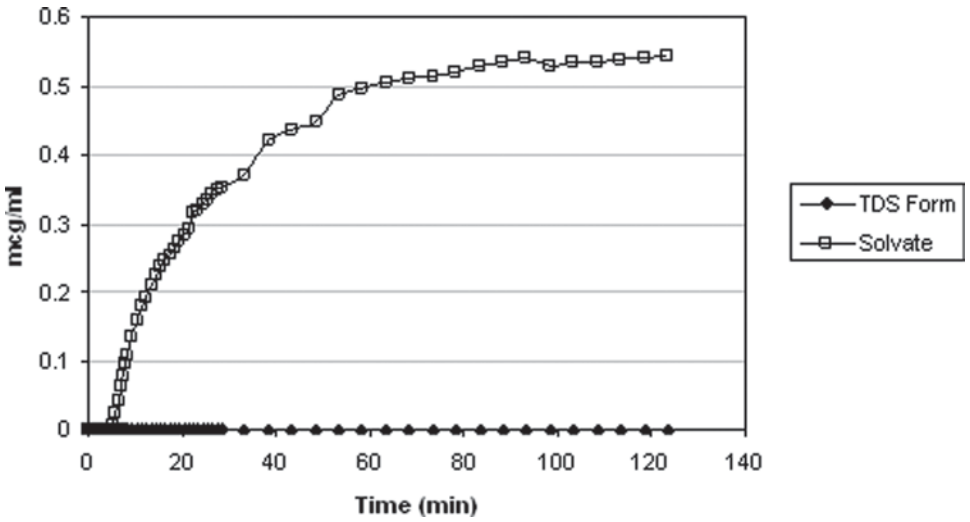

Figure 3. Dissolution of different physical forms of LCQ789 in 20 ml of 0.1N HCl, at 300 rpm.

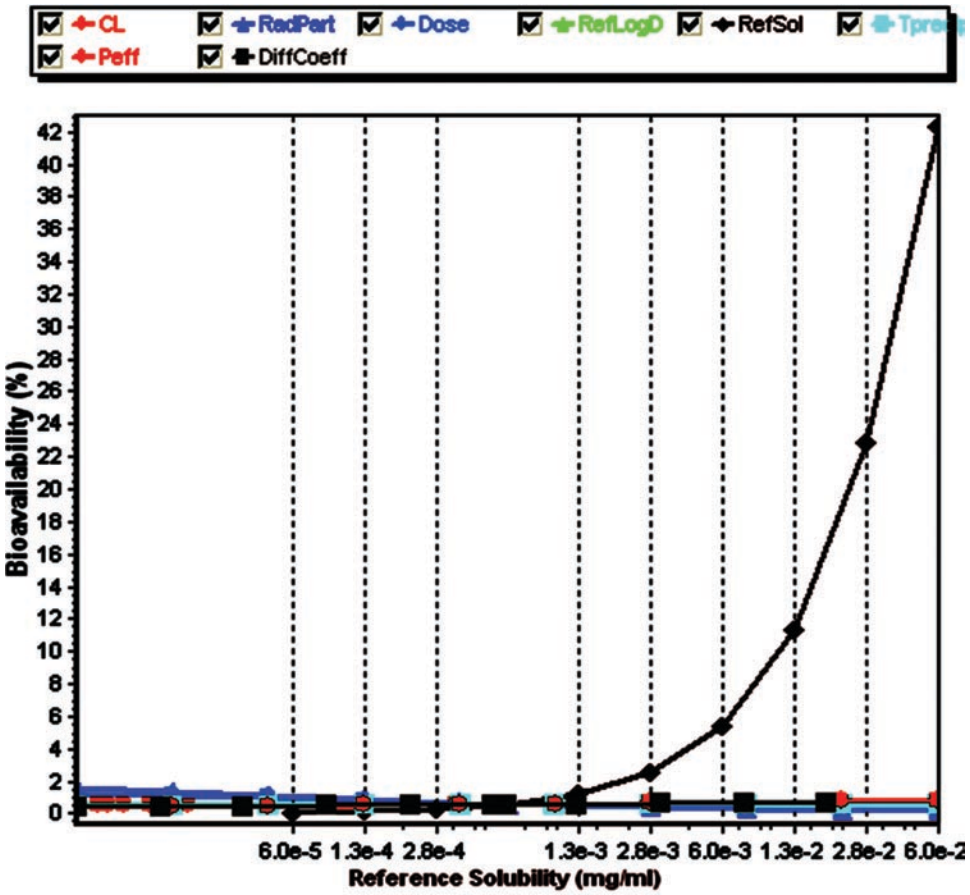

Figure 4. Gastro plus™ parameter sensitivity analysis on LCQ789.

groups, however, for neutral compounds like LCQ789 where salt formation is not feasible, co-crystal approach provides an alternative tool to enhance solubility/dissolution rate. A co-crystal screening was conducted for LCQ 789 with 27 co-crystal formers. A mesylate co-crystal was identified from this screening due to good crystallinity and reproducibility. However, *in- vitro* dissolution testing showed no improvement in dissolution rate with the co-crystal form, possibly due to rapid conversion of the co-crystal form to the poorly soluble neutral form in the dissolution medium (data were not shown).

Solid dispersion

(a) Solid dispersion screening and characterization. High-throughput screening was employed to facilitate the pre-formulation process for solid dispersions by solvent evaporation. This technique can simultaneously screen up to 96 combinations of polymer and surfactant with minimal consumption of the compound. It allows quick identification of the most promising solid dispersion system with minimal amount of material and time. Kinetic solubility values for the 64 solid dispersion variants, each containing 10% w/w LCQ789, at 15 and 60 min are presented in Figure 5. Solubility at two time points was measured to assess the potential of solid dispersions to achieve a high concentration and the ability to maintain the supersaturation for a length of time that would allow rapid absorption and increased bioavailability. As expected, the control well H-1 which does not contain any polymers or surfactants demonstrated low aqueous solubility in 15 min and no solubility was observed at 60 min. Based on the solubility values at 15 and 60 min (Figure 5), six lead variants containing one polymer and one surfactant (A-2, A-7, C-4, C-6, F-5 and F-7) were identified. These variants were scaled up via solvent evaporation using a rotary evaporator.

Solid dispersions consisting of vinyl based polymers (A) were free-flowing granules, and the particle size was successfully reduced by simple sieving. Solid dispersions containing cellulosic polymers (C) formed film-like solids, which presented a challenge for particle size reduction. Solid dispersions containing acrylate based polymers (F) formed a gel during scale up due to the low glass transition temperature (Tg), and were therefore eliminated.

Dissolution of the remaining four lead variants (A and C polymers) in simulated gastric fluid (SGF) is shown in Figure 6. Based on the higher dissolution rate and concentration as well as promising bulk properties, solid dispersion A-2 was selected as the lead variant for comparison with other formulation approaches.

(b) Dosing of solid dispersion in preclinical animal models. Solid dispersions are usually administered in a solid

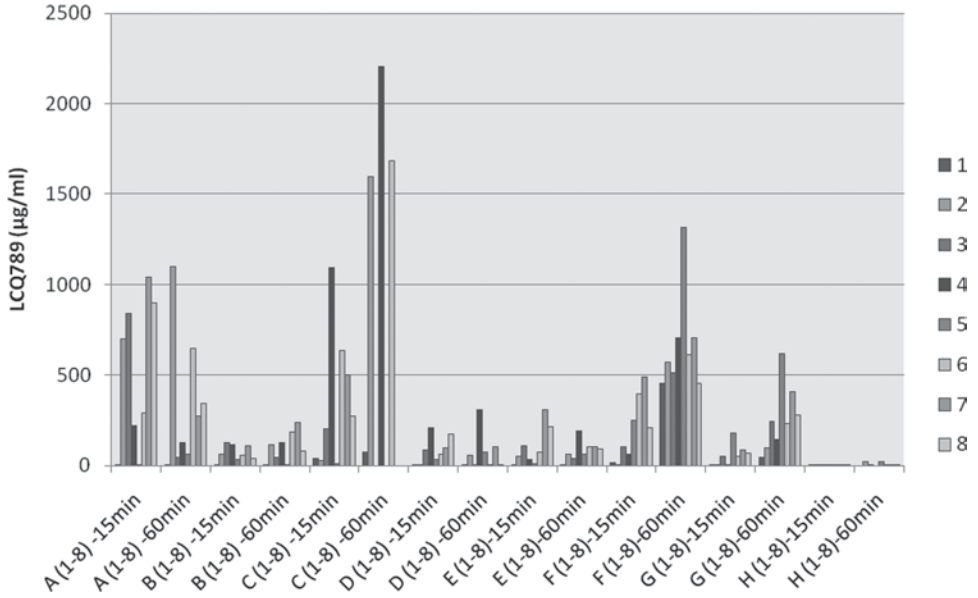

Figure 5. Kinetic solubility (µg/mL) of LCQ789 solid dispersions obtained from high-throughput screening (HTS) at 15 and 60 min in aqueous media.

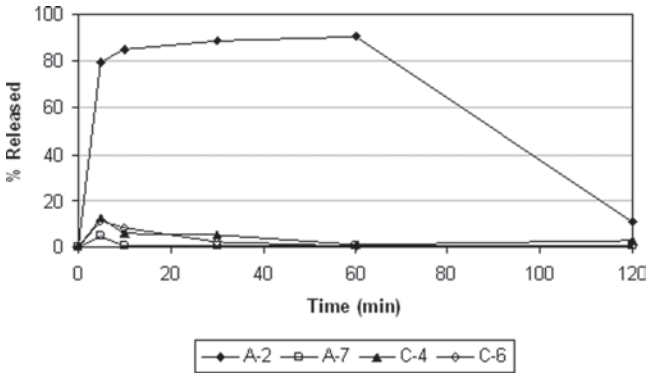

Figure 6. Dissolution of LCQ789 solid dispersions in SGF.

dosage form such as powder in capsule; however, such dosage forms are not feasible for rodents. In such instances, administering the lead solid dispersion as a suspension/solution is the only feasible alternative approach. A suitable vehicle for dosing a solid dispersion should maintain a high active concentration for a reasonable period of time (approximately 2 h for animal dosing) without precipitation of crystalline drug. In this study, purified water, 0.5% w/v methylcellulose (MC), 0.5% w/v MC/0.5% Tween-80, 0.5% w/v PVP, 0.5% w/v Tween-80 and 0.5% w/v SLS were evaluated as suspending agents for solid dispersion of LCQ789. Suspensions/solutions containing 10 mg/mL of the solid dispersion, which corresponds to 1 mg/mL active, were prepared using above vehicles, in duplicate. The concentration vs. time profile of LCQ789 in each vehicle is shown in Figure 7. As shown in the figure, a rapid dissolution of the solid dispersion was observed in all five vehicles. However, only three suspending agents (water, 0.5% PVP and 0.5% SLS) maintained the maximum concentration for at least 2 h. In the other two suspending agents (0.5% MC with and without 0.5% Tween-80), the maximum concentration reduced during the 2 h as the compound precipitated out of solution. As a result, water was selected as the vehicle to dissolve the solid dispersion prior dosing. In order to support higher doses in rodent safety studies with the solid dispersion, solutions with increasing concentrations of solid dispersion in water (10, 50 and 120 mg/mL) were prepared in duplicate and active concentrations were monitored over 2 h to ensure that the supersaturation behavior of solid dispersion was not compromised. As shown in Figure 7, target active concentrations were maintained for at least 2 h in all three solutions that appeared slightly translucent by visual assessment. Based on these results, it was concluded that, the 10% solid dispersion, when dosed as a 120 mg/mL suspension in a 10 mL/kg dosing volume, could support dosing of up to 120 mg/kg of LCQ789.

(c) Solid Dispersion Stability. Due to the presence of the amorphous form of drug substance in solid dispersions, their long-term physical stability is a major concern for further development. In order to identify any such risks with the solid dispersion containing LCQ789, its physical stability was investigated under ambient and accelerated conditions using XRPD and DSC.

Based on the results, LCQ789 maintained its amorphous form in the solid dispersion after storage for 1 week at 40°C/75% RH and 12 months at RT in a desiccator (Figures 8 and 9). However, the solid dispersion absorbed a significant amount of water due to the hygroscopicity of the polymer and formed a gel at 40°C/75% RH in an open container. On the DSC profile, the initial broad endotherm around 50–100° was attributed to the loss of moisture absorbed onto the polymer. As shown in Figure 8, the endotherm was more pronounced after storage at 40°C/75% RH and became less significant after storage in a desiccator. Modulated DSC was performed on the 12-month sample, and only one glass

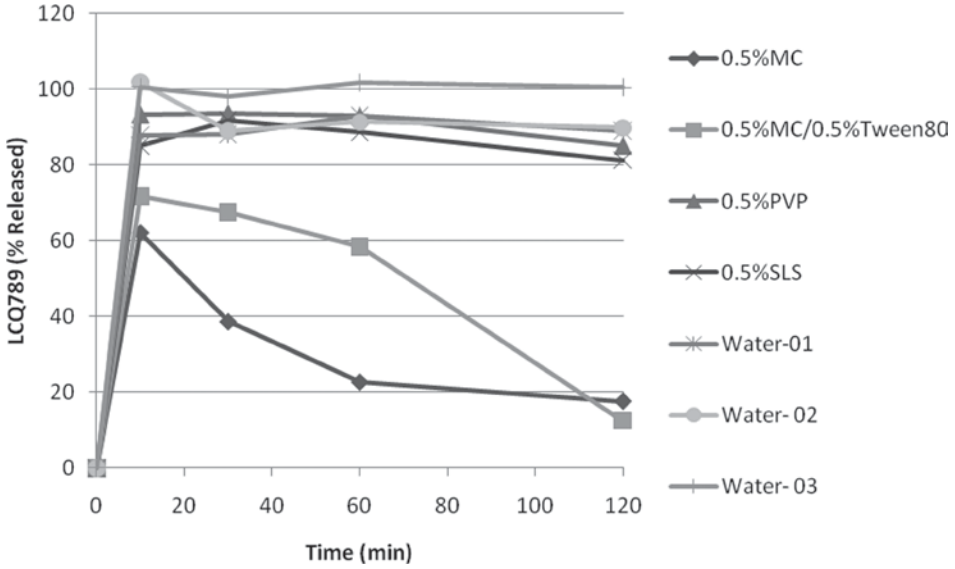

Figure 7. Dissolution of LCQ789 solid dispersion in suspending agents.

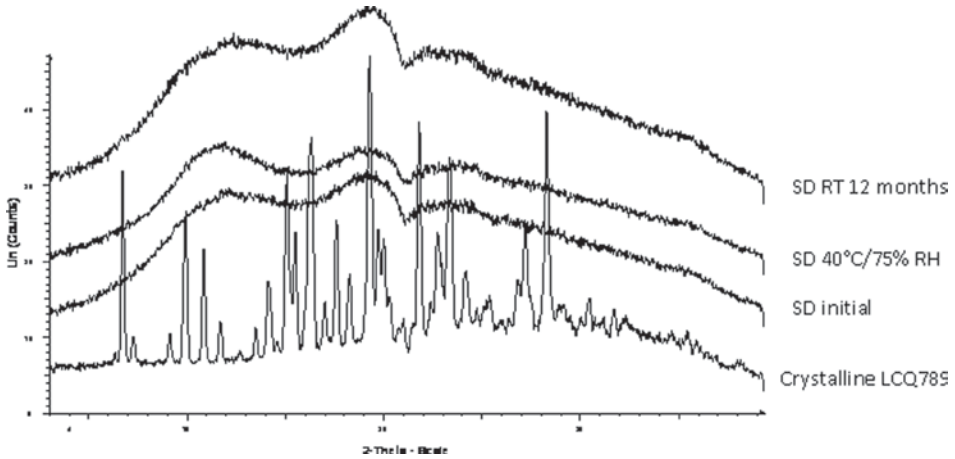

Figure 8. XRPD of LCQ789 solid dispersion after storage.

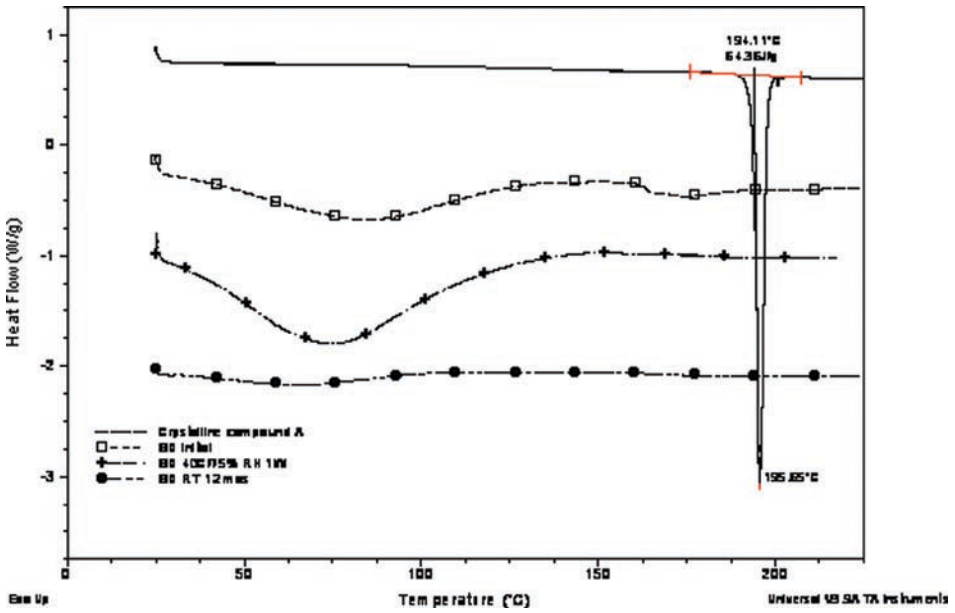

Figure 9. DSC profiles of LCQ789 solid dispersion after storage.

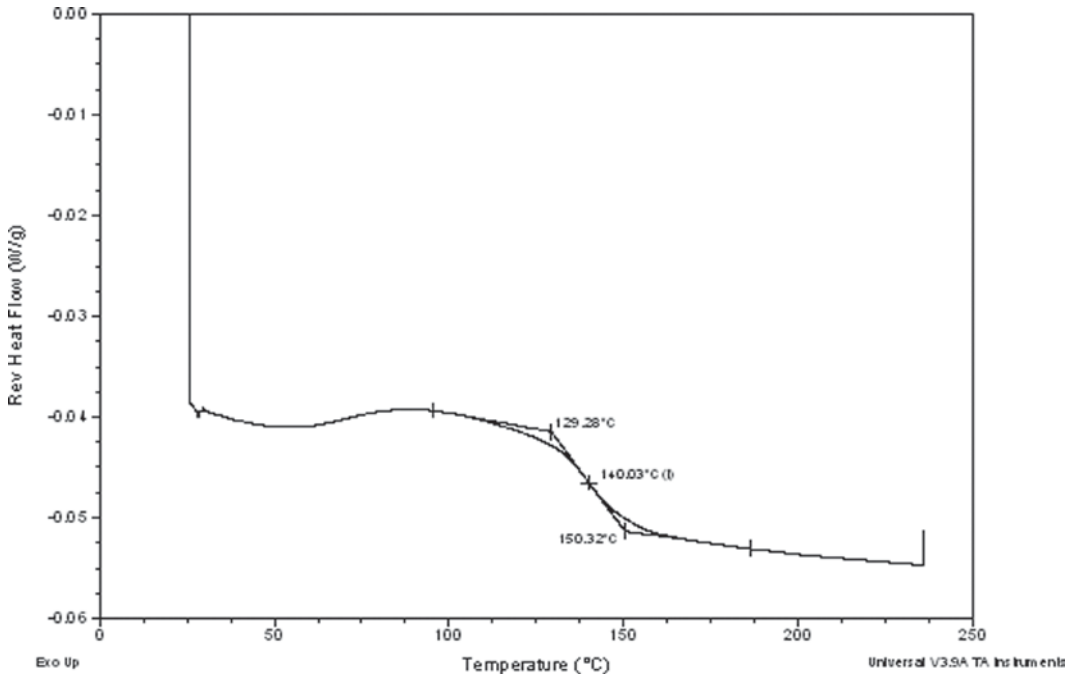

Figure 10. MDSC of LCQ789 solid dispersion after storage at RT for 12 months.

transition temperature (Tg) at 140°C was observed, indicating a completely miscible system and no phase separation (Figure 10). Tg of LCQ789 is 105°C and Tg of polymer is 160°C. In addition, LCQ789 was confirmed to be chemically stable under studied conditions by HPLC assay. Therefore, it was recommended to store the solid dispersion in a tightly closed container at room temperature.

Lipid-based formulation

Based on its high clog *P* value, it was postulated that LCQ789 is lipophilic and may have good solubility in lipids. Microemulsion preconcentrate (MEPC) systems were therefore evaluated as a formulation approach to increase the oral exposure of LCQ789.

In order to identify the components of the microemulsion system, solubility screening of LCQ789 in 25 excipients including solvents, surfactants, oils and lipids was performed. The solubility results are listed in Table 2. Based on the solubility in single component systems, physical stability after dilution and acceptable excipient amounts for toxicology studies, several MEPC systems were screened. Each MEPC consists of lipophilic, hydrophilic and surfactant phases. Solubility of LCQ789 in the studied MEPC systems is also shown in Table 2. MEPC1 was not further pursued since it had significantly lower solubility as compared to the other MEPCs. The other three MEPCs were selected for further studies based on solubility. MEPC4 was found to be the most stable preconcentrate amongst the three MEPCs based on 1-month physical and chemical stability at 4°C, RT and 50°C (Table 3). In addition, MEPC4 exhibited the most promising particle size distribution and physical stability after 10× dilution with water and simulated gastric

Table 2. Equilibrium solubility of LCQ789 in single and multiple-component lipid-based vehicles at room temperature (RT).

Vehicles	Equilibrium solubility (mg/mL)
Cremophor EL	21.13
Tween-80	4.22
Labrasol	10.22
Brij 98 50%w/w in ethanol	12.88
Gelucire 44/14 50%w/w in ethanol	13.79
Solutol HS 15 50%w/w in ethanol	14.34
Vitamin E TPGS 50%w/w in ethanol	17.03
Ethanol	1.68
Propylene Glycol	1.45
Polyethyleneglycol 400	37.45
Transcutol HP	49.06
Arlasolve (Dimethyl Isosorbide)	83.32
Corn oil	2.87
Maisoil glyceride	6.88
Olive oil	2.68
Sesame oil	2.34
Soybean oil	2.51
Miglyol 812	7.35
Peceol (glyceryl monooleate)	4.88
Maisine 35-1 (glyceryl monolinoleate)	5.72
Capmul MCM	13.87
Lauroglycol FCC	8.02
Labrafil M-1944 CS	8.22
Labrafil M-2125 CS	7.58
Triacetin (glycerol triacetate)	28.00
MEPC 1	6.6
MEPC 2	28.1
MEPC 3	20.1
MEPC 4	23.0

fluids among all the MEPCs evaluated. MEPC4 resulted in a translucent microemulsion (Figure 11) upon dilution with water and a narrow particle size distribution with mean particle size of 20 nm and polydispersity index of 0.045. Furthermore, the particle size and particle size distribution were found to be stable for at least 2 h. Similar physical appearance, particle size distribution and physical stability were observed in SGF.

Table 3. Chemical stability of LCQ789 in MEPCs after 1-month storage.

Temperatures	Vehicles	% Recovery
4°C	MEPC 2	99.6
	MEPC 3	99.5
	MEPC 4	99.7
RT	MEPC 2	99.4
	MEPC 3	94.5
	MEPC 4	99.6
50°C	MEPC 2	95.6
	MEPC 3	93.3
	MEPC 4	100

Therefore, MEPC4 was selected for further *in vivo* studies based on drug solubility, chemical stability, particle size distribution and physical stability of the resulting microemulsions.

In vivo evaluation of formulation

Bioavailability assessment

In the rat and dog PK studies comparing various formulation approaches (crystalline neutral form, co-crystal, solid dispersion and MEPC4), minimal exposure was observed with suspensions of crystalline LCQ 789 and co-crystal in rats, but the co-crystal showed improved exposure in dogs. In both species, significant improvement in bioavailability was achieved with the solid dispersion and MEPC formulations (Table 4 and Figure 12). The *in- vivo* pharmacokinetic studies indicated that the solid dispersion improved the oral bioavailability by 18-fold in rats and 50-fold in dogs, while the MEPC increased the oral bioavailability by 25-fold in rats and 80-fold in dogs (Table 5 and Figure 13). MEPC formulation achieved the highest exposure, and the solid dispersion demonstrated

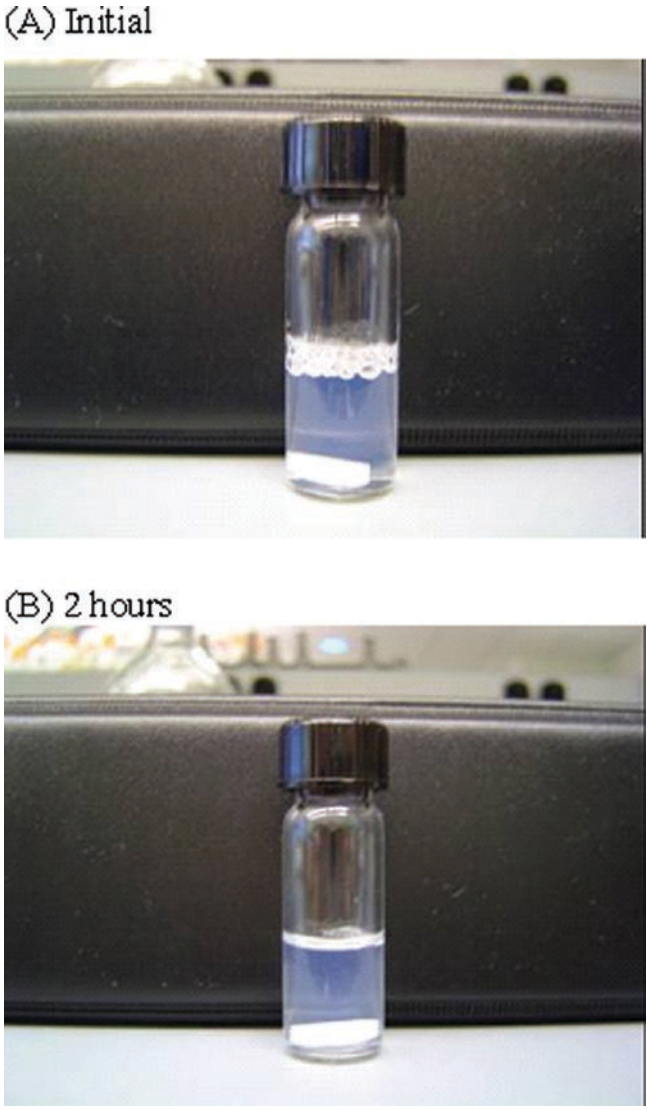

Figure 11. Physical appearance of LCQ789 microemulsion.

Table 4. Pharmacokinetic (PK) evaluation of LCQ789 formulation approaches in rat at 10 mg/kg.

Formulation	AUC/dose (ng·h/mL/mg/kg)	C_{max}/dose (ng/mL/mg/kg)	F%	T_{max} (hours)
Suspension	33.9 ± 7.2	4.9 ± 1.1	4.04	0.83
Co-crystal	50.1 ± 16.2	5.8 ± 2.7	5.91	0.83
ME	848.0 ± 354	127.7 ± 38	99.6	1.33
SD	629.5 ± 126	84.7 ± 1.7	74.0	1.33

(n = 3).

Table 5. Pharmacokinetic (PK) evaluation of LCQ789 formulation approaches in dog at 10 mg/kg.

Formulation	AUC/dose (ng·h/mL/mg/kg)	C_{max}/dose (ng/mL/mg/kg)	T_{max} (hours)
Suspension	4 ± 1	1 ± 0	0.5
Co-crystal	125 ± 98.3	14 ± 7	2
MEPC4	312 ± 63.6	132 ± 78	0.5
SD	210 ± 30.4	58 ± 17	1

(n = 3).

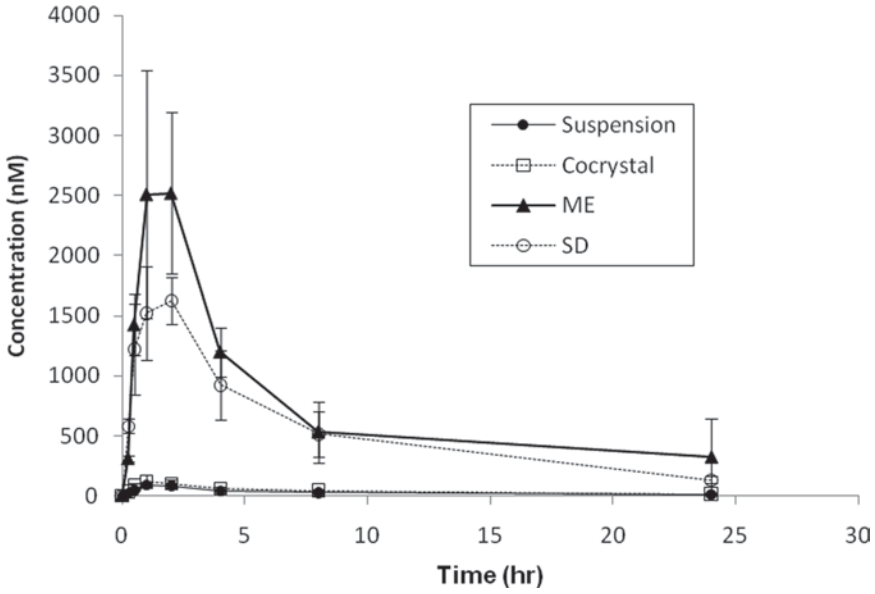

Figure 12. Plasma concentration of LCQ789 following oral administration of four formulations in rat.

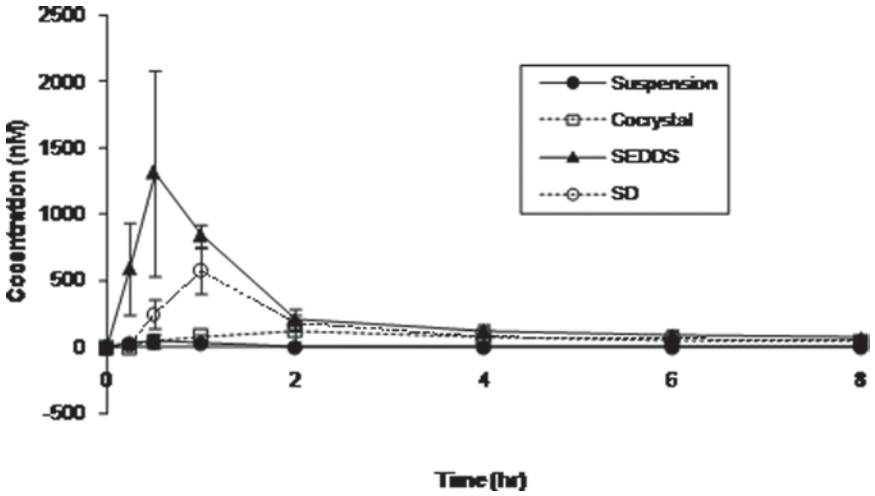

Figure 13. Plasma concentration of LCQ789 following oral administration of four formulations in dog.

less inter-subject variability. Therefore, both formulation principles were recommended as promising approaches to increase oral bioavailability of LCQ789 for preclinical as well as clinical studies.

Dose-rising studies

Based on promising bioavailability enhancement observed in the single low dose PK studies (10 mg/kg), the next step was to evaluate the performance of both solid dispersion and MEPC in a dose-rising study. The dose-rising study was performed in rats and a relatively linear increase in exposure was observed for both formulations upon dose escalation from 10 to 100 mg/kg. Higher exposure was achieved with the MEPC, but it also exhibited higher variability as compared to the solid dispersion. In addition, overall high organic content was a concern for the long-term safety studies. As a result, the solid dispersion formulation was selected for use in safety studies.

Conclusions

Utilization of high-throughput technologies and a decision-making process based on *in vitro* solubility screening and oral exposure in preclinical species, enabled early identification of bioavailability enhancing formulations for a poorly water-soluble research compound with significant savings of time and material. Early selection of the TDS form and formulation principle ensured that a single formulation could be used throughout preclinical studies, and could ultimately accelerate the clinical formulation development. A comprehensive developability assessment, as highlighted in this article, could be a very useful tool in providing timely feedback to the research chemists on structure modifications to improve solubility profile of lead candidates, and to development colleagues, on the preliminary clinical formulation strategy.

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Declaration of interest

The authors declare no conflicts of interest.

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