Date of Approval: March 30, 2022

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-551

Zenalpha®

medetomidine and vatinoxan hydrochlorides injection

Solution

Dog

Zenalpha® is indicated for use as a sedative and analgesic in dogs to facilitate clinical examination, clinical procedures and minor surgical procedures

Sponsored by:

Vetcare Oy

Executive Summary

Zenalpha® (medetomidine and vatinoxan hydrochlorides injection) is approved for use as a sedative and analgesic in dogs to facilitate clinical examination, clinical procedures and minor surgical procedures. The dose is based on the dog's body surface area and is calculated using 1 mg of medetomidine per square meter (m^2) of body surface area (BSA) or by using the dosing table provided in the labeling. The drug is administered once by intramuscular (IM) injection and the average duration of sedation is 38 minutes.

Medetomidine and dexmedetomidine (which is in the same class as medetomidine) are already approved for sedation and analgesia in dogs over 12 weeks of age to facilitate clinical examinations, clinical procedures, and minor surgical procedures not requiring muscle relaxation.

Proprietary Name	Established Name	Application Type and Number	Sponsor
Zenalpha®	medetomidine and vatinoxan hydrochlorides injection	New Animal Drug Application (NADA) 141-551	Vetcare Oy

Medetomidine is a potent, non-narcotic, alpha$_2$-adrenoceptor agonist which produces sedation and analgesia. These effects are dose dependent in both depth and duration. Within the central nervous system, medetomidine inhibits sympathetic neurotransmission and causes the level of consciousness to decrease. Respiratory rate and body temperature can also decrease. In the peripheral vasculature, medetomidine stimulates alpha$_2$-adrenoceptors within vascular smooth muscle which induces vasoconstriction and hypertension. This causes a decrease in heart rate and cardiac output. Medetomidine also induces a number of other alpha$_2$-adrenoceptor mediated effects, including piloerection, depression of motor and secretory functions of the gastrointestinal tract, diuresis, and hyperglycemia.

Vatinoxan is an alpha$_2$-adrenoceptor antagonist that selectively acts on the peripheral nervous system. Because vatinoxan's effects are limited to the peripheral nervous system, it prevents or attenuates the negative cardiovascular and other effects of medetomidine outside the central nervous system when the drugs are administered together. Vatinoxan does not alter the central effects of medetomidine, but it does reduce the duration of the sedation and analgesia, mainly by improving cardiovascular function which increases the clearance of medetomidine.

Safety and Effectiveness

Field Effectiveness Study

The sponsor conducted a field effectiveness study comparing Zenalpha® to dexmedetomidine in client-owned dogs that presented to the veterinary clinic for examinations or procedures that required restraint and sedation. The examinations or procedures were non-invasive and were either non-painful or mildly painful. The study included dogs of both sexes and various breeds and breed mixes. Enrolled dogs represented a range of weights and ages (all were at least 16-weeks old) and were in good general health. Dogs in the treatment group received one IM injection of Zenalpha® and dogs in the control group received one IM injection of an approved dexmedetomidine injectable solution. Dexmedetomidine was used as the control

drug because medetomidine was not commercially available when the field study was conducted.

There were two primary variables for effectiveness:

1) Zenalpha® must be non-inferior to (not worse than) dexmedetomidine for the ability of the veterinarian to complete the planned examination or procedure while the dog was sedated. The success rate for completing the examination or procedure was 94.5% in the treatment group compared to 90.9% in the control group. Therefore, Zenalpha® was non-inferior to dexmedetomidine for the ability of the veterinarian to complete the planned examination or procedure.

2) Zenalpha® must be superior to (better than) dexmedetomidine at causing less severe cardiovascular adverse effects, specifically less bradycardia. The mean heart rate of dogs in the treatment group remained within the normal range (defined as 60 to 140 beats per minute (bpm)), while the mean heart rate of dogs in the control group was below normal from 5 to 180 minutes post-treatment. Fewer dogs administered Zenalpha® experienced bradycardia (< 40 bpm) during sedation compared to the control group. Therefore, Zenalpha® was superior to dexmedetomidine at causing less severe cardiovascular adverse effects.

Several secondary variables were evaluated, including time to onset of sedation, duration of sedation, level of analgesia, respiratory rate, body temperature, and reactions at the time of injection. Overall, dogs treated with Zenalpha® had a faster time to onset of sedation and a shorter duration of sedation (a faster recovery) than dogs administered dexmedetomidine. The level of analgesia was assessed by using a handheld algometer that allowed a force to be applied at a controlled rate until either a pre-determined threshold point was reached or the dog responded to the force. Zenalpha® provided comparable analgesia to dexmedetomidine, and in both groups, the waning of the analgesic effect was associated with the waning of the sedative effect.

The mean respiratory rate in both groups decreased compared to pre-treatment. The mean respiratory rate in the treatment group decreased earlier and recovered faster than the control group. Following administration of the treatment and control drugs, the mean body temperature in both groups decreased. The treatment group reached its lowest mean temperature (99.1 °F) at 120 minutes post-treatment and returned to pre-treatment levels by 360 minutes. The mean temperature in the control group reached its lowest level (98.2 °F) at 240 minutes post-treatment and was still below pre-treatment levels at 360 minutes. Fewer dogs in the treatment group experienced hypothermia (< 99 °F) or hyperthermia (> 103.5 °F) compared to the control group. More dogs in the treatment group had moderate to severe reactions to the injection of Zenalpha® compared to the reactions to injection in dogs in the control group. The most common adverse reactions seen in dogs treated with Zenalpha® were diarrhea, muscle tremors, and colitis.

Compared to dexmedetomidine, Zenalpha® had an improved physiologic safety profile and did not cause prolonged sedation in dogs.

Atipamezole Reversal Study
The sponsor conducted a laboratory study in adult, male Beagle dogs to determine if atipamezole was effective in reversing the sedative and cardiovascular effects of Zenalpha®. Atipamezole is a specific alpha$_2$-adrenoceptor antagonist used to reverse the effects of dexmedetomidine and medetomidine in dogs.

All dogs received Zenalpha® at the labeled dose, and then 30 minutes later, half of the dogs were administered the approved dose of atipamezole. Atipamezole reversed the sedation caused by Zenalpha® within 10 to 20 minutes, and the dogs' heart rate and blood pressure returned to baseline within 20 to 30 minutes.

Target Animal Safety
The sponsor conducted a laboratory study in young Beagle dogs to evaluate the safety of Zenalpha® when administered intravenously (IV). The drug was administered IV to minimize potential issues with administering large volumes of the drug IM. Previous pilot studies indicated that IV administration would adequately assess the safety of IM administration of Zenalpha® in dogs.

The dogs were administered Zenalpha® at 0X, 1X, 3X, or 5X the labeled IM dose once daily for 4 days. Zenalpha® caused sedation, decreased blood pressure, hypothermia, and initial sinus bradycardia and later sinus tachycardia that all resolved within 24 hours (before the next scheduled dose). Dogs in the three Zenalpha® groups were often provided supplemental heat support. Two dogs in the 5X group had decreased serum blood glucose, and two dogs (one in the 1X group and one in the 3X group) had decreased serum potassium.

Several dogs in the three Zenalpha® groups had mucoid, soft, or watery feces. Clinical observations in dogs administered Zenalpha® included salivation, trembling, tremors, vocalization, pinpoint pupils, protrusion of the nictitating membrane, defecation or vomiting after dosing, injected sclera, struggling after dosing, and skin that was cool to the touch. These clinical findings are due to the drug's mechanism of action. The high plasma-to-cerebrospinal fluid ratio (36:1) for vatinoxan indicates that it has little appreciable activity in the central nervous system. Overall, Zenalpha® did not produce systemic toxicity and had an acceptable margin of safety in dogs.

Safety Warnings
The labeling for Zenalpha® includes detailed safety information for people who handle, administer, or are exposed to the drug. The labeling also includes a note to physicians in case a person is accidentally exposed to the drug via absorption through the skin, eyes, or mucosa or via oral intake or self-injection. Symptoms after systemic absorption or self-injection include dose-dependent sedation, respiratory depression, bradycardia, tachycardia, and hypotension.

People with cardiovascular disease and pregnant women should take special precautions to avoid any exposure to Zenalpha®. Uterine contractions and decreased fetal blood pressure may occur after accidental systemic exposure.

Veterinary staff should be careful when handling sedated animals, as handling or any other sudden stimuli, including noise, may cause an animal to react defensively even if it appears to be heavily sedated.

Conclusions
Based on the data submitted by the sponsor for the Zenalpha®, FDA determined that the drug is safe and effective when used according to the labeling.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-551

B. Sponsor

Vetcare Oy
P.O. Box PL 26 (Liedontie 45)
Mäntsälä, Uusimaa, 04601 Finland

Drug Labeler Code: 086155

U.S. Agent Name and Address:
David Petrick, VMD, JD
The Delta Consortium Regulatory Consulting
74 Leamington Lane
Hilton Head Island, SC 29928

C. Proprietary Name

Zenalpha®

D. Drug Product Established Name

Medetomidine and vatinoxan hydrochlorides injection

E. Pharmacological Category

Sedative, Analgesic

F. Dosage Form

Solution

G. Amount of Active Ingredient

Each mL contains 0.5 mg medetomidine hydrochloride and 10 mg vatinoxan hydrochloride

H. How Supplied

10 mL multi-dose glass vials

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

The dose is based on body surface area (BSA). Calculate the dose using 1 mg medetomidine /m^2 BSA or use the dosing table below. **Note that the mg/kg dosage decreases as body weight increases.**

Table 1. IM dose volume based on body weight

Dog body weight		Dose volume
lbs	**kg**	**mL**
4.4 to 7	2 to 3	0.3
7.1 to 9	3.1 to 4	0.4
9.1 to 11	4.1 to 5	0.6
11.1 to 22	5.1 to 10	0.8
22.1 to 29	10.1 to 13	1.0
29.1 to 33	13.1 to 15	1.2
33.1 to 44	15.1 to 20	1.4
44.1 to 55	20.1 to 25	1.6
55.1 to 66	25.1 to 30	1.8
66.1 to 73	30.1 to 33	2.0
73.1 to 81	33.1 to 37	2.2
81.1 to 99	37.1 to 45	2.4
99.1 to 110	45.1 to 50	2.6
110.1 to 121	50.1 to 55	2.8
121.1 to 132	55.1 to 60	3.0
132.1 to 143	60.1 to 65	3.2
143.1 to 154	65.1 to 70	3.4
154.1 to 176	70.1 to 80	3.6
> 176	> 80	3.8

After administration of Zenalpha®, the dog should be allowed to rest quietly until evidence of sedation has occurred (5-15 minutes). The average duration of sedation is 38 minutes. As with all $alpha_2$ adrenoceptor agonists, onset of sedation may be delayed or may be inadequate in some dogs.

K. Route of Administration

Intramuscular

L. Species

Dog

M. Indication

Zenalpha® is indicated for use as a sedative and analgesic in dogs to facilitate clinical examination, clinical procedures and minor surgical procedures.

II. EFFECTIVENESS

The effectiveness of Zenalpha® (medetomidine and vatinoxan hydrochlorides injection) for use as a sedative and analgesic in dogs to facilitate clinical examinations, clinical procedures, and minor surgical procedures was evaluated in a field study in client-owned dogs that presented to the veterinary clinic for a planned examination or procedure.

Vatinoxan hydrochloride was referred to as MK-467 in early product development.

A. Dosage Characterization

A dose of 1 mg medetomidine/m^2 BSA and 20 mg vatinoxan/m^2 BSA (1:20 ratio) administered by IM injection for sedation in dogs was selected based on two laboratory studies and a pharmacokinetic (PK)/pharmacodynamic (PD) modeling study.

Dose Determination of MK-467 in Combination with Medetomidine Following Intramuscular Administration in Dogs. (Study No. 14-410-02)
The laboratory study was conducted with a crossover design in 8 (4 male, 4 female) Beagle dogs, approximately 7 - 8 months of age, fitted with radiotelemetry devices. Dogs were randomized to treatment group order with a 3 - 7 day washout period between treatments. Group 1 was administered 1 mg/m^2 medetomidine, and groups 2 - 4 were administered 1 mg/m^2 medetomidine with vatinoxan hydrochloride at 15, 30, or 50 mg/m^2, respectively. All doses were administered by IM injection. All dogs were considered clinically sedated after dose administration for all dose groups. The medetomidine and vatinoxan combination provided a dose and time dependent attenuation of decreased heart rate, decreased respiratory rate, and occurrence of cardiac arrhythmias compared to medetomidine alone. Medetomidine and vatinoxan combinations also resulted in dose dependent decreased arterial blood pressures and decreased time to standing (quicker recovery from sedation). The attenuation of the adverse cardiovascular effects in the groups administered 15 mg/m^2 and 30 mg/m^2 of vatinoxan supported further evaluation of the dose of 20 mg/m^2 vatinoxan.

Plasma Concentration, Cardiovascular and Sedative Effects of Intramuscular Medetomidine With Three Doses of the Peripheral Alpha-2 Antagonist MK-467 in Dogs. (Study No. 14-410-01)

The laboratory study was conducted with a crossover design in 8 (6 male, 2 female) Beagle dogs approximately 1 year of age. Dogs were randomized to treatment group order with at least 14 days between treatments. Group 1 was administered 20 mcg/kg medetomidine, and groups 2 - 4 were administered 20 mcg/kg medetomidine with vatinoxan at 200 mcg/kg (1:10 ratio), 400 mcg/kg (1:20 ratio), or 600 mcg/kg (1:30 ratio), respectively. All doses were administered by IM injection. All dogs were considered clinically sedated after dose administration for all dose groups. The combinations of medetomidine and vatinoxan did not prevent the immediate cardiovascular depressive effects of medetomidine, which occurred within 5 minutes post dose. There was a time and dose dependent recovery to baseline for the cardiovascular parameters (heart rate and cardiac output) within 20 minutes post dose in the medetomidine and

vatinoxan combination groups. Sedation scores in dogs in the medetomidine and vatinoxan combination groups increased earlier and returned to baseline earlier compared to dogs in the medetomidine group. Seven dogs in group 1 required reversal of medetomidine with atipamezole; none of the dogs in the medetomidine and vatinoxan combination groups experienced prolonged sedation.

Pharmacokinetics and Pharmacodynamics (PK/PD) Modeling Report: PK Interaction and Effect of Dose Ratio. (Study No. 14-322-06)
A PK/PD model was developed to relate the plasma drug concentration data to the sedation and heart rate data collected in Study 14-410-01 and another PK study. Simulations with the PK/PD model were conducted using combinations of 1 mg/m^2 medetomidine and vatinoxan, with medetomidine to vatinoxan ratios ranging from 1:15 to 1:50. The medetomidine to vatinoxan ratio that maintained the mean heart rate closest to 70 beats per minute over a 2-hour period post dose was 1:20. Based on the results of the simulation, the 1 mg/m^2 medetomidine and 20 mg/m^2 vatinoxan dose (1:20 ratio) was selected for further evaluation in an effectiveness field study.

B. Substantial Evidence

1. Field Effectiveness Study

   Title: Field safety and efficacy study of a fixed combination MK-467/medetomidine product when used as a sedative in dogs. (Study No. 14-420-01)

   Study Dates: August 2017 to September 2018

   Study Locations: Six veterinary clinics from the following locations participated in the study.

Canandaigua, NY	Fort Collins, CO
Catonsville, MD	Quakertown, PA
Decatur, IL	Zachary, LA

   Study Design: This was a multicenter, double-masked, randomized, active-controlled field study.

   Objective: To evaluate the effectiveness and safety of Zenalpha® for use as a sedative and analgesic in dogs to facilitate clinical examinations, clinical procedures, and minor surgical procedures in client-owned dogs.

   Study Animals: The study enrolled 223 client-owned dogs that presented to the veterinary clinic for examinations or procedures that required sedation. There were 107 females and 116 males of various breeds and breed mixes, aged 5 months to 14.5 years, and weighing 5.1 to 154 lbs. (2.3 - 70 kg).

   Experimental Design: Dogs were randomized at a 1:1 ratio to receive Zenalpha® or dexmedetomidine (control group). There were 110 dogs in the Zenalpha® group and 113 dogs in the dexmedetomidine group. The field study was conducted in accordance with Good Clinical Practice.

Treatment Groups:

Table II.1. Treatment Groups

Group	Dose (IM injection)	Number of Animals
Zenalpha®	1 mg/m^2 BSA medetomidine 20 mg/m^2 BSA vatinoxan	110
Control	0.5 mg/m^2 BSA dexmedetomidine	113

BSA = body surface area

Inclusion Criteria: Dogs at least 16 weeks old, in good general health and American Society of Anesthesiologists (ASA) class I or II (healthy or mild systemic disease), that required a non-invasive, non-painful, or mildly painful procedure or examination which required restraint and sedation. Owner consent was provided prior to study enrollment.

Exclusion Criteria: Male dogs intended for breeding or female dogs that were pregnant or lactating; dogs less than 16 weeks old; or dogs that had marked systemic disease or hypovolemia, had marked cardiovascular or respiratory disease, had a known hypersensitivity to the active ingredients or excipients, were ASA class III or above, or were administered any medication which may have affected sedation, analgesia, or cardiovascular parameters.

Drug Administration: The Zenalpha® group received the final market formulation of medetomidine hydrochloride (0.5 mg/mL) and vatinoxan hydrochloride (10 mg/mL). The Zenalpha® dose was calculated based on the medetomidine in the combination at 1 mg/m^2 BSA or by using the dosing table below. The control group received an approved 0.5 mg/mL dexmedetomidine hydrochloride injectable solution and was dosed at 0.5 mg/m^2 or by using the dosing table provided with the product. The products were administered once by IM injection in the veterinary clinic. Dogs had been fasted for 12 hours prior to dose administration.

Table II.2. Zenalpha® Dosing Table by Dose Volume

Body weight in lbs.	Body weight in kg	Dose in mL
4.4 to 7	2.0 to 3.0	0.3
7.1 to 9	3.1 to 4.0	0.4
9.1 to 11	4.1 to 5.0	0.6
11.1 to 22	5.1 to 10	0.8
22.1 to 29	10.1 to 13	1.0
29.1 to 33	13.1 to 15	1.2
33.1 to 44	15.1 to 20	1.4
44.1 to 55	20.1 to 25	1.6
55.1 to 66	25.1 to 30	1.8
66.1 to 73	30.1 to 33	2.0
73.1 to 81	33.1 to 37	2.2
81.1 to 99	37.1 to 45	2.4

Body weight in lbs.	Body weight in kg	Dose in mL
99.1 to 110	45.1 to 50	2.6
110.1 to 121	50.1 to 55	2.8
121.1 to 132	55.1 to 60	3.0
132.1 to 143	60.1 to 65	3.2
143.1 to 154	65.1 to 70	3.4
154.1 to 176	70.1 to 80	3.6
>176	>80	3.8

Measurements and Observations: The primary variables for effectiveness were determined by 1) the ability to complete the planned examination or procedure while the dog was sedated, and 2) the demonstration of less severe cardiovascular adverse effects (determined by heart rate) compared to the control group. Respiratory rate, body temperature, analgesia, and adverse reactions were evaluated as secondary variables for the safety assessment. Dogs were evaluated at 5, 15, 30, 60, and 90 minutes and 2, 3, 4, 5, and 6 hours after drug administration. Baseline physical examination and clinical pathology were conducted to confirm the dog was healthy and suitable for inclusion in the study.

Analgesia was assessed by using Mechanical Nociceptive Threshold (MNT) testing. The assessment was conducted using a handheld algometer (Prod, Topcat Metrology Ltd) equipped with a 2 mm probe tip, which allowed a force to be applied at a controlled rate of 2 Newtons/second (N/s) until a response from the dog was seen. If no response was seen before the predetermined 20 N threshold, then the stimulation was stopped. MNT assessments were conducted at pre-treatment, and 15, 30, 60, 120, and 240 minutes post-drug administration.

Statistical Methods: Two variables were considered primary for demonstration of effectiveness: Non-inferiority (NI) of Zenalpha® (compared to control) was assessed for 'Ability to complete the procedure (yes/no)' using the generalized linear mixed effect model including the treatment group as the fixed effect and site and treatment by site interaction as the random effects. NI was established if the upper limit of the 95% confidence interval was less than the NI margin of 25%. Superiority was assessed for 'Heart Rate' during the first 3 hours after treatment, using the linear mixed-effect model including fixed effects of treatment group, time and treatment group by time interaction, subject as a random effect, and baseline value as a covariate. Statistical significance was evaluated at a two-sided alpha equal to 0.05. The p-values were adjusted using the Holm-Bonferroni method. Other variables were assessed as secondary or for evaluation of safety.

Results: Effectiveness was evaluated in 208 dogs (109 in the Zenalpha® group and 99 in the control group).

Ability to complete procedure: The success rate for the ability to complete the procedure was 94.5% in the Zenalpha® group and 90.9% in the control group. Zenalpha® met the criteria for success because it was non-inferior to the control product for the ability to complete the procedure while the dog

was sedated. The procedures in the study included: radiographic examination or diagnostic imaging, ear examination and treatment, eye examination and treatment, anal sac treatment, dermatological examination and procedures, dental examination, dental biopsy, dental minor extraction, fine needle aspiration/superficial biopsy, minor surgery to remove dermal masses, drain seroma or abscess, nail trimming, coat grooming, venous blood draw, and intravenous catheter placement. Many dogs underwent more than one procedure while sedated.

Heart rate: The Zenalpha® group mean heart rate remained within the normal range (normal range defined as 60 - 140 bpm), while the control group mean heart rate was below normal from 5 - 180 minutes post-treatment. The mean heart rate in the Zenalpha® group was lowest at 15 minutes (64 bpm) and the mean heart rate in the control group was lowest at 90 minutes (45 bpm).

Individual heart rates in the Zenalpha® group ranged from 24 - 240 bpm, and in the control group ranged from 22 - 180 bpm during sedation. There were 9 dogs (8.3%) in the Zenalpha® group and 54 dogs (52.4%) in the control group that had heart rates < 40 beats per minute (bpm) during sedation. The difference in heart rate between the Zenalpha® and control group was clinically relevant. Zenalpha® met the criteria for success for heart rate because it was superior to the control product for group mean heart rate (reduction in bradycardia).

Table II.3. Group Mean Heart Rates (beats per minute)*

Timepoint	Zenalpha®	Control	P-value
Pre-treatment	114	109	0.1584
5 min	70	69	0.9565
15 min	64	54	<0.0001
30 min	72	48	<0.0001
45 min	71	46	<0.0001
60 min	74	46	<0.0001
90 min	84	45	<0.0001
120 min	96	47	<0.0001
180 min	112	53	<0.0001
240 min	122	66	ND
300 min	118	79	ND
360 min	120	96	ND

*Based on the Effectiveness Population
ND = analysis not conducted

There were 24 (22%) dogs in the Zenalpha® group and 7 dogs (6.8%) in the control group (6.8%) that had tachycardia during the study (HR ≥ 160 bpm, based on the safety population). Rebound tachycardia has been reported in dogs administered an alpha$_2$ adrenoceptor agonist and occurs after the cardiovascular suppressive effects have abated. Data on heart rate was not collected past 360 minutes (6 hours), and therefore post-sedation rebound tachycardia in the control group may have been missed because the dogs were still sedated and experiencing cardiovascular suppression. One dog in the Zenalpha® group had tachycardia (HR of 160 bpm) that was noted at the

5-minute timepoint and lasted approximately 10 minutes; the tachycardia did not require treatment.

Time to onset of sedation and Duration of sedation: The group mean time to onset of sedation was 14 minutes in the Zenalpha® group and 18 minutes in the control group. The mean duration of sedation in the Zenalpha® group was 38 minutes (maximum of 90 min.) and in the control group was 90 minutes (maximum 5 hrs. 28 min.). Overall, the dogs in the Zenalpha® group had a faster time to onset of sedation and shorter duration of sedation (faster recovery).

Table II.4. Time to Onset of Sedation (hrs:min)

Parameter	Zenalpha®	Control
N	104	93
Mean	00:14	00:18
S.D.	00:10	00:13
Max	00:55	01:20
Median	00:10	00:14
Min	00:04	00:04

Note: Five dogs from the Zenalpha® group and 6 dogs from the control group were excluded due to missing data.

Table II.5. Duration of Sedation (hrs:min)

Parameter	Zenalpha®	Control
N	102	90
Mean	00:38	01:30
S.D.	00:20	01:01
Max	01:30	05:28
Median	00:36	01:22
Min	00:03	0:04

Note: Seven dogs in the Zenalpha® group and 9 in the control group were excluded due to missing data.

Analgesia: Analgesia assessments were similar in the Zenalpha® and control groups at pre-treatment, and at 15 and 30 minutes post treatment. From 60 minutes post treatment the pressure applied to elicit a response was significantly lower in the Zenalpha® group, indicating that the dogs were recovering from the analgesic effects of the sedative at these time points. At 4 hours post-treatment the mean MNT measurement in the Zenalpha® group had returned to pre-treatment levels. The results demonstrate that Zenalpha® has a comparable analgesic effect to the control product, and that the decrease in analgesic effect is associated with the waning of the sedative effect.

Table II.6. Mechanical Nociceptive Threshold Measurements (N)

Time	Treatment	N	Mean	S.D.	Min	Median	Max
Baseline	Zenalpha®	109	8.2	5.18	0	6.9	20.0
Baseline	Control	99	8.6	5.64	0.3	6.7	20.0
15 min	Zenalpha®	107	16.1	5.05	1.4	19.4	20.0
15 min	Control	99	15.4	5.61	1.47	18.5	20.0
30 min	Zenalpha®	107	15.8	5.00	1.4	18.2	20.0
30 min	Control	94	16.0	5.24	3.1	19.2	20.0
60 min	Zenalpha®	103	12.9	6.02	1.8	13.8	20.0
60 min	Control	89	15.9	4.97	4.4	18.8	20.0
2 hrs	Zenalpha®	96	10.1	6.03	0.4	8.9	20.0
2 hrs	Control	84	14.5	5.92	3.4	17.5	20.0
4 hrs	Zenalpha®	93	8.8	5.91	0.2	7.7	20.0
4 hrs	Control	88	11.1	5.83	1.4	9.4	20.0

Respiratory rate: Respiratory rate data were calculated using the safety population. The definition of normal respiratory rate was 10 - 30 breaths per minute (bpm). Following treatment, the mean respiratory rate in both groups decreased compared to pre-treatment. The group mean respiratory rate in the Zenalpha® group decreased earlier and recovered faster than the control group. The group mean respiratory rate in the Zenalpha® group was lowest at 30 minutes post-treatment (14 bpm) and in the control group was lowest at 90 minutes post-treatment (15.5 bpm).

Individual respiratory rates in the Zenalpha® group ranged from 2 - 102 bpm, and in the control group ranged from 2 - 96 bpm. Thirty-nine dogs (35.4%) in the Zenalpha® group and 33 dogs (29.2%) in the control group had respiratory rates < 10 bpm during the study. Eight dogs in the Zenalpha® group and 4 dogs in the control group had respiratory rates of 4 bpm. One dog in each group had a respiratory rate of 2 bpm at a single time point. One dog in the control group had apnea and received supplemental oxygen, and the event was reported as an adverse reaction. None of the other dogs in the study required supplemental oxygen. All dogs had normal respiratory rates (≥ 10 bpm) at 240, 300, and 360 minutes post-drug administration.

Table II.7. Group Mean Breaths per Minute by Timepoint

Timepoint	Zenalpha®	Control
Pre-treatment	58.1	51.3
5 min	25.8	36.4
15 min	15.7	23.8
30 min	14.0	18.0
45 min	16.0	15.8
60 min	18.9	16.1
90 min	23.4	15.5
120 min	25.7	16.6
180 min	31.8	19.4
240 min	35.2	24.4
300 min	37.3	27.2
360 min	39.9	31.0

Body temperature: Body temperature data were calculated using the safety population. Body temperature was recorded using a digital thermometer. The definition of normal body temperature for the study was 100 - 102.6 °F. Following treatment, the mean body temperature in both groups decreased with the Zenalpha® group reaching its lowest temperature at 120 minutes (99.1 °F) while the temperature in the control group continued to drop reaching its lowest level at 240 minutes post treatment (98.2 °F). The mean body temperature in the Zenalpha® group was below normal for the 90 - 180 minute timepoints and had returned to pre-treatment levels by 360 minutes post treatment. The mean body temperature in the control group was below normal from the 120-minute timepoint onwards and was still below pre-treatment levels at 360 minutes.

Table II.8. Group Mean Body Temperatures (°F)

Timepoint	Zenalpha®	Control
Pre-treatment	101.5	101.3
30 min	100.8	101.5
60 min	100.0	100.9
90 min	99.3	100.2
120 min	99.1	99.5
180 min	99.7	98.8
240 min	100.2	98.2
300 min	100.8	98.6
360 min	101.1	99.3

There were 57 dogs (51.8%) in the Zenalpha® group and 77 dogs (68.1%) in the control group that had a body temperature ≤ 99 °F. One dog in the Zenalpha® group and 13 dogs in the control group had hypothermia that required use of an external heat source and were reported as adverse events.

There were 5 dogs (4.5%) in the Zenalpha® group and 13 dogs (11.5%) in the control group that had a body temperature ≥ 103.5 °F. The instances of hyperthermia were limited to a single timepoint in 4 of the Zenalpha® dogs and were over 2 timepoints (at 60 and 90 min) in one Zenalpha® dog. The instances of hyperthermia occurred at a single timepoint for 5 dogs in the control group and over multiple timepoints in the other 8 control dogs.

Mucous membranes and Capillary refill time: Mucous membrane color and capillary refill time were normal in all dogs in both treatment groups throughout the study.

Reaction to Injection: Tolerance to the administration of Zenalpha® or control was assessed using the scoring scale below. To maintain the masking of personnel that conduct clinical observations and pain assessments, the injection reaction assessment was conducted by the treatment administrator. The summary data for injection reaction is based on the safety population.

There was no reaction to the administration of treatment in 56.4% of Zenalpha® dogs and in 71.7% of control dogs. There was a mild reaction in 18.2% of Zenalpha® dogs and in 20.4% of control dogs. There was a moderate reaction in 11.8% of Zenalpha® dogs and in 5.3% of control dogs.

There was a severe reaction in 13.6% of Zenalpha® dogs and in 2.7% of control dogs.

Table II.9. Injection Reaction Scores* by Treatment Group (N and %)

Group	Score 0	Score 1	Score 2	Score 3	Total
Zenalpha®	62 (56.4%)	20 (18.2%)	13 (11.8%)	15 (13.6%)	110
Control	81 (71.7%)	23 (20.4%)	6 (5.3%)	3 (2.7%)	113

*Injection Reaction Score:
0 = No reaction
1 = Mild reaction, non-specific change in posture, subtle movement without specific attention to the injection site
2 = Moderate reaction, specific change in posture and movement, specific attention to the injection site
3 = Severe reaction, attempt to move away from injection, vocalization, aggression, biting, growling, or barking

Concomitant Medications: Concomitant medications included: antibiotics (oral, otic, ophthalmic, and dermal topical), antianxiety/antidepressants, corticosteroids, H1 and H2 receptor blockers, estriol, levothyroxine, oclacitinib tablet, nutritional supplements, lokivetmab monoclonal antibody, lactulose, oral and topical parasiticides, and vaccines. Some dogs received additional analgesia as the effects of the sedation and analgesia waned, including non-steroidal anti-inflammatory drugs (NSAIDs), butorphanol, gabapentin, tramadol, and hydromorphone.

Adverse Reactions: Safety was evaluated in 223 dogs (110 in the Zenalpha® group and 113 in the control group). A total of 45 dogs experienced 51 adverse reactions during the study. Sixteen (31.4%) of the dogs were in the Zenalpha® group and 35 (68.6%) were in the control group.

One serious adverse reaction was reported during the study. One dog in the control group experienced severe bradycardia at approximately 28 minutes after treatment administration and became apneic about 14 minutes after the bradycardia developed. Atipamezole reversal was administered and the dog recovered within 5-10 minutes.

The most common adverse reactions in the Zenalpha® group were diarrhea (3.6%), muscle tremors (1.8%), and colitis (1.8%). The most common adverse reactions in the control group were hypothermia (11.5%), vomiting (5.3%), prolonged sedation (2.7%), and urinary incontinence (1.8%). If vomiting, nausea, and retching are combined then the occurrence was 6.2% in the control group and 1.8% in the Zenalpha® group.

Table II.10. Adverse Reactions Reported During the Field Study

Adverse Reaction	Zenalpha® (N = 110) n (%)	Control (N = 113) n (%)
Diarrhea	4 (3.6)	0 (0)
Muscle tremor	2 (1.8)	0 (0)
Colitis	2 (1.8)	0 (0)
Hypothermia*	1 (0.9)	13 (11.5)
Vomiting	1 (0.9)	6 (5.3)
Involuntary defecation	1 (0.9)	0 (0)
Nausea	1 (0.9)	0 (0)
Tachycardia, transient	1 (0.9)	0 (0)
Sedation (prolonged)	0 (0)	3 (2.7)
Urinary incontinence	0 (0)	2 (1.8)
Retching	0 (0)	1 (0.9)
Apnea	0 (0)	1 (0.9)
Bradycardia	0 (0)	1 (0.9)
Hyperthermia	0 (0)	1 (0.9)

*Hypothermia that necessitated use of an external heat source

Conclusions: Zenalpha® was effective and had an adequate safety profile for use as a sedative and analgesic in dogs to facilitate clinical examinations, clinical procedures, and minor surgical procedures when administered at the labeled dose. The combination of the alpha$_2$ adrenoceptor agonist medetomidine and the alpha$_2$ adrenoceptor antagonist vatinoxan had an improved physiologic safety profile and absence of prolonged sedation compared to the control product dexmedetomidine.

2. Atipamezole Reversal Study

A laboratory study in eight adult male Beagle dogs was conducted to determine whether atipamezole was effective in reversing the sedative and cardiovascular effects of Zenalpha®. The study was a 4-period cross over design with a 7-day washout period between treatments. Both treatment groups received Zenalpha® at the labeled dose and one treatment group was administered the approved dose of atipamezole hydrochloride (5 mg/m^2 IM) 30 minutes after administration of Zenalpha®. Dogs were monitored for 90 minutes after atipamezole hydrochloride administration. Administration of atipamezole hydrochloride resulted in reversal of sedation within 10 - 20 minutes and return to baseline values for heart rate and blood pressure within 20 - 30 minutes. Rectal temperature increased following atipamezole hydrochloride administration but had not returned to baseline within 90 minutes (end of study). Atipamezole hydrochloride administered at 5 mg/m^2 IM was effective in reversing the sedative and cardiovascular effects of Zenalpha®.

III. TARGET ANIMAL SAFETY

Zenalpha® was administered IV in the laboratory multi-dose safety study to minimize potential issues with administering large volumes of the product IM. Pilot studies indicated that IV administration for purposes of the safety study would adequately assess the systemic safety of Zenalpha® in dogs.

A. Laboratory Multi-dose Safety Study

Title: Target Animal Safety Evaluation of a Sedative Fixed Combination Drug in Dogs. (Study No. 14-410-03)

Study Dates: January 18, 2018 to January 29, 2019

Study Location: Mattawan, MI

Study Design:

Objective: To evaluate the safety of Zenalpha® (medetomidine and vatinoxan hydrochlorides injection) when administered IV once a day for 4 days.

Study Animals: Thirty-two Beagle dogs (16 males, 16 females), aged 4.5 to 6.5 months old, with a bodyweight range 5.4 to 10 kg, determined as healthy based on physical examination and clinical pathology.

Experimental Design: The study was masked, placebo-controlled, and conducted according to Good Laboratory Practices (GLP). Dogs were randomly assigned to 4 cohorts and then randomly assigned to the treatment groups within sex and cohort. Dogs were randomized to 0.9% saline or Zenalpha®. The dosing order was randomly determined within each cohort.

Table III.1. Treatment Groups and Dosages

Treatment Group	Medetomidine Dose (mg/m² BSA*)	Vatinoxan Dose (mg/m² BSA*)	Number of Dogs
Saline (Control)	0	0	4 male 4 female
Zenalpha® 1X	1.0	20	4 male 4 female
Zenalpha® 3X	3.0	60	4 male 4 female
Zenalpha® 5X	5.0	100	4 male 4 female

*BSA = Body surface area

Drug Administration: Zenalpha® contains 0.5 mg/mL medetomidine hydrochloride and 10 mg/mL vatinoxan hydrochloride. The final market formulation was used in the study. Doses were administered as intravenous boluses once on Days 1, 2, 3, and 4 through a peripheral intravenous catheter. Doses were administered in alternating limbs (left and right) each treatment day. The dose levels corresponded to 1X, 3X, and 5X the recommended label dose intended for IM administration. Dogs were fasted 12 hours prior to dose administration.

Study Schedule: To accommodate intensive observations and study schedule, the study was conducted in 4 cohorts (4 animals/sex/cohort). The acclimation period was 14 days, 27 days, 42 days, and 55 days for cohorts 1-4, respectively.

Measurements and Observations:

- Observations for mortality, morbidity, and injury were conducted twice daily.
- Detailed clinical observations were unscheduled and were conducted occasionally as needed.
- Physical examinations were conducted twice pre-study, prior to each dose, and at 0.33, 1, 2, 4, and 8 hours after dose administration on Days 1, 2, and 3 and once prior to necropsy on Day 4.
- Injection site evaluations (using scoring for erythema/eschar and edema) were scored daily on Days 1, 2, and 3.
- Sedation Scoring (10 cm Visual Analog Scale with 10 being most sedate) was performed twice pretest, and once prior to dosing on Days 1, 2, and 3 and post-dose at 0.25, 0.5, 1, 1.5, 2, 4, and 8 hours on Days 1, 2, and 3.
- The time from dose administration to lateral recumbency and subsequent time to standing was recorded on Days 1, 2, and 3.
- Ophthalmoscopic exams were conducted pre-study and on Day 3 by a veterinary ophthalmologist using tropicamide solution 1% for mydriasis.
- Body weights were measured and recorded pre-study and prior to each dose.
- Electrocardiographic examinations were performed pre-study and once prior to dose administration on Days 1, 2, 3, and 4, and at 0.25, 0.5, 1, 1.5, 2, 4, and 8 hours post-dose on Days 1, 2, and 3.
- Blood Pressure (indirect systolic, diastolic, and mean arterial pressure) was measured and recorded twice pretest, once prior to dose administration, and at 0.25, 0.5, 1, 1.5, 2, 4, and 8 hours post-dose on Days 1, 2, and 3.
- Blood and urine samples for clinical pathology evaluations were collected from all animals twice pre-study and on Day 3 approximately 4 hours post-dose.
- Buccal Mucosal Bleeding Time, C-Reactive Protein, prothrombin, fibrinogen, and activated partial thromboplastin time were evaluated as part of the clinical pathology evaluations.
- Urine Drug Excretion Analysis was performed on samples collected under cages twice pretest and once pre-dose on Day 4 (approximately 16 to 25 hours after the Day 3 dose).
- Plasma Analysis determination of plasma concentrations of vatinoxan, dexmedetomidine, and levomedetomidine was collected on Day 1 for all animals at 1, 5, and 20 minutes; at 1, 4, and 8 hours post-dose; and at only one timepoint on Day 4 for the control group dogs and 1X group dogs.
- Cerebrospinal fluid samples (CSF) and Central Nervous System (CNS) samples were collected from the control group and 1X group dogs on Day 4 at necropsy.
- Necropsy examinations were performed on Day 4, organ weights were recorded, and selected tissues were examined microscopically.

Statistical Methods: Repeated measures analysis of covariance (RMANCOVA) was applied to the continuous endpoints measured multiple times post-treatment. The models included treatment, time, sex, and their 2-way and 3-way interactions as fixed effects and the cohort as a random effect. Baseline value was included as a covariate. Covariance structure was selected from AR(1), ARH(1), CS, CSH, and SP(POW).* Model with the smallest corrected Akaike's Information Criterion (AIC) was chosen.

Linear mixed model was applied to the continuous endpoints measured once post-treatment. The model included treatment, sex, and treatment by sex interaction as fixed effects and the cohort as a random effect. Pre-treatment value was used as a covariate.

*Abbreviation definitions
AR(1): autoregressive(1)
ARH(1): heterogeneous AR(1)
CS: compound symmetry
CSH: heterogeneous CS
SP(POW): spatial power covariance structure

All fixed effects were tested at the significant level of 0.1 except the 3-way interaction, which was tested at the significant level of 0.05.

Results: Mortality and Morbidity: There were no deaths or clinically significant illness during the study.

Clinical observations and examinations: Unscheduled clinical observations that were noted included mucoid, soft, or watery feces reported in all groups during acclimation. There were no reported abnormalities for feces in the control group post-dose. Dogs administered Zenalpha® had reported mucoid, soft, or watery feces in the 1X (1 dog), 3X (2 dogs), and 5X (3 dogs) groups post-dose. One 3X female had explosive diarrhea 1 hour after dosing. One 5X female vomited once 4 hours after dosing. Observations that were not noted in the control group but occurred in the dogs in the Zenalpha® groups included observations of salivation, trembling, tremors, vocalization, pinpoint pupils, nictitans protrusions, defecation after dosing, injected sclera, struggling after dosing, and skin cool to touch.

Body weight: There were no clinically relevant effects on body weight for any of the groups during the study.

Physical Examination[1]:

Heart rate
The mean heart rate (beats per minute, bpm) in the Zenalpha® groups decreased shortly after dose administration and was lowest 1-hour post dose when compared to pre-dose and control group mean heart rates. Heart rate in the Zenalpha® groups returned to baseline or was increased above baseline by 2

[1] For all tables under physical examination, the pre-treatment value is arithmetic mean group value using descriptive statistics. The post-treatment value is least square means obtained from the statistical model.

hours post-dose. The mean heart rate was increased above 140 bpm on Days 1 and 2 at 2 or 4 hours post-dose in the 3X group and on Days 1, 2, and 3 at 2 or 4 hours post-dose in the 5X group. The instances of increased heart rate (Table III.3) were associated with the sinus tachycardia noted during the electrocardiograms results.

Table III.2: Minimum Heart Rates (HR)

Treatment Group	Minimum mean HR (bpm)[a]	Lowest reported individual HR (bpm)[b]
Control	94 at 1 hour post-dose Day 1 [118 pre-dose]	64 at 1 hour post-dose Day 1
Zenalpha® 1X	80 at 1 hour post-dose Day 1 [110 pre-dose]	48 at 1 hour post-dose Day 1
Zenalpha® 3X	71 at 1 hour post-dose Day 1 [110 pre-dose]	56 at 1 hour post-dose Day 1
Zenalpha® 5X	71 at 1 hour post-dose Day 2 [119 pre- dose]	56 at 1 hour post-dose for each Day

[a] Lowest mean heart rate observed post-dose for each treatment group.
[b] The lowest reported individual heart rate during the study.

Table III.3: Maximum Heart Rates (HR)

Treatment Group	Maximum mean HR (bpm)[a]	Highest reported individual HR (bpm)[b]
Control	136 at 4 hours post-dose Day 2 [122 pre-dose]	200 at 1 hour post-dose Day 2
Zenalpha® 1X	138 at 2 and 8 hours post-dose Day 3 [131 pre-dose]	192 at 2 hours post-dose Day 3
Zenalpha® 3X	145 at 4 hours post-dose Day 1 [110 pre-dose]	200 at 2 hours post-dose Day 3
Zenalpha® 5X	165 at 4 hours post-dose Day 1 [117 pre-dose]	208 at 4 hours post-dose Day 1

[a] The means in the table represent the highest heart rate observed post-dose for each treatment group.
[b] The highest reported individual heart rate observed during the study.

Respiratory rate
The mean respiratory rate (breaths per minute, bpm) for the dogs in the Zenalpha® groups was numerically lower than that for the dogs in the control group. Respiratory rate generally decreased by 20 minutes post-dose and began

to return to baseline by 2 hours post-dose and returned to baseline within 4 hours post-dose.

Table III.4: Minimum respiratory rates (RR)

Treatment Group	Minimum mean RR (bpm)[a]	Lowest reported individual RR (bpm)[b]
Control	29 prior to necropsy Day 4	12 at 1 hour post-dose Day 2
Zenalpha® 1X	14 at 20 minutes post-dose Day 3 [32 pre-dose]	8 at 20 minutes post-dose Day 2 and Day 3
Zenalpha® 3X	13 at 20 minutes post-dose Day 3 [32 pre-dose]	8 at 20 minutes post-dose Day 1,2, and 3
Zenalpha® 5X	13 at 20 minutes post-dose Day 3 [33 pre-dose]	8 at 20 minutes post-dose Day 1

[a] The means in the table represent the minimum respiratory rate (bpm - breaths per minute) observed post-dose for each treatment group.
[b] The lowest reported individual respiratory rate during the study.

Mucous membranes and capillary refill time
The mucous membrane color and capillary refill time were reported normal for all dogs during the study at all timepoints.

Body temperature
The mean body temperature for the dogs in the Zenalpha® groups was numerically lower than that for the dogs in the control group. As a result of clinical observations and body temperature recordings, dogs in the Zenalpha® groups were often provided supplemental heat support which began at either 1 or 2 hours post-dose and was discontinued at 4 hours post-dose. Body temperature generally normalized or increased by 4 hours post-dose. The highest reported individual body temperature after dosing in treated dogs was 103.4 °F.

Table III.5: Minimum Body Temperatures (°F)

Treatment Group	Minimum mean body temperature[a]	Lowest reported individual body temperature[b]
Control	100.7 at 20 minutes post-dose Day 3 [102 pre-dose]	99 at 1 hour post-dose Day 1
Zenalpha® 1X	98 at 1 hour post-dose Day 1 [101.8 pre-dose]	95.5 at 1 hour post-dose Day 3
Zenalpha® 3X	97.5 at 2 hours post-dose Day 1 [101.8 pre-dose]	95.9 at 2 hours post-dose Day 1
Zenalpha® 5X	95.7 at 2 hours post-dose Day 1 [102.1 pre- dose]	94.3 at 4 hours post-dose Day 1

[a] The means in the table represent the lowest body temperature observed post-dose for each treatment group.
[b] Lowest reported individual body temperature during the study.

Ophthalmoscopic exams: There were no abnormalities noted.

Electrocardiograms (ECG): The mean heart rate (lengthening of the RR interval) for the dogs in the Zenalpha® groups was numerically lower than that for the dogs in the control group. There were periods of sinus bradycardia that peaked 30 to 60 minutes post-dose that were followed by an acceleration of the heart rate (shortening of the RR interval) and instances of sinus tachycardia that peaked 1.5 hours (1X group) to 4 hours (5X group) post-dose. The heart rate changes were accompanied by physiologically appropriate changes in the PR and QT intervals. There was also evidence of QRS duration widening and QTc interval prolongation that corresponded to the intervals in which the heart rate was slowed. Second-degree atrioventricular (AV) block occurred infrequently (5 instances in 3 dogs) and was not dose related. The instances occurred once pre-test for a 1X male, 3 instances in one 3X female at 15 minutes post-dose on Day 3, and once in a 5X male at 1 hour post-dose on Day 3.

Blood Pressure: The mean blood pressure (mmHg) for the dogs in the Zenalpha® groups was numerically lower than for the dogs in the control group. Blood pressure generally decreased between 15 minutes and 1.5 hours post-dose, and fully recovered to baseline/control values by 2 to 4 hours post-dose.

Table III.6: Minimum Systolic Blood Pressures (SBP)

Treatment Group	Minimum mean SBP (mmHg)[a]	Lowest reported individual SBP (mmHg)[b]
Control	115 at 1.5 hours post-dose Day 1 [154 pre-dose]	70 at acclimation
Zenalpha® 1X	91 at 15 minutes post-dose Day 3 [153 pre-dose]	56 at 1 hour post-dose Day 3
Zenalpha® 3X	92 at 15 minutes post-dose Day 2 [158 pre-dose]	68 at 1.5 hours post-dose Day 3
Zenalpha® 5X	89 at 1 hour post-dose Day 3 [153 pre- dose]	67 at 2 hours post-dose Day 1

[a] The means in the table represent the minimum systolic blood pressure post-dose for each treatment group.
[b] Lowest reported individual systolic pressure during the study.

Table III.7: Minimum Diastolic Blood Pressures (DBP)

Treatment Group	Minimum mean DBP (mmHg)[a]	Lowest reported individual DBP (mm Hg)[b]
Control	57 at 4 hours post-dose Day 1 [100 pre-dose]	27 at acclimation
Zenalpha® 1X	37 at 30 minutes post-dose Day 3 [91 pre-dose]	20 at 15 minutes post-dose Day 2
Zenalpha® 3X	37 at 15 minutes post-dose Day 2 [90 pre-dose]	22 at 15 minutes and 30 minutes post-dose Day 1
Zenalpha® 5X	37 at 15 minutes post-dose Day 1 [101 pre-dose]	24 at 1.5 hours post-dose Day 1

[a] The means in the table represent the minimum diastolic blood pressure post-dose for each treatment group.
[b] Lowest individual diastolic pressure during the study.

Table III.8: Minimum Mean Arterial Pressures (MAP)

Treatment Group	Minimum average MAP (mmHg)[b]	Lowest reported individual MAP (mmHg)[c]
Control	81 at 4 hours post-dose Day 1 [122 pre-dose]	46 at 8 hours post-dose Day 2
Zenalpha® 1X	58 at 30 minutes post-dose Day 3 [117 pre-dose]	36 at 15 minutes post-dose Day 2
Zenalpha® 3X	58 at 15 minutes post-dose Day 1 [123 pre-dose]	38 at 15 minutes post-dose Day 1
Zenalpha® 5X	59 at 15 minutes post-dose Day 1 [123 pre- dose]	38 at 1 hour post-dose Day 3

[a] The means in the table represent the minimum average mean arterial blood pressure post-dose for each treatment group.
[b] Lowest reported individual mean arterial pressure during the study.

Clinical Pathology: The mean serum glucose concentration for the dogs in the 3X and 5X groups was numerically lower than that for the dogs in the control group. Two male dogs in the 5X group had serum blood glucose less than 65 mg/dL on Day 3 at 3 hours post-dose (56 mg/dL and 62 mg/dL). Blood glucose was normal at acclimation and other study time points for these two dogs.

Two dogs had slightly decreased serum potassium post-dose, a 1X female (4.1 mEq/L, reference range 4.2-5.2 mEq/L) and a 3X female (4.0 mEq/L).

There were no other clinically relevant findings for hematology or urinalysis variables in any of the treatment groups. There were no test-article effects on prothrombin time, activated partial thromboplastin time, buccal mucosal bleeding time, or C-Reactive Protein.

Injection Site Evaluation: There were no findings of erythema/eschar or edema.

Sedation Scoring: The mean sedation scores for dogs in the Zenalpha® groups were significantly different and numerically higher than that for the dogs in the control group. Dogs in the Zenalpha® groups achieved sedation level 9 or 10 (out of 10) at 15 minutes post-dose dosing. In general, the sedation score returned to near baseline (score 1 or 2) or baseline (score 0) at 2 to 4 hours post-dose for the 1X group and 4 to 8 hours for the 3X and 5X groups. Time in lateral recumbency was calculated as the time that standing occurred minus the time that lateral recumbency occurred. The mean duration of time in lateral recumbency for the Zenalpha® groups increased as the dose increased.

Table III.9. Mean Duration of Lateral Recumbency in Minutes (standard deviation) by Treatment Group and Study Day

Treatment group	Day 1	Day 2	Day 3	Pooled days
Zenalpha® 1X	82.6 (45.0)	66.7 (47.0)	51.9 (21.6)	67.1 (39.6)
Zenalpha® 3X	102.0 (66.0)	87.8 (65.6)	83.9 (31.8)	91.2 (54.8)
Zenalpha® 5X	124.3 (53.2)	108.3 (20.8)	133.0 (85.4)	121.8 (57.6)

There was individual dog variability within each treatment group for duration of lateral recumbency in minutes. Dogs in the control group did not become sedated.

Table III.10. Individual Dog Minimum and Maximum Duration of Lateral Recumbency (minutes) by Treatment Group

Treatment group	Minimum	Maximum
Zenalpha® 1X	19	125
Zenalpha® 3X	27	232
Zenalpha® 5X	61	340

Necropsy Examination: All groups had occasional red discoloration of the skin at the injection site noted on gross pathology that correlated with hemorrhage, mixed leukocyte infiltration, or mononuclear cell infiltration on histopathology. There were no treatment related effects on histopathology or organ weights.

Toxicokinetics: Plasma, urinary, and CSF concentrations of dexmedetomidine, levomedetomidine, and vatinoxan were measured using a validated liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) bioanalytical method. The maximum observed concentration (C_{max}) and estimated concentration at time zero (C_0) were less than dose-proportional for vatinoxan, and approximately dose-proportional for dexmedetomidine and levomedetomidine after one dose on Day 1. The area under the plasma concentration-time curve from time zero to 8 hours ($AUC_{0\text{-}8h}$) was approximately dose-proportional for vatinoxan and was more than dose-proportional for both dexmedetomidine and levomedetomidine after one dose on Day 1. The systemic exposure was similar among male and female dogs.

Following administration of the 1X dose for 4 consecutive days, adjusted for plasma protein binding, the plasma unbound fraction: CSF ratio was approximately 36:1 for vatinoxan, and close to 1:1 for dexmedetomidine. It was considered that the plasma: CSF ratios showed vatinoxan would be unlikely to exert appreciable CNS effects; therefore, CNS tissue levels were not determined.

Urine drug analysis between the Day 3 and 4 doses showed that vatinoxan was excreted in low concentration (ng/mL) and variable amounts in the urine. Taking into account the urine volumes collected and total dose of vatinoxan received in each dog, the amount of the Day 3 dose detected in the urine was approximately 2% (1X group) to 5% (3X and 5X groups).

Conclusion: Zenalpha® administered as an intravenous injection once daily for 4 days at doses of 1 mg medetomidine and 20 mg vatinoxan/m^2 BSA, 3 mg medetomidine and 60 mg vatinoxan/m^2 BSA, or 5 mg medetomidine and 100 mg vatinoxan/m^2 BSA did not produce systemic toxicity and had an acceptable margin of safety. The administration of Zenalpha® resulted in sedation, decreased blood pressure, hypothermia, and initial sinus bradycardia and later sinus tachycardia that all resolved prior to the next scheduled dose. Clinical observations during the study were related to the mechanism of action of the product. Toxicokinetic analysis showed approximately dose-proportional systemic exposure to the active ingredients and very low levels of urinary excretion of vatinoxan. The results of the plasma: CSF ratio analysis suggest that vatinoxan lacks appreciable activity in the CNS. This study supports the safe use of Zenalpha® when administered intramuscularly, according to the label, as a sedative for dogs.

IV. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to Zenalpha®:

Not for use in humans. Keep this and all medications out of reach of children and pets.

Avoid skin, eye or mucosal contact. Use caution while handling and using filled syringes. Absorption of the active ingredients is possible following exposure via the skin, eye or mucosa. In case of accidental eye exposure, flush eyes with water for 15 minutes, remove contact lenses then continue to flush. In case of accidental skin exposure, wash with soap and water and remove contaminated clothing. If symptoms occur, seek the advice of a physician.

In case of accidental oral intake or self-injection, seek medical advice immediately and show the package insert to the physician. DO NOT DRIVE as sedation, loss of consciousness, and changes in blood pressure may occur.

Persons with cardiovascular disease (for example, hypertension or ischemic heart disease) should take special precautions to avoid any exposure to this product.

Pregnant women should exercise special caution to avoid exposure. Uterine contractions and decreased fetal blood pressure may occur after accidental systemic exposure.

Persons with known hypersensitivity to any of the ingredients should avoid contact with Zenalpha.

Caution should be exercised when handling sedated animals. Handling or any other sudden stimuli, including noise, may cause a defense reaction in an animal that appears to be heavily sedated.

Note to physician: Zenalpha contains medetomidine, an alpha$_2$-adrenoceptor agonist, in combination with vatinoxan, a peripherally selective alpha$_2$-adrenoceptor antagonist. Symptoms after absorption or accidental self-injection may include dose-dependent sedation, respiratory depression, bradycardia, tachycardia, and hypotension.

V. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that Zenalpha®, when used according to the label, is safe and effective for use as a sedative and analgesic in dogs to facilitate clinical examination, clinical procedures and minor surgical procedures.

A. Marketing Status

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required for proper dosing and administration of the product and to monitor the safe use of the product.

B. Exclusivity

Zenalpha®, as approved in our approval letter, qualifies for FIVE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(i) of the FD&C Act because this is the first time we are approving vatinoxan hydrochloride in a new animal drug application submitted under section 512(b)(1) of the FD&C Act. Any applicable exclusive marketing rights and exclusivity for this drug run concurrently.

C. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.