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RESEARCH PAPER

Oral Dosage Development of a Human Rhinovirus and Non-Polio Enterovirus Inhibitor

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ABSTRACT

WIN 63843, a Picornavirus replication inhibitor, is physically and chemically stable in the solid state, to light, elevated temperature, and humidity. This 3-aryl-5-trifluoromethyl disubstituted 1,2,4-oxadiazole compound has very low water solubility but is highly soluble in ethanol and in safflower seed and corn oils. Solubility in the vegetable oils is doubled by the synergistic effect of ethanol at the 16% alcohol concentration. Vegetable oil solutions of WIN 63843 are thermally stable but react slowly in the presence of light resulting in an amidoxime compound (WIN 65489) formed by opening of the 1,2,4-oxadiazole ring. This reaction does not occur in oil solutions containing small concentrations of ethanol. Of biopharmaceutical interest, the addition of ethanol or PEG-400 solutions of WIN 63843 to human gastric fluid resulted in oily droplet formation whereas crystals form upon addition of these solutions to water. Also, the compound is greater than 8,000 times more soluble in human gastric fluid.

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INTRODUCTION

WIN 63843 is one of a class of potent, viral specific inhibitors of the replication of human rhinoviruses (HRVs) and non-polio enteroviruses (NPEVs: Coxsackie viruses and echoviruses) (1). Potential indications include prophylaxis and treatment of respiratory tract infections (i.e., common cold) and the treatment of diseases resulting from NPEVs infection (e.g., aseptic meningitis). It exists in at least three low melting forms (61–64°C) (2).

These studies were performed to determine key chemical and physical properties of WIN 63843 and for clinical formulation development. Since solubility results and decomposition profiles of other compounds in biological fluids differed substantially from the simple acid and oxidation profiles routinely used in preformulation evaluations, the drug substance was also tested in human gastrointestinal (GI) fluids to provide a unique biopharmaceutical profile useful for oral formulation development and evaluation (3,4,5).

MATERIALS AND METHODS

HPLC Method

The HPLC system and method have been previously described in detail (6). The peak areas were integrated at 230 nm.

Solubility

WIN 63843 was added to four mL vials with teflon lined screw caps, containing 1 mL of solvent. The capped vials were placed on a laboratory rotator (GlasCol, Terra Haute, IN) for 40 hr. Aliquots were taken at 20 and 40 hr and filtered through 0.45 μm membrane filters for HPLC analysis. The corn oil, safflower seed oil and oleic acid solutions were first dissolved with acetone and then diluted with acetonitrile in a 3:1 ratio. All other samples were diluted with acetonitrile. The pH was measured with a Beckman ϕ71 pH meter (Beckman Instruments Inc., San Ramon, CA).

After 40 hr, the saturated solutions were sonicated for 30 to 40 min, then allowed to stand at room temperature overnight. Each solution was filtered and assayed as above.

Solid State Stability

WIN 63843, in an open petri dish, was placed in a 40°C oven at 75% relative humidity. A second sample, in a glass-covered petri dish was placed in a cabinet of 1000 foot-candle fluorescent and incandescent light. Portions of the samples were removed for analysis at various intervals during the stress period.

Suspension Stability

Approximately 20 mg of WIN 63843 was accurately weighed in separate, tared four dram, screw cap glass vials. Buffers (0.05 M . 0.15 ionic strength) were added in five mL volumes to each vial. The capped vials were placed in a shaking water bath (model 25, Precision Scientific, Chicago, Il) set at 50°C and 114 cycles/min. The entire vial contents were qualitatively transferred to a volumetric flask with methanol rinses and the resultant solutions were assayed by HPLC. Human gastric fluid was used in place of buffers in a similar test. The pH of each suspension was determined on the day of analysis.

Oil and Ethanol/Oil Solution Stability

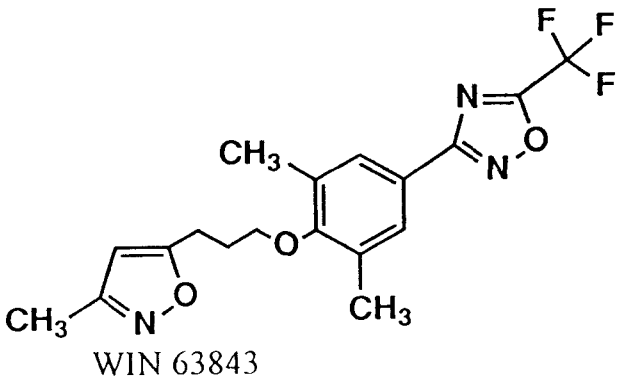
Corn oil and safflower seed oil solutions of WIN 63843 were placed in 4 mL, glass vials with teflon lined screw caps. Vials of the solutions were placed in the 30°C, 40°C, and 50°C ovens and in the 1000 foot-candle light cabinet. Similar samples were protected from light in a closed cardboard container at room temperature in a closed cupboard.

Solubilty and Characterization in Human GI Fluids

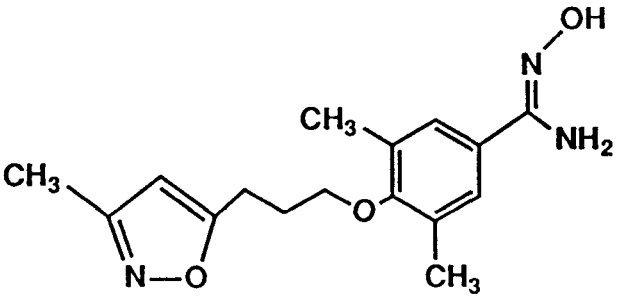
Solutions of WIN 63843 in ethanol were prepared at 10 mg/mL concentrations and in PEG-400 at 1 and 2 mg/mL. Human GI fluids were collected by the Sterling Pharmacology Study Unit personnel (Albany Medical Center Hospital, Albany, NY) from paid healthy volunteers. The procedure was described previously (3). The fluids were heated to 37°C in two mL volumes and used immediately. The heated fluids were spiked with PEG-400 solutions of WIN 63843 (2 mg/mL) until precipitates were observed by microscopy, using the reported method and equipment (3).

RESULTS AND DISCUSSION

WIN 63843 and it primary decomposition product WIN 65489 are shown in Figure 1.



WIN 63843



WIN 65489

Figure 1. WIN 63843 and the primarily decomposition product WIN 65489.

HPLC Method

WIN 63843 peak areas eluted at 7.02 minutes and were integrated at 230 nm. Two standard reference series were used, ranging from 20 to 200 μg/mL (linearity = 0.999, slope = 0.00041, intercept = 0.00019) and from 0.02 to 2 μg/mL (linearity = 0.996, slope = 0.0005, intercept = 0.00004).

Solubility

WIN 63843 solubility was tested at 25°C using sonication to ensure solution saturation. The results (Table 1) show a high solubility in corn and safflower seed oils. The same results were obtained with oleic acid. The drug concentration in water was below quantifiable limits (< 20 μg/mL).

Ethanol was miscible up to 16% with corn oil, safflower seed oil, and oleic acid. Solutions of WIN 63843 prepared in ethanol/oil showed a saturation solubility synergism at all ethanol concentrations (Table 2, Figure 2).

Table 1

WIN 63843 Solubility at Room Temperature Following Sonication and Recrystallization

Solvent	WIN 63843 Concentration (mg/mL)
Water	< 0.00002
22.5% HP-β-CD*	0.28
45% HP-β-CD*	1.26
Ethanol	113
PEG-400	28.9
Glycerol	0.13
Corn Oil	71.2
Safflower Seed Oil	69.2
Oleic Acid	81.5

*Hydroxy propyl-beta-cyclodextrin.

WIN 63843 solubility is directly proportional to the percent ethanol in the oil (approximately a five mg/g increase per percent addition of ethanol) (Figure 2). Since the oils are not miscible with more than 16% ethanol, the limit of the solubility is near 145 mg/g. The addition of 16% ethanol results in a 28% increase over that in 100% ethanol, and a 100% increase over than in pure oil. Increasing the ethanol concentrations would increase the solvent dielectric constant and result in a more favorable environment for solutions to form. Considering the large lipophilic moiety and the more hydrophilic 5-trifluoromethyl-1,2,4-oxadiazole portion of the molecule, the solubility increase above that of each pure solvent is not surprising.

Solid State Stability

WIN 63843 was shown to be a stable solid under light conditions (1000 foot-candles) and at elevated tem-

Table 2

WIN 63843 Solubility in Corn Oil and Safflower Seed Oil That Contain Ethanol

	WIN 63843 Concentration (mg/g)	
Percent Ethanol	Corn Oil	Safflower Seed Oil
5	92.2	90.2
10	114	116
15	130	130

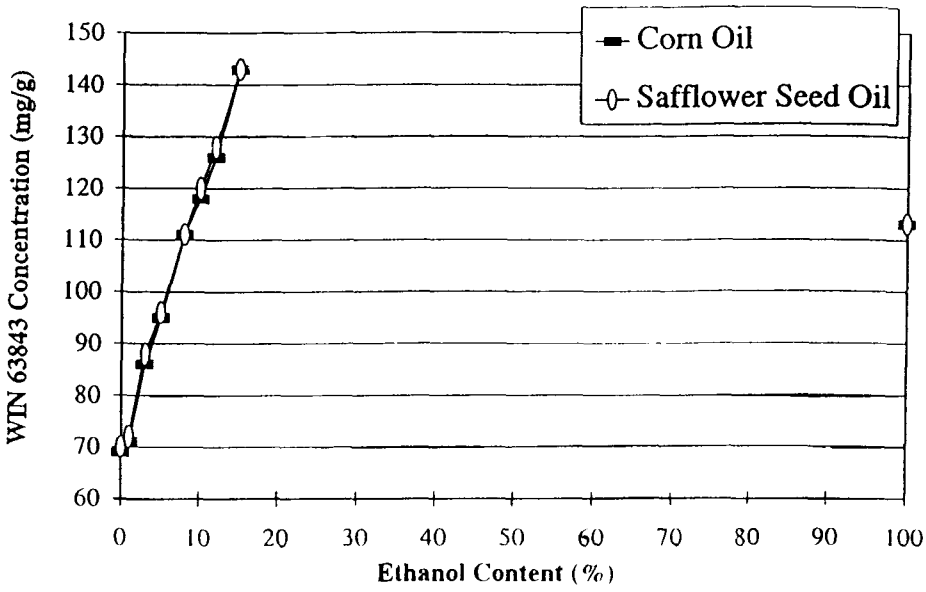


Figure 2. WIN 63843 concentration in corn oil solutions that contain ethanol concentrations ranging from 0 to 16%.

perature and humidity (40°C/75% RH) for five weeks with 100% of the powder as WIN 63843. There was no chemical change or change in texture or color.

Suspension Stability

An intranasal or oral suspension dosage was under consideration. This made the study of drug suspension necessary. Since the molecule's stability was anticipated, it was stressed at 50°C for test acceleration. Constant mixing ensured even exposure of the drug surface to the buffer solutions. WIN 63843 is stable as a suspension in aqueous buffers at 50°C for six weeks at pH values of 2, 3, 5, 6.5, and 7.4. No chemical or physical changes occurred, probably due to the compound's low aqueous solubility.

Corn Oil Stability

Since WIN 63843 concentrations in oils were favorable, stability of a preparation was determined. Accelerated conditions of heat and light were compared to a control kept in the dark at room temperature. The 30°C samples were depleted after six weeks, terminating that station (Table 3).

Table 3

WIN 63843 Concentration (mg/g) in Corn Oil Solutions Subjected to Thermal and 1000 Foot-Candle Light Stress

Time (weeks)	Room Temperature	30°C	40°C	50°C	Light Cabinet
Initial	73.9	74.9	74.4	74.9	65.5
2	74.3	74.2	74.8	74.8	63.9
4		75.0	74.6	75.2	61.4
6	74.6	75.2	74.9	74.6	60.6
8					59.4
9					59.8
13					56.7
14	74.0		74.7	74.0	

WIN 63843 was thermally stable for 14 weeks but showed a slow, light mediated decomposition of about 1% per week (Figure 3). A more accurate degradation value cannot be estimated due to the lack of half-life determination, however a decomposition trend is seen. Buffered aqueous solutions that contained ethanol showed decomposition not seen in oil/ethanol solutions (6). The amidoxime compound, WIN 65489, appeared as 1.3% of the drug substance by HPLC after 13 weeks in the light cabinet. This compound is formed via an oxadiazole ring opening reaction (6). Other compounds of this class also degrade by the same ring opening process (5, 7, 8). Several other compounds appeared as small peaks on the chromatograms in addition to the known degradation products of WIN 63843. Therefore, corn oil solutions of WIN 63843 should be adequately protected from light.

When a corn oil solution (68.2 mg/g) was exposed to 1 N HCL or 30% hydrogen peroxide at 37°C for 2 hr, the compound's concentrations were unchanged. Oxidation and acid hydrolysis did not occur.

Ethanol/Oil Solution Stability

Since the addition of ethanol to corn oil or safflower seed oil increased WIN 63843 concentrations in the formulation, the stability of these solutions was tested. WIN 63843 was shown to be stable to thermal stress (50°C) or light for 50 days in the oils containing between one and 16% ethanol (Figure 3). The light mediated decomposition seen in the oil solutions did not occur when ethanol was added even in very low concentrations. Ethanol functioned as an enhancing agent for solubilization and appeared to preserve WIN 63843 concentrations in oil solutions. WIN 63843 solutions in ethanol/oil have the necessary stability and concentration for a clinical dosage form.

Characterization in Human GI Fluids

Since solubility is usually higher in biological fluids due to protein binding and the presence of surfactant-like compounds, short time stability was investigated in addition to the acid hydrolysis and oxidation tests. WIN 63843 was stable for 2 hr in human gastric fluid when added as a solid or in a corn oil solution, (Table 4). No chemical reaction is expected to occur in gastric contents upon administration of WIN 63843, therefore no special enteric formulation strategies are necessary for these dosage forms.

WIN 63843, when added as a PEG-400 or ethanol solution to water, precipitated as crystals. The same solutions formed oily droplets in human GI fluids. The phenomena was previously reported and may explain the

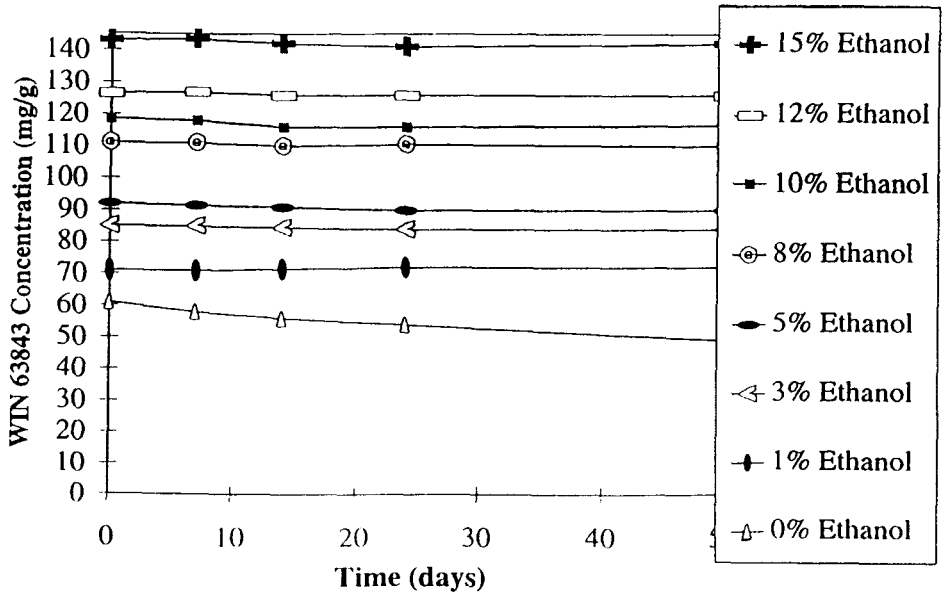


Figure 3. WIN 63843 concentrations in corn oil that contained various amounts of ethanol and was exposed to 1000 foot-candles of incandescent-fluorescent light.

Table 4

WIN 63843 Suspended in Human Gastric Fluid

Gastric Fluid pH	Initial mass (mg)	Mass after 2 hours (mg)
2.0	26.4	26.5
2.8	20.5	20.6
	In a corn oil solution	
Gastric Fluid pH	Initial mass (mg)	Mass after 2 hours (mg)
2.0	60.2	60.0
2.8	46.6	45.3

bioavailability of poorly water soluble compounds (3,4). Drugs in droplet form dissolve more rapidly than in the crystal form and therefore are more rapidly absorbed. The solubility ranges differ among individuals and between the fed and fasted states. The volunteers in the fasted state were given Kinevac® (CCK) to stimulate the gall bladder. The subsequently collected fluid was similar to intestinal fluid from the fed state by the presence of bile.

The physical form that any compound takes when a solution is added to GI fluids is of biopharmaceutical interest. When a PEG-400 solution (2 mg/mL) or an ethanol solution (10 mg/mL) of WIN 63843 was added to human GI fluids at 37°C, oily droplets of WIN 63843 formed. This occurred when the WIN 63843 concentration was greater than 160 μg/mL in gastric fluid, and also occurred in intestinal fluids in concentrations ranging from 50 μg/mL to 190 μg/mL (Table 5). Below these concentrations, WIN 63843 was soluble in these fluids during the 15 min observation period. The great capacity of these fluids to enhance solubility is apparent from the greater than 8,000 times concentration increase in gastric fluid and greater than 2,500 times in intestinal fluid compared to the compound's solubility in water of less than 20 ng/mL.

CONCLUSIONS

WIN 63843 is stable to light, heat, and humidity in the solid state and should be suitable for formulation in a solid dosage form.

Aqueous solubility is extremely low (<20 ng/mL). In contrast, solubility in lipid solvents like corn oil is high. These solutions are stable to heat but sensitive to light. The light sensitivity is alleviated with the addition of ethanol, which also permits higher drug concentrations in oil solutions. Thus, an oil solution is a potential clinical formulation.

WIN 63843 is also stable when added as a powder or oil solution to human GI fluids. Addition of either a PEG-400 or ethanol solution to gastric fluid resulted in oily droplet formation whereas crystals formed when the same solutions were added to water. Formation of oily

Table 5

WIN 63843 Solubility in Human Gastric and Intestinal Fluids at 37°C

Fluid	Measured pH	WIN 63843 Solubility Range (μg/mL)
Gastric	2.8	160–190
Gastric	2.0	160–190
Intestinal (fasted)	2.0	190–210
Intestinal (fed)	1.9	50–80
Intestinal (fasted)	6.7	70–90
Intestinal (fed)	6.6	80–100
Intestinal (fasted)	7.1	120–150
Intestinal (fed)	2.2	50–80

droplets may increase the bioavailability of poorly water soluble drugs.

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