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Physicochemical Characterization of Lupeol and Development of a Preclinical Formulation for Oral Administration

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Abstract

Purpose: The aim of this research was to characterize the physicochemical properties of lupeol, a highly lipophilic (calculated log P = 11), low solubility compound (calculated S = $3x10^{-6}$ mg/mL), and to devise a formulation that would enable oral dosing for preclinical animal studies. **Methods:** Lupeol (>98% purity, Sigma) was characterized for its purity by HPLC as well as some solid state properties by x-ray powder diffraction, differential scanning calorimetry, thermogravimetric analysis, gravimetric moisture sorption and hot-stage microscopy. Solubility of lupeol was measured in 11 pharmaceutically acceptable cosolvents and lipids, from which selected formulations were advanced to dissolution/precipitation testing and stability assessment. **Results:** Lupeol is a crystalline solid exhibiting a volatiles content of ~0.6% (mainly moisture) and a melting point of 212°C. The solubility of lupeol was highest in medium chain mono/diglycerides (Capmul® MCM) and mono/dipropylene glycol esters (Capmul® PG-8), and next highest in triglycerides (Captex® 355 and Canola oil). Formulations composed of 95% Capmul PG-8/5% Tween 80 and 60% Captex 355/35% Capmul MCM/5% Tween 80 were further advanced and found to be more compatible with hard gelatin than HPMC capsules. Both formulations were placed on stability for 1 month at 30°C/65% RH and 40°C/75% RH, where it was found that they were chemically stable, but exhibited a decrease in dissolution (greater precipitation) in 0.01 M HCl. Additional experiments revealed that the dissolution decrease was likely due to pellicle formation of the capsule shell and not the formulation. The overall assessment led to the selection of 60% Captex 355/35% Capmul MCM/5% Tween 80 as a viable formulation to move forward to animal studies. **Conclusions:** Characterization and preclinical formulation assessment of lupeol led to the identification of a lipid-based vehicle (60% Captex 355/35% Capmul MCM/5% Tween 80) to achieve highest solubility, excellent stability over 1 month, and minimal precipitation upon dilution in 0.01 M HCl. This study represents the first report of developing a lipophilic vehicle deliver system for lupeol, and will offer a significant advantage to the previous formulations that included a 1:1 corn oil and ethanol vehicle.

Introduction

Lupeol [Lup-20(29)-en-3β-ol] (Figure 1) is a dietary triterpene found in fruits and is the proposed active constituent of various medicinal plants. Multiple traits have been attributed to Lupeol that include antioxidant, anti-inflammatory, antiarthritic, antimutangenic and antimalarial activity. Lupeol modulates the activity of protein kinases and serine proteases and inhibits the activity of topoisomerase II, a known target for anticancer chemotherapy. Animal studies have shown that Lupeol possesses anti-cancer activity against various cancers that include prostate, melanoma, and head and neck cancer. Lupeol is currently under development for chemoprevention and chemotherapy.

Lupeol is lipophilic, has no ionizable moiety in the physiological pH range, is insoluble in water and the the calculated log P = 11 and calculated S = $3x10^{-6}$ mg/mL (ACD Labs v8.19); therefore, Lupeol is expected to exhibit poor bioavailability by the oral route.

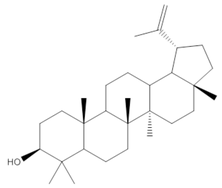

Figure 1 – Structure of Lupeol

Materials

Lupeol (Sigma lots 076K1464, 076K1736, 018K1213), polyethylene glycol 400 (PEG400) (Fluka lot 1120128 43304095), propylene glycol (Sigma lot 114K0180), ethanol (AAPER lot 06H17WA), dimethylacetamide (Sigma lot 10057JD), N-methylpyrrolidone (Omnisolv lot 46102), Transcutol HP (diethylene glycol monoethyl ether, Gattefosse Lot 450542007), Captex® 500 (triacetin) (Abitec lot 051002), Captex® 355 (glycerol caprylate caprate, an MCT) (Abitec lot 070505-7), Pureco® canola oil (an LCT) (Abitec lot 061010-1), Capmul® MCM (glyceryl mono and dicaprate) (Abitec lot 070529-6), Capmul® PG-8 (propylene glycol monocaprylate) (Abitec lot 070322), Labrasol® (caprylocaproyl polyoxyl-8-glycerides) (Gattefosse lot 08257), Labrafil® M 1944 CS (oleoyl polyoxyl-6-glycerides) (Gattefosse lot 106739), Tween® 80 (Polysorbate 80) (Fisher lot 032097), gelatin capsules, natural transparent size 00LLC (Capsugel lot 70063271), HPMC capsules, natural transparent size 00 (Vcaps, Capsugel lot 273891). Additional chemicals were HCl (Fisher lot 073966), Acetonitrile (Fisher lot 076146), and Isopropanol (Fisher lot 071519).

Methods

Powder X-Ray Diffraction was measured using a Bruker D8 Advance diffractometer operated at 40 kV/40 mA in Bragg-Bretano Θ-Θ geometry with a Si(Li) scintillation detector. Samples were prepared on zero-background Si plates and scanned from 2-50°2Θ in 3 s steps of 0.02°. The XRD pattern of Lupeol is presented in Figure 2 indicating the material is crystalline.

Thermal and Moisture Analysis: DSC was run on a Q2000 (TA Instruments) at a scan rate of 10°C/min with N2 purge at 50 mL/min. Duplicate samples were prepared in Al crimp-sealed pans. TGA was run on a Q5000IR (TA Instruments) in a platinum pan at a scan rate of 10°C/min with N2 purge at 5 mL/min. Moisture sorption was measured on a Q5000SA (TA Instruments) automated apparatus operated at ambient pressure with RH varied by mixing dry N2 and water-saturated N2 gas streams. The method was run at 25°C with an RH sequence 40-90-10-90-40% RH in 10% increments. Equilibrium criterion was not more than a 0.005% weight change over 60 min.

Hot-Stage Optical Microscopy: Used the BH2-UMA Olympus microscope with 20x magnification and 5°C/min heating rate. Duplicate samples were prepared by dispersing on a microscope slide and adding a coverslip. Images were captured using Adobe Photoshop Elements version 5.1 service pack 2 (Adobe).

HPLC Assay: Samples were analyzed by HPLC using a Waters Symmetry Shield RP18 (3.5µm, 4.6x150 mm) column operated at 25°C. The isocratic method used acetonitrile mobile phase, 1.0 mL/min flow rate, 200 nm UV detection, and 10 µL injection volume. The sample diluent was isopropanol/acetonitrile (75:25).

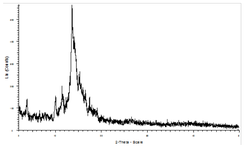

Figure 2 – Powder X-Ray Diffractogram of Lupeol

Solubility was determined by the shake-flask method. Approximately 100 mg of Lupeol was weighed into 4-mL glass vials. A 1.0 mL aliquot of vehicle was added to each vial, then vortexed. Vials were rotated (Thermo Scientific) in an incubator (Fisher Scientific) at 25ºC. At 1 week intervals, vials were removed from the incubator. A portion of supernatant from each vial was transferred into a filtering microcentrifuge tube (Costar Spin-X) and centrifuged (Fisher Scientific) at 10,000 rpm until all of the liquid passed through the filter (0.22 µm nylon) to the bottom of the tube. HPLC samples were prepared by diluting 100 µL of these solutions with 75/25 IPA/ACN to a sample concentration bracketed by Lupeol standards. Samples were tested every 7 days until the analysis results showed that the concentration was equilibrated (e.g., change is less than ±10%) or until significant degradation was observed.

Capsule Preparation: Gelatin and hypromellose (HPMC) capsules were filled manually and sealed by applying a drop of water to the seam with a syringe. Capsules were assayed using the HPLC method.

Stability of the capsule formulations was assessed over a 1 month period by storing the capsules in a plastic container (open) in environmental chambers (Caron, model 6010) at 30°C/65% RH and 40°C/75% RH. The capsule formulations were evaluated for appearance, assay, and precipitation.

Precipitation on Dilution of each formulation was assessed using USP Apparatus 2 dissolution equipment (VanKel VK7000) with 200-mL vessels containing 0.01 M HCl at 37°C. Paddles were operated in the top third of the vessels (approximately 6 inches from the surface) at 250 rpm to produce a homogenous dispersion. Capsules were added using sinkers (Quality Lab Accessories). Aliquots (3-mL) were taken from the center of the vessels, using a 16 g x 5 in needle (Air-Tite Products Co., Inc.) and 3-mL syringe (BD), and filtered through 0.2 µm PTFE filters. Filtrates were diluted 1:1 with 100% IPA for HPLC analysis.

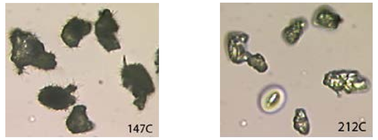

Figure 4 – Hot-Stage Optical Microscopy of Lupeol

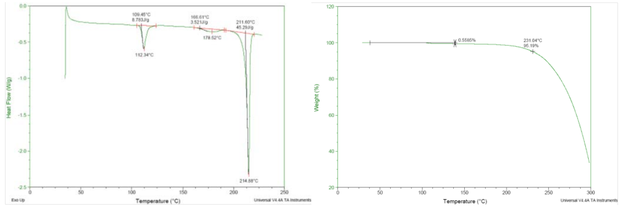

Figure 3 – DSC and TGA Thermo-grams of Lupeol

Results & Discussion

The XRD pattern of Lupeol is presented in Figure 2, indicating the material is crystalline.

DSC and TGA results are presented in Figure 3 and hot-stage optical microscopy (HSOM) photomicrographs are provided in Figure 4. Thermal data support the XRD finding that the material is crystalline. Three thermal events occur in the DSC scan. The event at 109°C is associated with a weight loss of 0.56% by TGA. This may be due to a low and not well-defined hydrate stoichiometry (0.13 mol H_2O to 1 mol Lupeol). HSOM did not show any changes in the range of this thermal event. After this endotherm, weak exothermic activity occurs, during which needle-like projections start to appear on the surface of the larger particles, as seen in HSOM at 147°C (Figure 4). The endotherm at 167°C is accompanied by the disappearance of these needle-like crystallites. The endotherm at 212°C is at the melting point of Lupeol. No further characterization of the thermal transitions was conducted.

The moisture sorption profile of Lupeol is presented in Figure 5, where it is observed that the powder sorbs 0.6% water over the 10-90% RH range on the first cycle. A smaller water uptake of 0.4% is observed on the second cycle. The powder is expected to contain ~0.4% water at ambient conditions of 50% RH, which is close to the weight loss observed by TGA.

The solubility results are presented in Table 1. Based on these solubility results, two formulations were advanced: Capmul PG-8 and 60% Captex 355/35% Capmul MCM/5% Tween 80. The Capmul PG-8 formulation was amended to include 5% Tween 80 to promote better dispersion in an aqueous environment (based on preliminary precipitation experiments). Compatibility of the two formulation vehicles was assessed with gelatin and HPMC capsules stored open at 30°C/65% RH. After one day, the HPMC capsules were wet on the outside, implying the vehicle had significantly migrated into the capsule shell.

Hard gelatin capsule formulations were placed on stability (open) for one month at 30°C/65% RH and 40°C/75% RH. The Capmul PG-8/Tween 80 formulation appeared to sorb into the capsule shell. The Captex 355/Capmul MCM/Tween 80 formulation did not appear to sorb into the capsule shell, but was distorted. Both formulations were chemically stable, but exhibited greater precipitation upon dilution after 1 month compared to initial. This was determined to be an artifact of gelatin crosslinking. Based on these results, the Captex 355/Capmul MCM/Tween 80 formulation is more stable for evaluation in a proof-of-concept animal testing program.

Conclusions

Lupeol is a crystalline solid. The moisture sorption profile illustrated that Lupeol sorbs 0.6% over 10-90% RH. Solubility of Lupeol is high in oil, most notably medium chain mono and diglycerides and propylene glycol monocaprylate. Low levels of Tween 80 (5%) combined with oil phases enabled the formulations to maintain the highly lipophilic Lupeol in solution when dispersed in 0.01 M HCl. In particular, the formulation composed of 35 mg Lupeol/mL in 60% Captex 355 (MCT)/35% Capmul MCM/5% Tween 80 was able to maintain 80-90% of Lupeol dispersed in 0.01 M HCl. When stored in glass vials for 1 month at 30°C/65% RH, this formulation was stable with respect to appearance, content and impurities, and precipitation upon dilution in 0.01 M HCl. The formulation was filled into hard gelatin capsules. Based on the high solubility of Lupeol in the formulation (35 mg/mL) and the stability assessment, the 60% Captex 355/35% Capmul MCM/5% Tween 80 formulation was selected to advance Lupeol for oral dosing in animal studies.

Table 1 – Solubility of Lupeol

Vehicle	Solubility (mg/mL)
PEG400	<0.9
Propylene glycol	<0.3
Ethanol	6.7-10*
Dimethylacetamide	12-25*
N-methylpyrrolidone	>103
Transcutol HP	10-20*
Captex 355 (MCT)	25.2
Pureco canola oil (LCT)	18.6
Capmul MCM	40.2
Capmul PG-8	45.9
Labrasol	13.0
60% Captex 355/35% Capmul MCM/5% Tween 80	35.0
60% Canola oil/35% Capmul MCM/5% Tween 80	31.4

* Visual estimate determined at ~22°C.

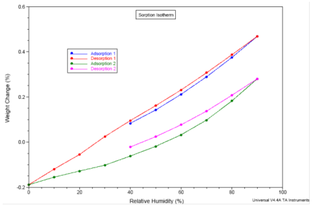

Figure 5 – Moisture Sorption Profile of Lupeol

Acknowledgement

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