NATURAL FIELD

Compiling Organization
NATURAL FIELD CO.,LTD.

2026

Healthy Sleep Solutions

WHITE PAPER

Comprehensive Guide
to Restful Sleep

Natural FIELD

Catalogue

1. Global Sleep Health Trends and From Sleep Needs to Scientific Questions

Changes in modern lifestyles and demographic structures are triggering a global sleep-health crisis. Sedentary office work, chronic stress and disrupted circadian rhythms, coupled with accelerating population ageing, have made sleep disorders a major public-health issue impairing people’s quality of life and long-term metabolic health.

According to Fortune Business Insights, the global sleep-supplements market reached USD 8.55 billion in 2025. It is projected to grow from USD 9.27 billion in 2026 to USD 17.65 billion in 2034, representing a compound annual growth rate (CAGR) of 8.38% over the forecast period.

Traditional sleep-care concepts have long centered on “falling asleep fast”, focusing on short-term relief of insomnia symptoms. In contrast, multi-pathway synergistic sleep-health solutions, which focus on circadian rhythm regulation, neurotransmitter balance and stress-adaptation support, precisely address modern consumers’ urgent demand for deep sleep and full-cycle sleep wellness, and are gradually becoming a core development trend in the nutrition and health industry.

Over-the-counter products intended to improve sleep onset, prolong sleep duration, enhance relaxation and overall sleep quality mainly include melatonin supplements, herbal sleep-aid products, magnesium preparations, amino acid and neurotransmitter-supporting supplements, etc. Rising stress, screen exposure, irregular work schedules, travel-related sleep disruption, and consumers’ growing preference for natural health products are driving increased consumption of such supplements. Sleep health is gradually shifting from simple “sleep-aid” purposes toward comprehensive sleep-health management.

2. Co-loaded Liposomes Based on Ashwagandha: A New Exploration Direction

2.1 Experimental Data-Characterization of Ashwagandha Liposomes

Ashwagandha liposomes enable stable encapsulation of melatonin to form a homogeneous and stable nano-delivery system. They help improve the stability and bioavailability of active ingredients and provide technical support for the development of innovative functional health-food products.

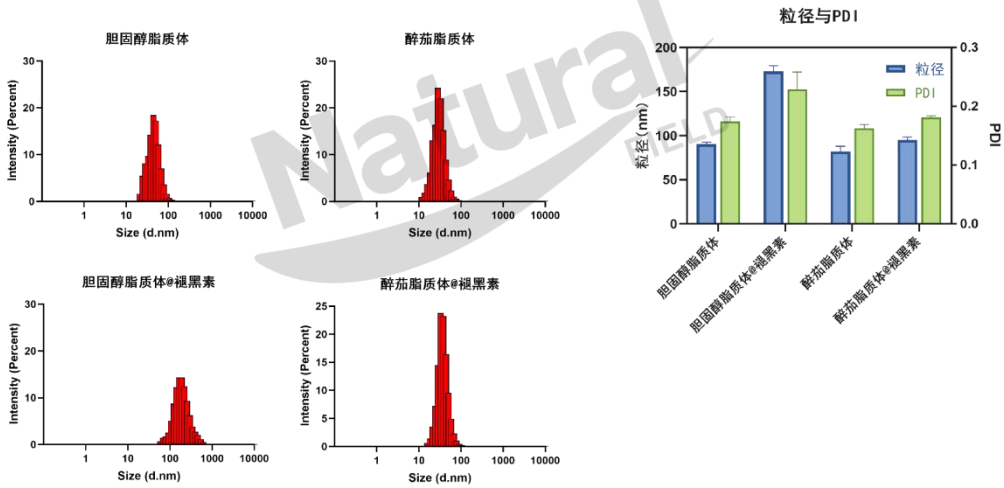

Figure 2-1.Characterization of Ashwagandha Liposomes

2.2 Experimental Data-In-vitro Targeting Verification

To evaluate the cellular internalization and targeted-delivery capacity of ashwagandha liposomes, cerebral vascular endothelial cells and neuronal cells were selected for in-vitro cellular-uptake experiments. Conventional cholesterol liposomes were set as the control group, and Coumarin 6 fluorescent labeling was applied to trace the cellular uptake behavior of carriers.

Fluorescence imaging results intuitively reflect the intracellular distribution

differences between the two groups of carriers. DAPI stains cell nuclei with blue fluorescence, while Coumarin 6 labels liposome carriers with green fluorescence. Merge overlay images enable observation of intracellular localization of carriers. Comparisons show that the cholesterol-liposome group exhibits faint green-fluorescence signals with low intracellular carrier accumulation. By contrast, the ashwagandha-liposome group displays markedly intensified intracellular green-fluorescence signals, indicating that abundant carriers have entered cerebral vascular endothelial cells and neuronal cells.

Flow-cytometry quantitative detection further quantifies cellular-uptake efficiency. MFI (Mean Fluorescence Intensity) data demonstrate that the ashwagandha-liposome group achieves far higher fluorescence intensity than the cholesterol-liposome control group in both cerebral vascular endothelial cell and neuronal cell models, with statistically significant differences between groups. It confirms that ashwagandha liposomes can be efficiently internalized by these two types of brain cells.

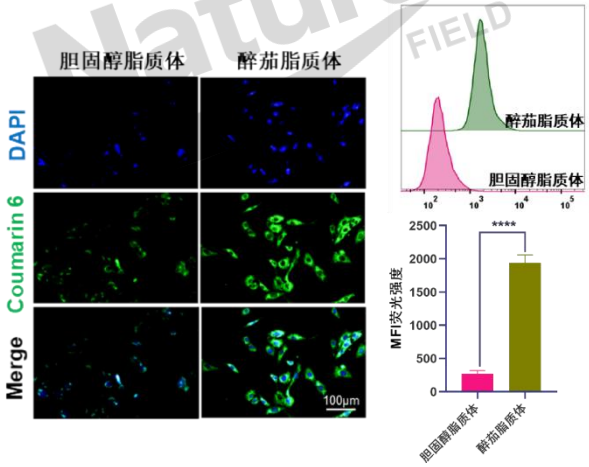

Figure 2-2.Cerebral Vascular Endothelial Cells

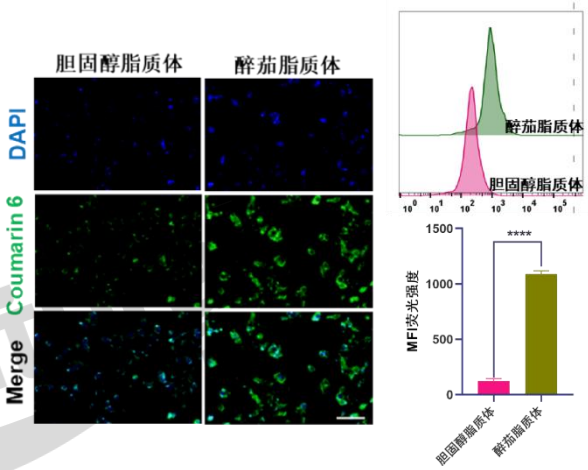

Figure 2-3.Neuronal Cells

2.3 Experimental Data-In-vivo Targeting Verification (In-vivo Fluorescence Imaging)

To further verify the brain-targeting capability of ashwagandha liposomes in-vivo, in-vivo animal imaging and ex-vivo tissue distribution experiments were carried out in this study. Using cholesterol liposomes as control, liposome carriers were labeled with IR780 fluorescent dye, and the in-vivo carrier distribution was tracked at time points of 0.5 h, 1 h, 2 h and 4 h.

Whole-body in-vivo imaging results show that hardly any obvious fluorescent signal was detected in the head region of the cholesterol-liposome group. By contrast, prominent fluorescent accumulation could be observed in the head of the ashwagandha-liposome group as early as 0.5 h after administration. Relatively high brain fluorescent signal was sustained throughout the observation period from 1 h to 4 h, indicating effective accumulation of carriers towards the brain.

Ex-vivo organ imaging further confirms the differences in tissue distribution. For organ samples collected at 2 h and 4 h post-administration, brain tissue from the ashwagandha-liposome group exhibited significantly higher fluorescence intensity compared with the cholesterol-liposome control group. When compared with peripheral organs including heart, liver, spleen, lung and kidney, this carrier enables

directional enrichment of more active substances in the target organ of the brain.

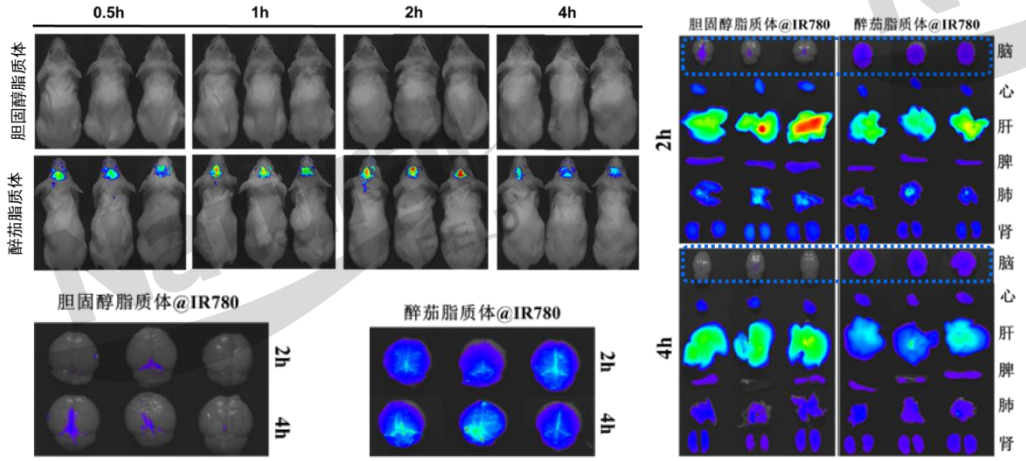

Figure 2-4.In-vivo Targeting Verification

2.4 Experimental Data-In-vivo Targeting Verification (Quantitative Tissue Distribution)

The drug distribution levels of ashwagandha liposomes versus free withanolides in various organs of animals were compared at different time points (1 h, 2 h, 4 h, 8 h). The concentrations of withanolides in brain, heart, liver, spleen, lung and kidney tissues were quantitatively determined.

The data demonstrate that within the entire observation window of 1-8 h, the drug concentration in brain tissue of the ashwagandha-liposome group was significantly higher than that of the free-withanolide group, with statistically significant differences between groups. This confirms that the liposome carrier can effectively enhance the enrichment of active ingredients in the brain. As the major metabolic organ, the liver showed high drug levels in both groups. No abnormal accumulation of ashwagandha liposomes was observed in peripheral organs including heart, spleen, lung and kidney.

Quantitative tissue-distribution data corroborate the in-vivo imaging results. It further validates the brain-targeted-delivery advantages of ashwagandha liposomes from the perspective of drug concentration, increases the exposure of active

substances at the brain target site, and provides quantitative in-vivo experimental evidence for sleep-regulating efficacy.

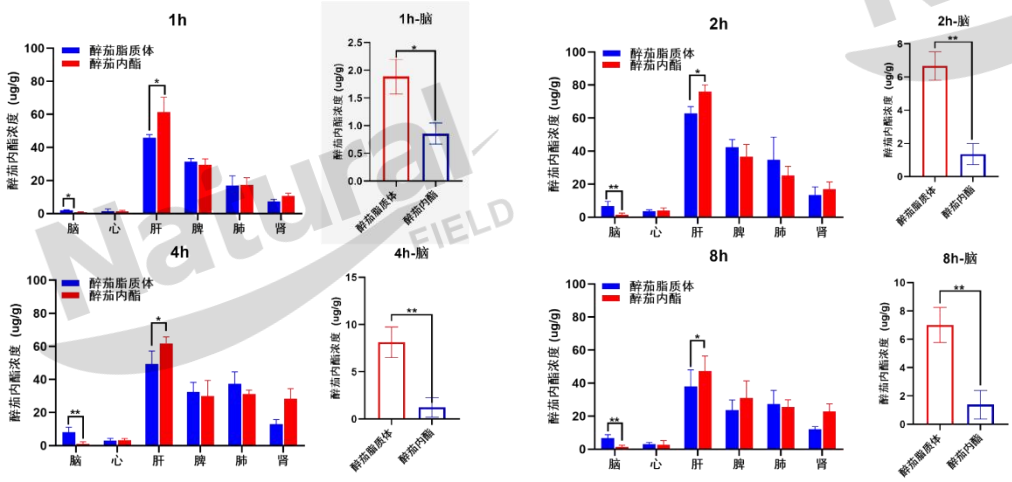

Figure 2-5.In-vivo Tissue Distribution

2.5 Experimental Data-In-vitro Pharmacodynamic Assays (Neuronal Cells)

An in-vitro neuronal stress-injury model was established using cortisol (CORT). Multiple control groups were set up to evaluate the regulatory effect of the ashwagandha-liposome plus melatonin system on neuroinflammation and neurotrophic factors.

Four key biomarkers including BDNF, TNF-α, IL-6 and IL-1β were determined. Compared with the blank control group, the CORT-induced stress group showed significantly elevated pro-inflammatory factors (TNF-α, IL-6, IL-1β) and marked down-regulation of the neurotrophic factor BDNF, indicating the successful construction of the neuronal-injury model. In comparison with the melatonin-only group and cholesterol-liposome plus melatonin group, the ashwagandha-liposome plus melatonin group significantly up-regulated BDNF expression and effectively suppressed the release of multiple pro-inflammatory factors, with statistically significant differences among groups.

The ashwagandha-liposome delivery system can alleviate neuronal inflammatory damage induced by stress and improve neurotrophic status. It confirms at the cellular

level that this formula holds the potential to protect neurons and modulate neuronal homeostasis, and provides in-vitro pharmacodynamic evidence for sleep- and emotion-regulating functions.

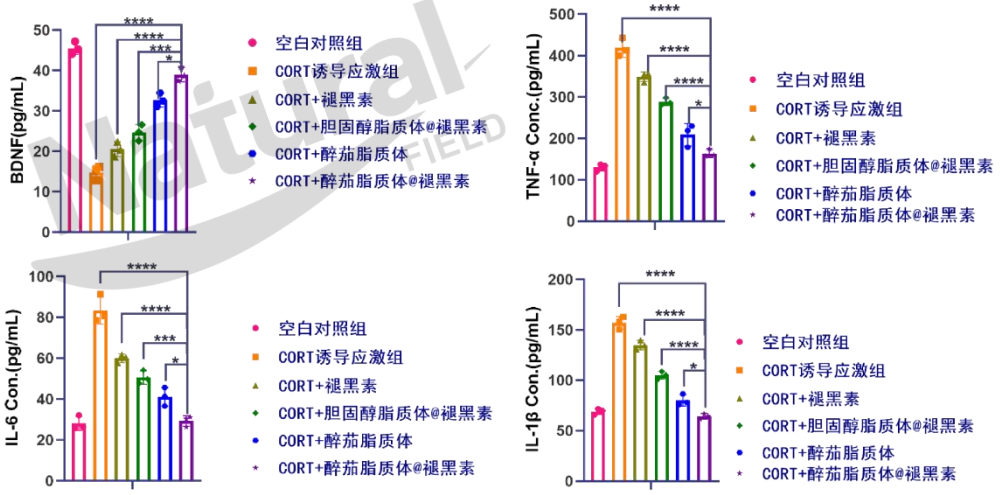

Figure 2-6.In-vitro Pharmacodynamic Assays (Neuronal Cells)

2.6 Experimental Data-In-vivo Pharmacodynamic Evaluation (Open-Field Test + Elevated-Plus-Maze Test)

A chronic restraint-stress paradigm was used to establish an animal anxiety-depression model. Behavioral evaluations were conducted using the open-field test and elevated-plus-maze test. A healthy control group, model group and multiple drug-administration intervention groups were established, and animal movement trajectories together with activity heat-map distribution were recorded.

The track plots and heat-maps from the open-field test revealed that spontaneous locomotor activity and central-zone exploratory behaviors were markedly decreased in the chronic restraint stress model group. Results from the elevated plus-maze test also demonstrated a significant increase in anxiety-like behaviors in model animals. Compared with the pure melatonin group and cholesterol-liposome@melatonin group, animals receiving ashwagandha-liposome@melatonin intervention exhibited movement tracks and heat-map distribution closer to those of the healthy control group, with notably improved exploratory behaviors.

Behavioral experimental results confirm that the ashwagandha-liposome plus melatonin formula can effectively ameliorate stress-triggered neurobehavioral abnormalities and possesses favorable anxiolytic and antidepressant potential. It validates the practical effect of the formula for synergistic emotion-sleep regulation at the animal-behavior level.

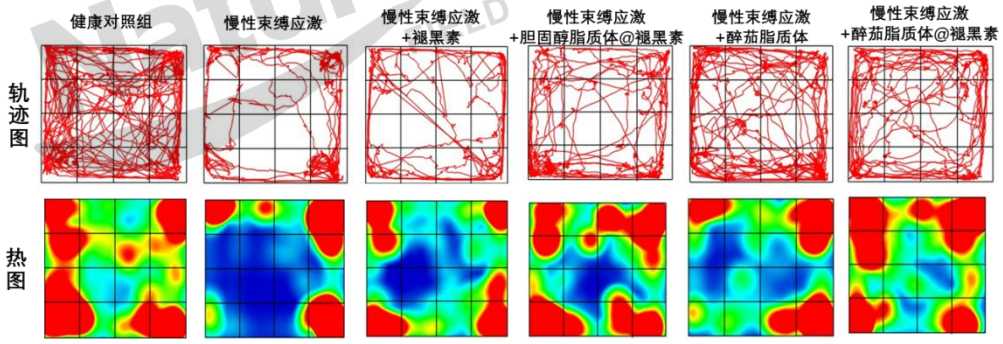

Figure 2-7 Open-Field Test

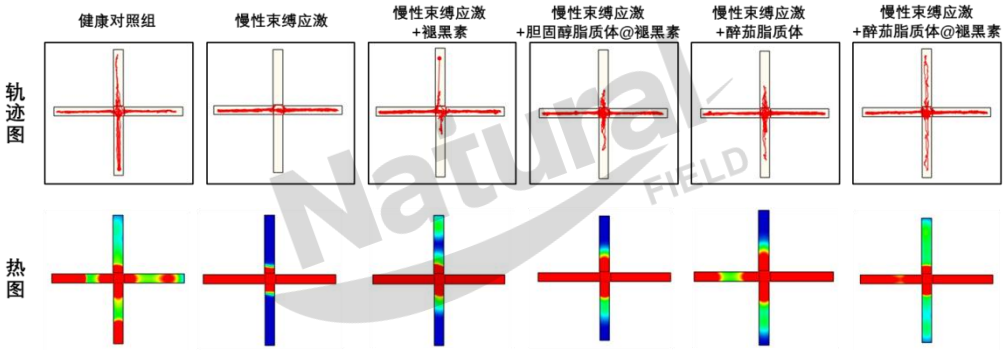

Figure 2-8.Elevated-Plus-Maze Test

3. NF TriSolve®: Scientifically Formulated Sleep Health Solutions

3.1 Active Ingredients + Delivery Technology + Scientific Evidence

NF TriSolve® Sleep-Improving Solution adopts a multi-dimensional sleep-regulating formulation system to build a four-layer stereoscopic framework consisting of core technology layer, nerve relaxation layer, sleep circadian rhythm layer and auxiliary

support layer. It breaks through the limitations of conventional single-target sleep-aid ingredients. Centering on stress adaptation, circadian rhythm, nerve soothing and neurotransmitter metabolism, it achieves multi-target synergistic regulation, and delivers full-chain sleep-health intervention covering sleep onset, sleep maintenance and circadian rhythm restoration.

Table 3-1.Four-Tier Stereoscopic Formula System of NF TriSolve® Sleep-Improving Solution

Tier	Core Ingredients	Functional Direction
Core Technology Layer	Ashwagandha-melatonin co-loaded liposome system	Stress adaptation support, sleep circadian rhythm support
Nerve Relaxation Layer	GABA, Magnesium Glycinate, L-Theanine	Nerve relaxation support
Sleep Circadian Rhythm Layer	Melatonin	Circadian rhythm regulation
Auxiliary Support Layer	5-HTP, Vitamin B6, Apigenin	Neurotransmitter-related support

The four-tier system works in interconnected synergy instead of simple ingredient stacking. The core technology layer guarantees absorption and stress regulation; the nerve relaxation layer delivers immediate soothing effects; the circadian rhythm layer calibrates the biological clock; the auxiliary support layer consolidates neurotransmitter metabolism. Multi-layer collaboration forms a complete logic of "stress relief-calming effect-circadian rhythm calibration-homeostasis maintenance". Different from single-function formulas on the market that only target sleep onset, it provides a systematic nutritional solution for modern people's complex sleep concerns, including high-level stress, frequent dreaming with easy awakening and circadian rhythm disruption.

3.2 Scientific Design Concept of NF TriSolve® Sleep-Improving Formula: Three-Tier Synergy, Multi-Pathway Intervention

Following the scientific design concept of three-tier synergy and multi-pathway

intervention, NF TriSolve® addresses complex sleep issues among modern populations, including difficulty falling asleep, frequent sleep interruptions and stress-induced sleep deterioration. It establishes an integrated three-in-one sleep-health management system supporting sleep onset, sleep maintenance and sleep quality. Breaking away from the conventional approach where single ingredients only target sleep onset, it realizes nutritional regulation for the full sleep cycle.

Table 3-2.Three-Pathway Synergistic Sleep Intervention System of NF TriSolve®

Pathway	Target Objective	Mechanism of Action	Core Ingredients
Pathway 1: Sleep-onset Support	Circadian rhythm regulation	Calibrate the biological clock	Melatonin
Pathway 2: Sleep-maintenance Support	Neurotransmitter balance	Reduce neuronal excitability	GABA, L-Theanine, Magnesium Glycinate
Pathway 3: Sleep-quality Support	Stress adaptation	Alleviate the root cause of anxiety	Ashwagandha

The three pathways do not operate independently but interact and synergize with one another. The circadian-rhythm pathway facilitates sleep onset; the neurotransmitter pathway safeguards uninterrupted sleep; the stress-adaptation pathway eliminates sleep disturbances caused by emotional stress. Together they form a closed-loop system, delivering a systematic nutritional solution for sleep troubles induced by multiple factors in modern populations.

4. Experimental Verification Using Zebrafish Model

All experiments were performed by Hunter Biotech,an AAALAC-international-ac credited CRO institution, in strict compliance with IACUC ethical review requi rements and the 3R principles for animal experimentation.All experiments were performed by Hunter Biotech,

4.1 RNA Extraction Results and Primer Sequence Information

At the experimental endpoint, total RNA was extracted from zebrafish. The RNA concentration and A260/A280 ratio were determined using an ultraviolet-visible spectrophotometer (Table 4-1). All A260/A280 ratios ranged from 1.8 to 2.2, indicating that the extracted zebrafish total RNA was of good quality and suitable for subsequent q-PCR experiments.

Table 4-1.Concentration and A260/A280 ratio of total RNA（n = 3）

Group	Concentration (μg/mL)	RNA concentration(μg/μL)			A260/A280		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Control	-	0.745	0.795	0.798	1.98	1.95	1.96
Model	-	0.841	0.851	0.856	1.99	1.98	1.92
Melatonin	125	0.849	0.836	0.869	1.95	1.96	1.93
Control	0.833	0.865	0.814	0.822	1.92	1.91	1.90
Benchmark product 1	2167	0.879	0.846	0.866	1.95	1.95	1.93
Benchmark product 2	115	0.912	0.901	0.925	1.99	1.95	1.94
Target Formula 6	141	0.952	0.921	0.945	1.95	1.96	1.94
Target Formula 9	80.8	0.874	0.876	0.843	1.95	1.98	1.92

Table 4-2.Primer Sequence Information

gene		Primer Sequences
β-actin	Forward	5’-TCGAGCAGGAGATGGGAACC-3’
	Reverse	5’-CTCGTGGATACCGCAAGATTC-3’
hcrt	Forward	5’-GGACTGCACAGCTAAGAAGC -3’
	Reverse	5’-AATGTCTGGCGACGGAAGAG-3’
mtnr1aa	Forward	5’-TATTTCACCGGGGCTGGAAC-3’
	Reverse	5’-CATACTGCAAGGAGCCCACA-3’
gabra1	Forward	5’-CATTCCGGGTGAGTCAGAGAC-3’
	Reverse	5’-GTGACCAGTAAACAGGCCCA-3’

4.2 Evaluation of Sleep-Improving Efficacy (Behavioral)

Under the experimental conditions of this study, Control Group 1, Control Group 4, benchmark product 1, benchmark product 2, and Target Formulations 1–9 all exert sleep-improving efficacy, which is reflected in decreased awakening movement distance and total awakening duration.

Table 4-3.Experimental Results of Behavioral Evaluation on Sleep-Improving Efficacy of Test Samples (n = 10)

Group	Concentration (μg/mL)	Awakening movement distance (mm, mean ± SE)	Sleep-improving efficacy(%)	Total awakening duration (s,mean ± SE)	Sleep-improving efficacy(%)
Control	-	102 ± 16.0***	-	4.49 ± 0.716***	-
Model	-	1732 ± 65.7	-	43.0 ± 1.70	-
Melatonin	125	1109 ± 56.5***	36	26.7 ± 1.16***	38
Control Group 1	0.833	1273 ± 84.8***	27	31.9 ± 2.00***	26
Control Group 4	12.8	1226 ± 75.0***	29	31.8 ± 1.57***	26
Benchmark product 1	2167	1436 ± 99.8*	17	36.0 ± 2.08*	16
Benchmark product 2	115	1161 ± 73.4***	33	31.7 ± 1.46***	26
Target Formula 1	50.3	1492 ± 75.0*	14	36.2 ± 1.95*	16
Target Formula 2	80.3	1447 ± 111*	16	35.7 ± 2.28*	17
Target Formula 3	85.3	1338 ± 61.7***	23	35.0 ± 1.66**	19
Target Formula 4	90.3	1433 ± 100*	17	35.1 ± 1.82**	18
Target Formula 5	91.2	1200 ± 68.5***	31	29.7 ± 1.77***	31
Target Formula 6	141	1048 ± 67.3***	39	28.2 ± 1.60***	34
Target Formula 7	91.0	1492 ± 51.0**	14	38.2 ± 1.29*	11
Target Formula 8	116	1343 ± 90.9**	22	36.3 ± 1.76*	16

Group	Concentration (μg/mL)	Awakening movement distance (mm, mean ± SE)	Sleep-improving efficacy(%)	Total awakening duration (s,mean ± SE)	Sleep-improving efficacy(%)
Target Formula 9	80.8	1406 ± 113*	19	36.2 ± 2.59*	16

Compared with the model control group , *p < 0.05, **p < 0.01, ***p < 0.001

Figure 4-1.Representative Trajectory Diagrams of Zebrafish after Sample Treatment

Note: Black lines represent slow movement distance, green lines represent medium movement distance, and red lines represent fast movement distance.

All test groups 1-9 exhibited sleep-improving effects, manifested as decreased awakening activity and shortened total awakening duration.

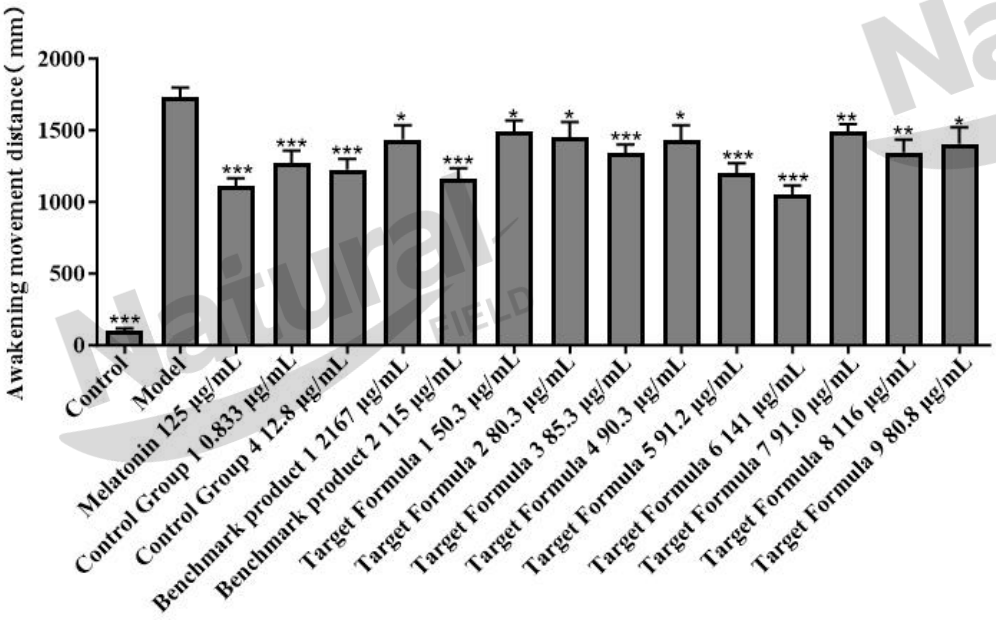

Figure 4-2. Awakening movement distance of zebrafish after sample treatment

Compared with the model control group,*p < 0.05,**p < 0.01,***p < 0.001

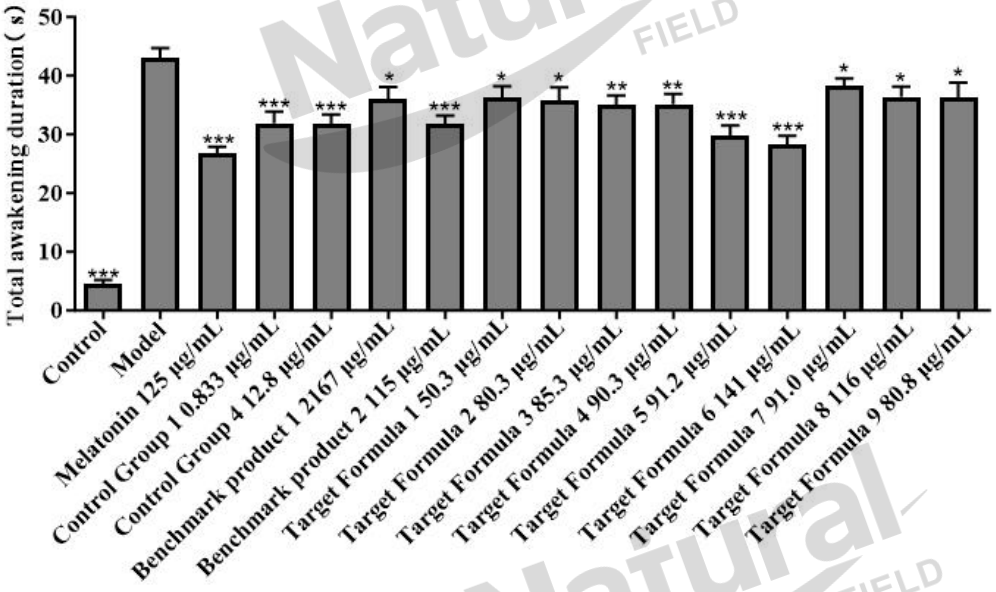

Figure 4-3.Total awakening duration of zebrafish after sample treatment

Compared with the model control group,*p < 0.05,**p < 0.01,***p < 0.001

4.3 Evaluation of Sleep-Improving Efficacy (Gene)

Under the experimental conditions of this study, Control Group 1, benchmark product

1, benchmark product 2, Target Formula 6 and Target Formula 9 all exhibit sleep-improving efficacy, as evidenced by upregulated relative expression levels of gabra1 and mtnr1aa genes and downregulated relative expression level of the hcrt gene.

Table 4-4.Experimental Results of Genetic Evaluation on Sleep-Improving Efficacy of Test Samples(n = 3)-1

Group	Concentration (μg/mL)	Relative Expression Level of *hcrt* Gene(mean ± SE)	Sleep-improving efficacy(%)
Control	-	0.449 ± 0.011***	-
Model	-	1.00 ± 0.008	-
Melatonin	125	0.593 ± 0.014***	41
Control Group 1	0.833	0.783 ± 0.025**	22
Benchmark product 1	2167	0.891 ± 0.014**	11
Benchmark product 2	115	0.818 ± 0.030**	18
Target Formula 6	141	0.682 ± 0.023***	32
Target Formula 9	80.8	0.829 ± 0.050*	17

Compared with the model control group,*p < 0.05,p < 0.01,***p < 0.001**

Table 4-5.Experimental Results of Genetic Evaluation on Sleep-Improving Efficacy of Test Samples(n = 3)-2

Group	Concentration (μg/mL)	Relative Expression Level of *gabra1* Gene（mean ± SE）	Sleep-improving efficacy（%）	Relative Expression Level of *mtnr1aa* Gene（mean ± SE）	Sleep-improving efficacy（%）
Control	-	1.67 ± 0.035**	-	1.81 ± 0.058***	-
Model	-	1.00 ± 0.106	-	1.00 ± 0.055	-
Melatonin	125	1.54 ± 0.043**	54	1.58 ± 0.026***	58
Control Group 1	0.833	1.43 ± 0.008*	43	1.48 ± 0.042**	30
Benchmark product 1	2167	1.33 ± 0.030*	33	1.27 ± 0.030*	18
Benchmark product 2	115	1.39 ± 0.026*	39	1.43 ± 0.062**	34
Target Formula 6	141	1.52 ± 0.011**	52	1.59 ± 0.032***	41
Target Formula 9	80.8	1.30 ± 0.013*	30	1.24 ± 0.016*	15

Compared with the model control group,*p < 0.05,p < 0.01,***p < 0.001**

The gabra1 gene encodes the γ-aminobutyric acid (GABA) receptor. As the major inhibitory neurotransmitter in adults, γ-aminobutyric acid (GABA) plays a critical role via suppressing excitatory signal transmission. Its biological activity is mediated by GABA receptors, and activation of GABA(A) receptors has been confirmed to facilitate sleep.

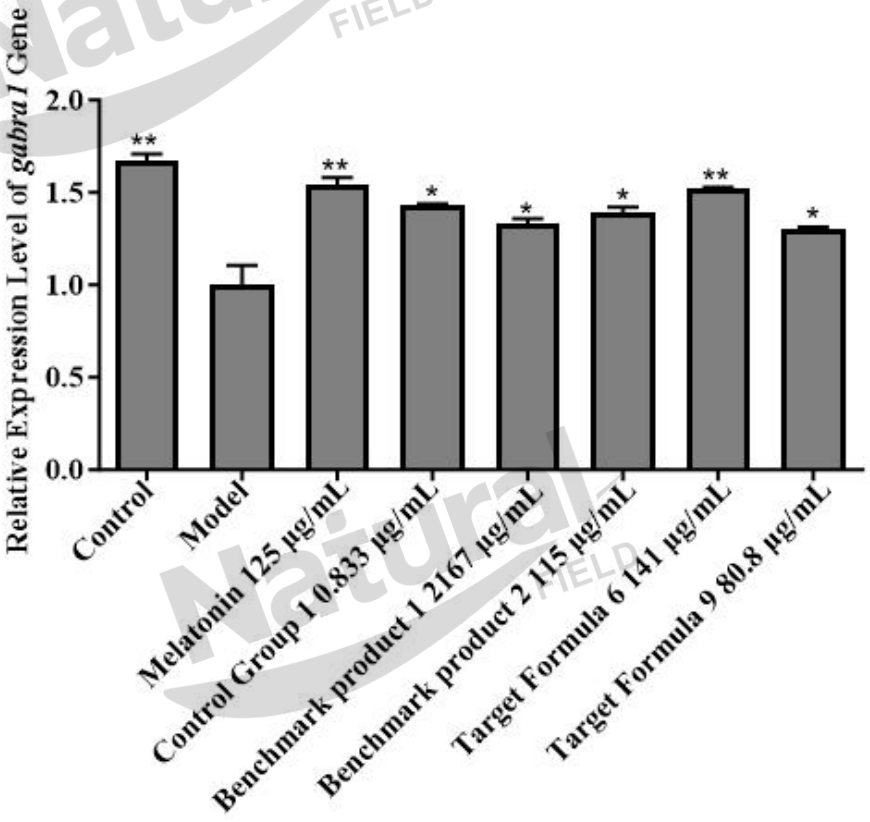

Figure 4-4.Relative Expression Level of gabra1 Gene

Compared with the model control group,*p < 0.05,**p < 0.01

The mtnr1aa gene encodes melatonin receptor MT1, which serves as a core regulator of sleep-wake cycles and circadian rhythms. This receptor is highly expressed in the suprachiasmatic nucleus of the hypothalamus. Melatonin secreted by the pineal gland at night binds to MT1, inhibiting adenylate cyclase and activating BK channels (large-conductance calcium-activated potassium channels). These effects reduce the excitability of neurons in the central circadian clock, thereby promoting sleep initiation, maintaining NREM and REM sleep, and stabilizing circadian rhythms.

Gene deletion or mutation leads to defective receptor function and attenuated melatonin signaling, manifested as delayed sleep onset, sleep fragmentation, circadian rhythm disruption and impaired sleep homeostasis. Polymorphisms of this gene are associated with sleep disorders including insomnia and shift work intolerance.

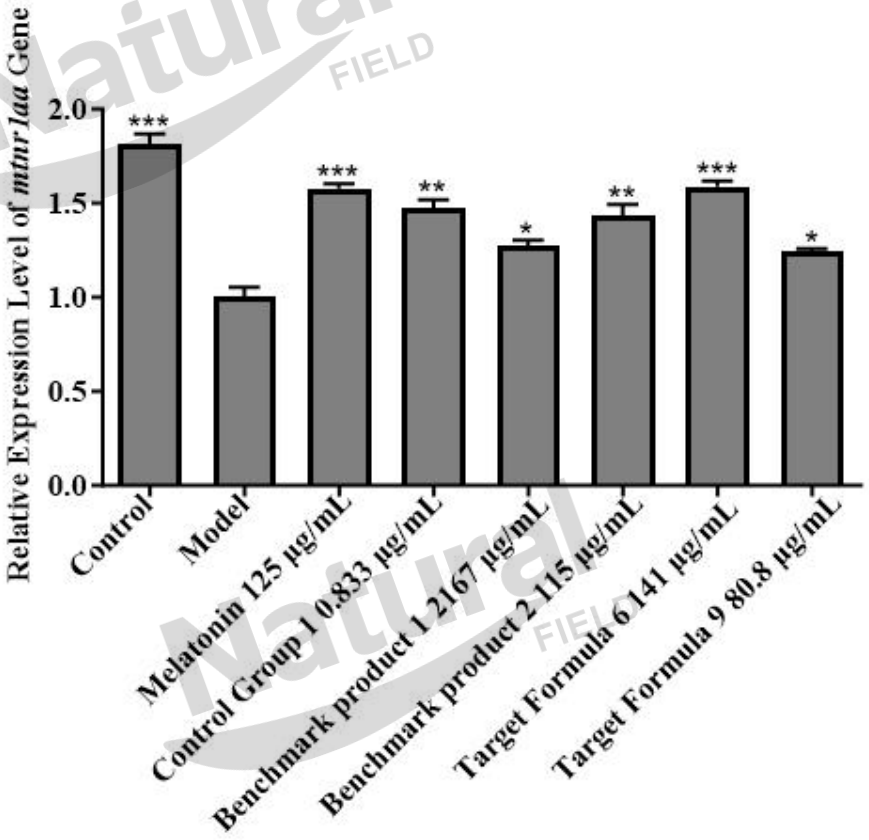

Figure 4-5.Relative Expression Level of mtnr1aa Gene

Compared with the model control group,*p < 0.05,**p < 0.01,***p < 0.001

hcrt (hypocretin/orexin precursor gene) encodes a neuropeptide precursor mainly expressed in the hypothalamus, which regulates sleep-wake homeostasis, feeding and energy metabolism via HCRTR receptors. This gene is evolutionarily conserved and present as a single copy in zebrafish. Overexpression of hcrt induces persistent wakefulness and sleep fragmentation, while inhibition of hcrt signaling alleviates PTZ-induced sleep disorders. Thus, hcrt acts as a pivotal gene mediating excitatory imbalance and sleep disturbance.

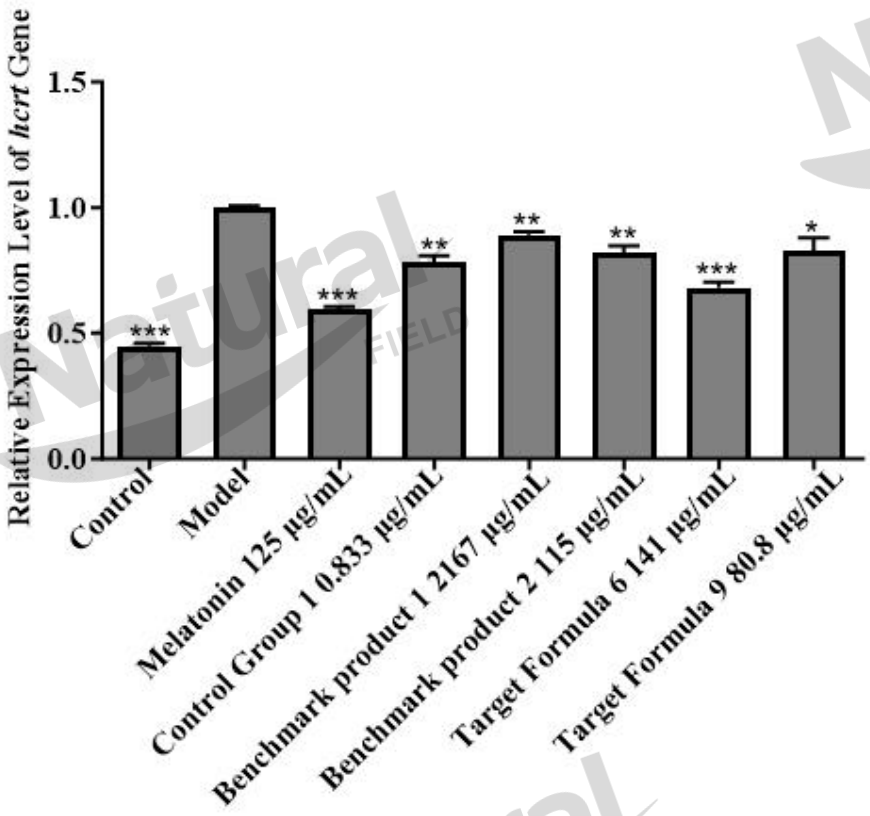

Figure 4-6.Relative Expression Level of hcrt Gene

Compared with the model control group,*p < 0.05,**p < 0.01,***p < 0.001

5. Analysis of Market Competitiveness

5.1 Comparative Analysis Between NF TriSolve® and Mainstream Commercial Products

To objectively evaluate the comprehensive efficacy of NF TriSolve® Formula 6, multiple parallel control groups were established in this study, including commercial Product A containing melatonin, commercial Product B without melatonin, and 5 mg high-dose pure melatonin. Horizontal comparative experiments were conducted across three dimensions: behavioral improvement effect, molecular mechanism verification, and core differences in formulation logic.

Table 5-1.Horizontal Comparison of NF TriSolve® Formula 6 with Commercial Products and

Group	Commercial Product A(Melatonin-containing)	Commercial Product B(Melatonin-free)	Pure 5 mg Melatonin	NF TriSolve® Formula 6
Behavioral Improvement	Limited	Significantly weaker than Formula 6	36%	39%
Mechanism Verification	Relatively weak	More pronounced difference	Single-mechanism action	Synchronized improvement of three key genes
Core Differences	Single mechanism with limited improvement ceiling	Lack of core regulatory components	High dosage, potential side-effect risks	Low dosage, high efficacy, multi-target synergy

Pure Melatonin

Behavioral data reveal that commercial Product A delivers limited sleep-improving effects; the overall efficacy of commercial Product B is markedly weaker than that of NF TriSolve® Formula 6. The sleep-improvement rate of the 5 mg pure melatonin group is 36%, while NF TriSolve® Formula 6 achieves an improvement rate of 39%, outperforming the high-dose pure melatonin control group.

At the mechanism-validation level, most mainstream commercial products only produce mild physiological improvements without supporting molecular-level evidence. The 5 mg pure melatonin group functions via a single pathway only. By contrast, NF TriSolve® Formula 6 synchronously modulates three key sleep-related genes and realizes multi-target intervention on sleep pathways at the molecular level, presenting a more complete efficacy mechanism.

In terms of core formulation differences, commercial Product A features a single mechanism of action with an obvious efficacy ceiling; commercial Product B lacks core active components for circadian-rhythm regulation. High-dose pure melatonin obtains effects through dosage escalation, which carries potential risk of side effects. Adopting a low-dose design, NF TriSolve® Formula 6 balances safety and efficacy

and achieves multi-target synergistic action.

Key experimental conclusions demonstrate that NF TriSolve® Formula 6, built upon merely 2 mg melatonin, achieves better sleep-improving outcomes than 5 mg pure melatonin. This fully verifies the "1+1 > 2" synergistic gain brought by scientific compounding. It proves that superior sleep-health support can be realized through multi-component and multi-pathway synergy, instead of simply increasing the dosage of a single ingredient. This provides new research insights for the development of sleep-nutrition raw materials.

5.2 NF TriSolve®: Exploring More Health-oriented Solutions

The NF TriSolve® Functional Ingredient Delivery Platform has established a complete R&D chain: From Ingredients → To Technologies → To Solutions, achieving a leap from single-ingredient supply to the output of systematic health solutions.

Rooted in innovations of plant-derived actives, the platform selects high-quality natural active raw materials to guarantee the purity and stability of functional components, consolidating the foundational source for product R&D. Relying on core delivery technologies, it realizes stable encapsulation and targeted delivery of active substances, overcomes absorption barriers of conventional raw materials, and effectively enhances ingredient bioavailability. Through systematic scientific research and verification, laboratory data is further converted into implementable industrial outcomes.

Beyond sleep health, the NF TriSolve® technology platform covers diversified health tracks such as anti-aging and joint & bone care, and is no longer restricted to a single application scenario. Breaking away from the entrenched industry mindset of simple ingredient stacking and high-dose superposition, it leverages scientific compounding and delivery technologies to deliver multi-target synergistic gains at low dosage levels.

Looking toward the future of the functional-food industry, NF TriSolve® upholds

research-driven innovation and facilitates the translation of scientific achievements from laboratory to industrial applications. It provides scenario-oriented, customizable and all-dimensional health solutions to address the market's diversified health-management demands, and offers brand-new raw-material and technical R&D ideas for product development in brain health, sleep wellness and broader big-health sectors.

6. Annex

6.1 Evaluation of Sleep-Improving Efficacy (Behavioral)

Wild-type AB strain zebrafish at 5 days post fertilization (5 dpf) were randomly selected and placed in 6-well plates, with 30 zebrafish per well for each experimental group. Samples were administered via aqueous immersion at concentrations listed in Table 1-1; the positive control melatonin was set at a concentration of 125 μg/mL. A normal control group and a model control group were established simultaneously, with a liquid volume of 3 mL per well. After 1 day of incubation at 28 ℃, 10 zebrafish were randomly picked from each experimental group and transferred to a 96-well plate. Except for the normal control group, all other groups were treated with PTZ via aqueous immersion to construct the zebrafish insomnia model. The plates were immediately loaded into the behavioral analysis system to record the awakening movement distance (A1) and total awakening duration (A2) of zebrafish within 1 h. The sleep-improving efficacy of samples was evaluated based on statistical analysis of the above indicators. All statistical data were expressed as mean ± SE. The calculation formula for sleep-improving efficacy is shown as follows:

$$\text{Sleep-improving efficacy}\ (\%) = \frac{\text{A}\ (\text{Model Group}) - \text{A}\ (\text{Sample Group})}{\text{A}\ (\text{Model Group})} \times 100\%$$

Statistical analysis was performed using SPSS software. A difference with $p < 0.05$ was considered statistically significant.

6.2 Evaluation of Sleep-Improving Efficacy (Gene)

Wild-type AB strain zebrafish at 5 dpf were randomly distributed into 6-well plates, with 30 zebrafish treated per well for each experimental group. Samples were administered by aqueous immersion at concentrations shown in Table 1-4 and Table 1-5; melatonin at 125 μg/mL served as the positive control. A normal control group and a model control group were set up concurrently, with a liquid volume of 3 mL per well. All experiments were performed in triplicate. After 1 day of incubation at 28 ℃, PTZ was added to all groups except the normal control group via aqueous immersion to establish the zebrafish insomnia model. Following an additional 1 h incubation at 28 ℃, total RNA was extracted from zebrafish of each group using the Universal RNA Extraction TL Kit C. The concentration and purity of total RNA were determined with a UV-Vis spectrophotometer. A total of 2.00 μg total RNA from each zebrafish sample was used to synthesize 20.0 μL cDNA in accordance with the instructions of the First-Strand cDNA Synthesis Kit. The expression levels of β-actin, hcrt, gabra1 and mtnr1aa genes were detected by qPCR. β-actin was used as the reference gene to calculate the relative RNA expression levels (B) of hcrt, gabra1 and mtnr1aa. Statistical data were presented as mean ± SE. The calculation formula for sleep-improving efficacy is listed below:

$$\text{Sleep-improving efficacy}\ (\%) = \frac{\text{B}\ (\text{Model Group}) - \text{B}\ (\text{Sample Group})}{\text{B}\ (\text{Model Group})} \times 100\%$$

Statistical analysis was performed using SPSS software. A difference with $p < 0.05$ was considered statistically significant.

7. Disclaimer

This white paper is for industry exchange and informational reference only. It does not constitute any medical advice, diagnosis or treatment plan. Persons suffering from sleep disorders shall seek medical consultation at formal medical institutions and

follow the guidance of qualified physicians.

The research data and conclusions cited herein are based on available scientific evidence and shall not be construed as performance guarantees for any specific product. Actual results may vary due to individual differences, usage patterns and other factors.

Information regarding the regulatory status of raw materials is based on the regulations in effect at the time of writing. Regulations are subject to update. Please refer to the latest requirements issued by local regulatory authorities for specific compliance obligations.

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