NEW DIETARY INGREDIENT (NDI) SAFETY INFORMATION

Instructions

- In this template, which supplements the data entry screens in the NDI notification electronic submission portal, you will describe the scientific information on which you base your conclusion that the dietary supplement containing the NDI will reasonably be expected to be safe. Safety information includes, among other things, (1) information showing that the NDI is identical or related to substances documented as having a history of use as food; (2) information showing that the NDI is identical or related to test articles used in safety studies; (3) information showing that a substance or product has a history of use as food; and (4) safety data, including the results of genetic toxicology studies, pharmacokinetic studies, animal toxicology studies and human clinical studies. This template asks for details about the identity of the NDI, verification of that identity, information about history of use as food, and any other evidence relevant to the safety of the NDI under its proposed conditions of use in the dietary supplement. After filling in the template, you will upload the completed template as an attachment to your online NDI notification and attach files containing the scientific publications cited in your notification.

- For a notification that concerns the use of an NDI in a dietary supplement that contains no other ingredients, the safety of the NDI and the dietary supplement would be synonymous.

In other situations, however, that may not be the case. For example, when an NDI is used in a dietary supplement with one or more other NDIs, the safety of the dietary supplement may not be the sum of the safety of the individual NDIs. In such circumstances, you should document your basis for concluding that the dietary supplement will reasonably be expected to be safe and explain why that conclusion is reasonable. For example, if two botanical extracts have separate histories of use in traditional medicine, but no history of being used together, the safety of the combination may not be clear from the safety information pertaining to the individual NDIs. On the other hand, if an extract of a medicinal herb is combined with an extract of a material that has a long history of safe use as food, then it may be reasonable to conclude that the combination is safe based on information about the safety of the individual NDIs. If you wish to submit a notification for the use of an NDI in a dietary supplement with other NDIs, the FDA recommends that you confer with a member of the New Dietary Ingredient Review Team in FDA's Division of Dietary Supplement Programs about how to proceed. If you have any questions concerning this matter, please contact the New Dietary Ingredients Review Team by email at NDITEAM@fda.hhs.gov.

- If a section or subsection is not applicable to your notification, mark "N/A" in your response.

- Sections marked as "Required" in the template's section headings must have complete responses in all subsections for which you have data. If you leave a "Required" section blank or respond "N/A," FDA will consider your notification incomplete for failure to

comply with 21 CFR 190.6(b). An incomplete notification does not satisfy the requirement to submit an NDI notification. You may not introduce your NDI or a dietary supplement containing the NDI into interstate commerce, or deliver the NDI or dietary supplement for introduction into interstate commerce, until at least 75 days after you have submitted a complete notification to FDA.

- Please include full citations for all published and unpublished sources cited or relied on in your notification in the Reference List (Section 5). You will be prompted to attach e-copies of these sources when you return to the electronic submission portal after filling in this template.

- The template includes some sections identified as "Recommended." These sections solicit information that FDA considers helpful in evaluating NDI notifications. You are encouraged but not required to respond to template sections that are identified as "Recommended." However, if you leave a "Recommended" section blank or respond "N/A" and FDA determines that the information is needed to establish safety, your notification may be considered inadequate to conclude that the NDI will reasonably be expected to be safe under its proposed conditions of use in the dietary supplement.

Table of Contents

1. New Dietary Ingredient Identity Information (Recommended)

1.1 Description of the identity of the NDI

1.1.1 Ingredient Name

- Vernonia Mother Tincture

1.1.2 Common Name

- Alecrim de Campo; Vernonia

1.1.3 Botanical Name

- *Vernonanthura nudiflora* (Less.) H. Rob. - Asteraceae

1.1.4 Taxonomy and distribution

- *V. nudiflora* is commonly known as "vernonia". This herb is native to South America, particularly Southern Brazil, Eastern Argentina, and Uruguay. It grows naturally in the region's prairies, thriving in undisturbed areas without requiring specific soil conditions. Vernonia is also highly resistant to pests, contributing to its widespread growth (Alonso, 2024) (EXHIBIT 01).

  V. nudiflora taxonomic classification:

  Division: Spermatophyta
  Subdivision: angiospermae
  Class: Dicotyledoneae or Magnoliopsida
  Order: Asterals
  Family: Asteraceae
  Subfamily: Cichorioideae
  Tribe: Vernonieae
  Genders: Vernonanthura H.Rob.
  Species: Vernonanthura nudiflora (Less.) H. Rob

1.1.5 Part Used

- Dry and grinded leaves

1.1.6 Extract Type

- Standardized hydro alcoholic extract (mother tincture)

1.1.7 Identification Characteristics

- Physical Appearance: Dark brown liquid
- Odor: Characteristic of Vernonia
- Taste: Typical of Vernonia

1.1.8 Active Compounds

- Major previously described phytochemical constituents include sesquiterpene lactones, norisoprenoids, sesquiterpenoids, pentacyclic triterpenes, flavonoids, fatty acid derivatives, and caffeic acid esters such as rosmarinic acid (Alonso, 2024) (EXHIBIT 01).

1.1.9 Potential Adulterants

- Although no adulterants have been identified or found in the areas where vernonia is typically collected (Alonso, 2024) (EXHIBIT 01), it is important to note that the plant can occasionally be confused with other species in the Asteraceae family due to similarities in flower structure and leaf shape.

  Some potential species that may be mistaken for it include:

- Vernonanthura spp.: Other species in the same genus can have similar morphological traits.
- Eupatorium spp.: Certain members of this genus share similar flower characteristics and habitat preferences.
- Ageratum spp.: These plants can also have similar flowering patterns and foliage, especially in tropical regions.

  Identifying *V. nudiflora* accurately often requires close examination of specific features, such as leaf arrangement, flower structure, and habitat. Consulting local floras or using field guides can be helpful for distinguishing between these species.

1.2 Description of the evidence verifying the identity of the NDI

1.2.1 Sourcing and Botanical Authentication

- Source Documentation:

  The vernonia botanical material is collected by personnel from Osigma Cell & Genetics (OSG) from wild fields located on various ranches in northern Uruguay, specifically in the departments of Rivera and Tacuarembó. During the collection process, detailed records of the plant's origin, location, and date of harvest are maintained (EXHIBIT 02). Harvesting takes place between November and March, and proper identification requires close examination of key morphological features such as leaf arrangement, flower structure, and habitat. To confirm botanical identity, flowering material is collected and compared with voucher specimens held at the National Museum of Natural History and verified against regional floras.

Recently, OSG collected new plant material to establish a proprietary voucher specimen (number MVM 23518), which has been deposited in the aforementioned museum for future reference (EXHIBIT 03).

Identification includes:

- Morphological Analysis: Visual inspection of the morphological details confirms key characteristics, including size, color, and shape of different aerial parts of vernonia, consistent with *V. nudiflora*.

- Morphological and Microscopic Analysis: Visual and microscopic examination of the vernonia leaves and reproductive organs to ensure authenticity.

The following parameters are used for comparison to determine the identity of *V. nudiflora*:

a. Habit and General Morphology

Life form: Shrub or small tree (commonly up to 3–5 meters).
Branching pattern: Often with erect branches; glabrescent or slightly pubescent.

b. Leaves

Phyllotaxy: Alternate.
Leaf shape: Oblong to lanceolate.
Margins: Entire or slightly undulate.
Venation: Pinnate.
Surface texture: Varies from glabrous to slightly pubescent beneath.
Petiole: Usually short or subsessile.

c. Inflorescence and Flowers

Inflorescence type: Terminal corymbiform or paniculate clusters.
Capitula (flower heads): Small, numerous.
Involucral bracts: Several series, narrowly lanceolate, slightly imbricate.
Florets: All tubular and bisexual.
Corolla color: Typically purplish to lavender.
Style: Bifid (with two branches).
Anthers: Syngenesious (fused into a tube), with basal auricles.

d. Fruits and Seeds (when present)

Fruit type: Cypsela (a dry, one-seeded fruit typical of Asteraceae).
Cypsela surface: Often with longitudinal ribs, glabrous or shortly hairy.
Pappus: Present; bristly, whitish to brownish.

e. Micromorphological Traits

Trichomes: Type and distribution on leaves, stems, and involucre may help distinguish species.
Pollen morphology: Triporate or tricolporate, echinate (spiny); useful under SEM.
Leaf anatomical traits: Stomata type, presence of secretory structures.

Plant material is collected by OSG personnel and processed at the company's facility located at Camino a Fraile Muerto No. 101, Treinta y Tres. This facility is dedicated to the preparation of botanical material, including processes such as drying and grinding.

1.2.2 Standardization

- Active Ingredients: Rosmarinic acid: ≥ 0.008 mg/mL (assayed by high performance liquid chromatography method described in the United States Pharmacopeia Reference Standard - HPLC) (EXHIBIT 04 and 05).

1.3 NDI manufacture

Please note*: In a typical NDI notification, the description of the NDI's manufacture contains trade secrets (TS) and/or confidential commercial information (CCI). You may indicate to FDA your designation of information as TS or CCI in Section 2 of the NDI portal. You also may indicate in that section whether you are attaching a redacted copy of some or all of the notification. If you provide a redacted copy of the notification or a list of information that you believe to be TS or CCI, you should upload and attach it in Section 5 of the NDI portal.*

1.3.1 Raw materials

- Dried and grinded leaves of V. nudiflora

1.3.2 Formulation ingredients

- 70% ethanol

1.3.3 Manufacturing process

- **Drying:** Once the plants are collected in the field and transported to OSG's facility in Treinta y Tres (as described in Section 1.2.1), the leaves are separated from the rest of the plant. Wilted leaves are discarded, and the selected leaves are washed to remove any dirt or debris. Drying is carried out naturally, in the shade and under a roof, to prevent microbial growth and preserve the plant's active components. The leaves are spread in thin layers on racks and turned regularly to ensure even drying.

The drying process typically takes between 3 and 10 days, depending on the moisture content of the batch and prevailing weather conditions.

- **Grinding:** The grinding of the leaves is carried out using a mechanical mill adapted to obtain particles no larger than 4 cm in length, resulting in finely shredded plant material.

The manufacture of the mother tincture is outsourced to the Faculty of Chemistry of the University of La República, located near Montevideo, Uruguay (EXHIBIT 06), and analysis of the resulting product is performed at Laboratorio Industrial Montevideo (EXHIBIT 04 and 05) as described below:

- **Maceration:** The ground leaves are placed in stainless steel containers along with 70% ethanol. For every 200 g of leaves, 1000 ml of 70% ethanol are added. The mixture is left to macerate for 21 days at room temperature, with periodic manual agitation, to enhance the extraction of the components. Once the extraction is completed, the resulting hydro-alcoholic tincture is separated from the plant material using a metal or plastic strainer. The 70% ethanol used to manufacture the proposed NDI is obtained from diluting 95% NORTESUR Kosher-certified ethanol (EXHIBIT 07) with distilled water. We also include a technical sheet for the NORTESUR SA alcohol used in the process (EXHIBIT 08) together with a translation of the document (EXHIBIT 09). The use of the extraction protocol here described ensures that the extraction process will be optimized to minimize batch-to-batch inconsistencies.
- **Filtration:** The resulting tincture is initially filtered using a 300-micron filter, followed by a 100-micron filter. This process ensures a final product of excellent quality, guaranteeing its stability, as indicated by the International Pharmacopoeia. For both filtrations, a filtration unit is used, consisting of a stainless-steel container, a pump, and a piston-like container where the filters are placed.
- **Analysis:** As stated above, analysis of the resulting product is performed at Laboratorio Industrial Montevideo (EXHIBIT 04 and 05). During this analysis, the presence of rosmarinic acid was detected (unpublished data)
- **Flowchart:** Below is a graphical outline of the described procedure including collection, post-harvest management, and extraction.

The process herein described – plant collection, botanical material processing and extraction - have been verified to comply with Good Handling Practices applicable in this case, referring to identification, insect presence control, conservation, traceability, and packaging. The process is carried out under the supervision of the General Directorate of Agricultural Services (DGSA) through the Agricultural Protection Division, which certifies that Standard 3.7 is approved (EXHIBITS 10-13). This standard concerns harmonized phytosanitary requirements by risk category for the entry of regulated articles (Decree No. 204/022 of 06/24/2022, Article 2). According to this standard, CATEGORY 1 products are of plant origin and have been processed to the point that they are no longer capable of being infected/infested by quarantine pests. Therefore, products in this category do not require phytosanitary measures, and a phytosanitary certificate is not required regarding pests that may have been present in the products prior to processing. Among the Category 1 products are hydroalcoholic herbal extracts, such as *Vernonanthura nudiflora*.

The resulting mother tincture is then exported to the USA where the dietary supplement containing the NDI is manufactured by the Liquids Division of American Nutraceuticals Inc. The resulting dietary supplement will be distributed by OSG in the USA.

1.3.4 NDI specifications

To gather further information on the safety and security of the NDI, the following additional tests were performed:

- A microbiological study, to determine presence of pathogens. Tested parameters were:

  Appearance and colour
  Total Aerobic Count
  Total Yeast and Moulds
  Escherichia coli

Salmonella spp.
Pseudomonas aeruginosa.

All results were acceptable and are presented in the EXHIBIT 14.

- A heavy metals study. Tested parameters were:

  Lead (Pb): < 0.1 ppm
  Arsenic (As): < 0.1 ppm
  Cadmium (Cd): < 0.1 ppm
  Mercury (Hg): < 0.1 ppm

  All results were acceptable and are presented in EXHIBIT 14.

1.3.5 Methods of analysis

- **Analysis (HPLC Profile):** High-Performance Liquid Chromatography analysis demonstrates a distinct chromatographic profile with characteristic peaks corresponding to key active compound and marker rosmarinic acid (EXHIBIT 15 and 16), confirming previous findings (EXHIBIT 04 and 05).

1.3.6 Analysis of potentially toxic processes

- Heavy metals: STP LB705.
- USP 43 (61) (62)

  (EXHIBIT 14)

1.3.7 Disintegration and dissolution profile

- N/A

1.3.8 Shelf-life and conditions of storage

- Once filtered, the resulting mother tincture is placed in 25-litre opaque and hermetically sealed containers and kept at room temperature in cool and ventilated spaces, away from solar radiation. One of these containers is set aside for a quality hold, allowing for the recording of the manufacturing date and batch number, together with handling and processing conditions. Typically, alcohol-based tinctures can last anywhere from 5 to 10 years when manufactured and stored as described.

1.3.9 Detailed description of the identity specifications in table form

The vernonia mother tincture assay results included in the specifications is described below, ensuring it meets safety and quality standards for use in dietary supplements:

Certificate of Analysis

Product:	**Vernonanthura Nudiflora 240ml (8oz)**
Production Date:	**febrero-23**
Expiry Date:	**noviembre-28**
Capsule lot Number:	**008C**

Item	Specification	Results		Method
Appearance	Liquid	Liquid		Organoleptic
Color	Light Brown	Light Brown		Organoleptic
Odor	Charateristic	Charasteristic		Organoleptic
Taste	Charateristic	Charasteristic		Organoleptic
pH	<7.0	-		USP<791>
Assay (Rosmarinic Acid) mg/ml	≥0.00090	0.00093		HPLC
Heavy Metals(ppm)	NMT 10	<10	ppm	STPLB705
Lead - Pb (ppm)	NMT 0.5	0.00021	ppm	STPLB705
Arsenic - AS (ppm)	NMT 1.5	0.0001	ppm	STPLB705
Mercury -Hg (ppm)	NMT 0.1	0.001	ppm	STPLB705
Cadmium-Cd (ppm)	NMT 0.5	0.0001	ppm	STPLB705
Microbial Limits				
Total bacterial count (cfu/g)	NMT 1,000	<10		USP 43<61>
Mold & Yeast (cfu/g)	≤100	<10		USP 43<61>
E.Coli (MPN/100g)	Negative	Negative		USP 43<62>
Salmonella	Negative	Negative		USP 43<62>
Staphyloccus aureus	Negative	Negative		USP 43<62>
Pseudomonas aeruginosa	Negative	Negative		USP 43<62>

Packing and Storege Store in tighly closed container. Keep away from the light and the humidity.

Shelf Life

2. Dietary Supplement Manufacture (Recommended)

The final product is a dilution of the NDI, prepared at the following ratio: 24 grams of *Vernonia nudiflora* tincture (the NDI) are diluted with purified water to a final volume of 240 ml (8 ounces). The maximum ethanol content permitted in the dietary supplement is 6%. The manufacturing process is outlined below, with detailed information on aspects relevant to the product's safety and identity.

2.1. Raw materials

The *V. nudiflora* mother tincture, stored in 25-liter opaque and hermetically sealed containers (as described in 1.3.8).

2.2. Formulation ingredients other than the NDI

Purified water

2.3. Manufacturing process

The manufacturing process of the dietary supplement is performed in the Liquids Division of American Nutraceuticals Inc., following the instructions described below:

2.3.1 General instructions

a. The use of masks, hairnets, eye protection, gloves, protective clothing, and other safety equipment is mandatory during all operations.
b. Review the Master Formula, the Liquid Production Order, and the Manufacturing Instructions to verify the correct mixing of components. Note any special instructions.

2.3.2 Processing instructions

a. Have all product components in the Liquid Mixing Room.
b. Clean and disinfect the stainless-steel mixer, the agitator, and other required equipment with approved water and disinfectant.
c. Measure raw materials separately in individual containers and add them to the stainless-steel mixer as specified.
d. Active ingredients/preservatives may be weighed and provided by QC before mixing.

2.3.3 Mixing process

a. The Liquid Production Technician must verify the number of raw materials, the supplier, and the lot number of each raw material to be used for that order.

b. The Liquid Production Technician must record the lot number and the exact quantity of each raw material added. All work must be approved (initialled) on the Liquid Work Sheet.
c. Fill the liquid mixing tank as described in the formula sheet. Load all ingredients appropriately, as indicated.
d. Close the lid and mix for the specified time.
e. When the mixing time is reached, send a sample to Quality Control for approval.
f. When Quality Control has signed off on the mix based on pre-established specifications, proceed with bottling.

2.3.4 Bottling Process

a. The Supervisor collects the following information from the Order, the Bottle Weight Work Sheet, and the Liquid Specifications Work Sheet; and prepares the Liquid Work Sheet with the Product Name, Lot #, Packaging Type, Product Description, Total Bottles, Start Time, Production Date, Bottling Date, and Fill Volume Range.
b. The Supervisor calculates the packaging weight limits, then signs and dates the work sheet. The total number of bottles produced is recorded when the information is available. The liquid filling work sheet is handed over to the liquid filling technician.
c. If filling is done manually, clean the container with an approved water and disinfectant rinse.
d. Fill the bottles by weight, randomly checking the weight of the bottles and adjusting the fill volume to meet weight requirements if necessary. Record the weights on the Liquid Work Sheet every 15 minutes.
e. After bottling, ensure that the necks and sides of the bottles are clean.
f. All product must be bottled. If packaging is done simultaneously, then: cap, pass through the induction sealer, label, and then pass through the heat tunnel to apply the shrink wrap, according to specifications.
g. The automatic labeller will be set with the product lot number and expiration date, as approved by QC.

2.3.5 Quality Control Process

a. Continuously monitor the bottling process for dirty or damaged caps and bottles.
b. Confirm that the cap seals are secure and there are no leaking bottles.
c. Check the final product performance and record it.
d. The Supervisor collects the Work Sheets, which are then sent to QC for final approval. After Quality Control (QC) has approved the finished product, QC personnel will complete the approval form, conduct the product "inspection," and finally deliver the approval form to the QC Director.
e. Set aside and retain 2 samples. The QC Director will collect 2 sample bottles and record the same information on the bag. This reference sample will be placed in the sample storage area.
f. The QC Director will prepare an Analysis Certificate to describe the product's identity, content, lot numbers, control numbers, and manufacturing, testing, and product release dates. The Analysis Certificate will include:

    01. Product name.

02. Product code number.
03. Lot number.
04. Formula description.
05. Colour and appearance.
06. Manufacturing date.
07. Testing and/or release date, active ingredients, milligrams, inactive ingredients.
08. Testing methods used, acceptable limits, and test results (if applicable).
09. Recommended date for lot re-evaluation.
10. Signed and dated by authorized QC personnel.

2.3.6 Packaging

01. Package according to customer specifications.
02. Label the boxes with tags showing the product name, lot #, and quantity per box.
03. Seal the boxes with tamper-proof tape.
04. Deliver the boxed product ready for shipment.

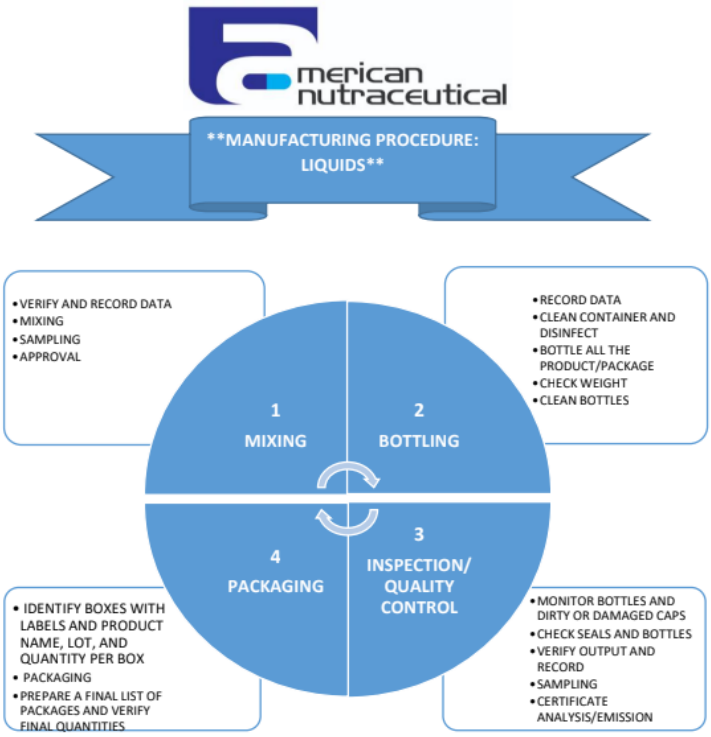
Prepared By: DM_____
Date: 11/20/2024_____

2.3.7 Labelling

Label attached as EXHIBIT 17. Recommended dose is 4ml, four times a day. Duration of the bottle, if used according to the recommended dose, is 30 days. Pregnant and breastfeeding women. Transplanted patients or patients taking any medication are advised to consult their doctor before use.

2.3.8 Flowchart

The summary of the process described is presented in the following figure:



2.4. Product specifications

2.4.1 Ingredient Name

- Vernonia Tincture (*Vernonanthura nudiflora*)

2.4.2 Identity

- Common Name: Vernonia
- Botanical Name: *Vernonanthura nudiflora*
- Part Used: Leaves
- Extract Type: Tincture

2.4.3 Composition

- Active Ingredients (marker): Rosmarinic acid: ≥ 0.0005 mg/mL (assayed by high performance liquid chromatography method described in United States Pharmacopeia Reference Standard - HPLC) (EXHIBITS 18 and 16)

2.4.4 Purity

- Heavy Metals:
    - Lead: < 0.1 ppm
    - Arsenic: < 0.1 ppm

- Cadmium: < 0.1 ppm
- Mercury: < 0.1 ppm

All results were acceptable and are presented in the EXHIBIT 19.

- Microbial Limits:
  - Total Aerobic Microbial Count: < 1000 CFU/g
  - Yeast and Mold: < 100 CFU/g
  - Pathogens: Negative for E. coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa.

All results were acceptable and are presented in the EXHIBIT 20.

2.4.5 Physical Characteristics

- Appearance: Light brown liquid.
- Odor: Characteristic of vernonia.
- Taste: Earthy and slightly bitter.

2.4.6 Storage Conditions

- Store in a cool, dry place, away from direct sunlight and moisture.

2.4.7 Shelf Life

- Results of accelerated stability study along with the manufacturing history of the product support a three-year expiration date, as described in EXHIBIT 20.

2.4.8 Detailed description of the identity specifications in table form

The vernonia supplement is standardized to contain a minimum of 0.0005 mg/mL rosmarinic acid, with assay results included in the specification described below, thus ensuring it meets safety and quality standards for use in dietary supplements.

merican nutraceutical

Certificate of Analysis

Product:	**Vernonanthura Nudiflora 240ml (8oz)**
Production Date:	**noviembre-23**
Expiry Date:	**noviembre-26**
Capsule lot Number:	**A14423**

Item	Specification	Results	
Appearance	Liquid	Liquid	
Color	Light Brown	Light Brown	
Odor	Charateristic	Charasteristic	
Taste	Charateristic	Charasteristic	
Foreign Particles	None Present	None Present	
pH	4.5-8.5	6.39	
Assay (Rosmarinic Acid) mg/ml	≥0.00045	0.00053	
Name	Label Claim per 2ml		
Vernonanthura Nudiflora 240ml (8oz)	200mg		
Heavy Metals(ppm)	NMT 10	<10	ppm
Lead - Pb (ppm)	NMT 0.5	0.0008	ppm
Arsenic - AS (ppm)	NMT 1.5	0.0013	ppm
Mercury -Hg (ppm)	NMT 0.1	0.019	ppm
Cadmium-Cd (ppm)	NMT 0.5	0.0001	ppm
Microbial Limits			
Total bacterial count (cfu/g)	NMT 1,000	<10	
Mold & Yeast (cfu/g)	≤100	<10	
E.Coli (MPN/100g)	Negative	Negative	
Salmonella	Negative	Negative	
Staphyloccus aureus	Negative	Negative	
Pseudomonas aeruginosa	Negative	Negative	

Packing and Storege Store in tighly closed container. Keep away from the light and the humidity.

Shelf Life 3 years if sealed and store away from direct sun light.

Conclusion: The specification conformed with the enterprise standart.

2.5. Methods of analysis

2.5.1 Identity

- **Analysis (HPLC Profile):** High-Performance Liquid Chromatography analysis demonstrates a distinct chromatographic profile with characteristic peaks corresponding to key phytochemical marker rosmarinic acid (EXHIBIT 18 and 16)

2.5.2 Composition

- As described in 2.5.1.

2.5.3 Purity

- Heavy metals: STP LB705.

- USP 43 (61) (62)

  (EXHIBIT 19)

2.5.4 Physical Characteristics

- Sensory Evaluation: For appearance, odor, and taste.

2.5.5 Storage Conditions and Shelf Life

- Accelerated stability studies were conducted to evaluate shelf life under various conditions, as described in EXHIBIT 20.

Using these methods will help ensure that the vernonia supplement meets its specified quality and safety standards.

2.6. Analysis of potentially toxic processes

When analyzing a botanical dietary supplement like vernonia extract, several potentially toxic processes or concerns can arise. Here are some key considerations:

2.6.1 Heavy Metal Contamination

- Soil and Water Contamination: Vernonia may absorb heavy metals from contaminated soil or water sources, leading to unsafe levels of lead, arsenic, cadmium, or mercury in the final product.

- Processing Equipment: Use of non-food-grade equipment can introduce harmful metals into the extract.

  Risk mitigation strategy: Botanical material is sourced in farmlands and wild areas, where no industrial activities have been identified. The potential content of heavy metals is regularly analysed in the raw material (mother tincture) and finished product, as described above.

2.6.2 Microbial Contamination

- Improper Harvesting and Handling: Poor hygiene practices during harvesting, processing, or storage can result in contamination by pathogenic bacteria or spoilage organisms.

- Natural Microflora: Some botanical materials may harbor harmful microbes, necessitating rigorous testing.

  Risk mitigation strategy: In order to minimize the potential impact of microbial contamination, the collection and processing of the botanical material of vernonia

follows the "WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants" published by WHO (2003). The potential presence of microbial contamination is regularly analyzed in the raw material (mother tincture) and finished product, as described above.

2.6.3 Adulteration

- Intentional or Unintentional Adulteration: The addition of cheaper or potentially harmful substances can occur, leading to toxicity or reduced efficacy.

- Misidentification: The use of incorrect plant parts or other botanicals that may have harmful effects.

  Risk mitigation strategy: As stated in 1.2.2, plant material is compared to voucher specimen MVN 23518 deposited at the herbarium of the National Museum of Natural History of Uruguay (EXHIBIT 03). As stated in 1.1.9. no potential adulterants have been identified or found growing around the collection area.

2.6.4 Storage Conditions

- Decomposition or Deterioration: Improper storage can lead to the breakdown of active compounds, potentially generating harmful byproducts.

- Mould Growth: Inadequate drying or humidity control can result in mould contamination.

  Risk mitigation strategy: Storage conditions are those described in GMP manufacture procedures for dietary supplements. As described in EXHIBIT 20, results of accelerated stability study along with the manufacturing history of the product do support a three-year expiration date.

  At all stages of the process, stringent quality control measures are implemented for sourcing and processing, and suppliers are verified to adhere to good agricultural and manufacturing practices. By addressing these potential toxic processes, we ensure the safety and quality of the vernonia extract dietary supplement.

2.7. Disintegration and dissolution profile

N/A

3. History Of Use Or Other Evidence Of Safety (Required)

3.1. History of use

3.1.1 Description of the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

- Vernonia (*V. nudiflora*) has been used for centuries as a medicinal herb by both native and creole communities in Uruguay, Southern Brazil, and Eastern Argentina. The aerial parts are traditionally prepared as an oral infusion, decoction or tincture, primarily for the prevention and treatment of flu, bronchitis, and other respiratory conditions. (Anonymous, 2020; Braga et al, 2007; Castro et al, 2024; Leitão et al, 2014) (EXHIBITS 21 -24).

  In traditional use, the most common preparation of *Vernonia* involves making an infusion or decoction of its leaves:

  Infusion (milder use)
  Parts used: leaves (fresh or dried)
  Preparation: Place approximately 1 to 2 tablespoons of dried leaves (or a handful if fresh) in 1 liter of hot water. Let it steep, covered, for 10 to 15 minutes. Strain and drink warm.
  Traditional dosage: 1 cup, 2 to 3 times a day.

  Decoction (more concentrated use)
  Boil the leaves (generally 20–30 g per liter) for 5 to 10 minutes.
  Then let it steep, covered, for another 10 minutes and strain.

  Tincture
  1:2 ratio (1-part fresh plant to 2 parts alcohol 95% by weight/volume).

- A dietary supplement containing vernonia hydroalcoholic tincture and standardized in rosmarinic acid aims to harness the health benefits of the product used traditionally in a standardized, and therefore safer and more easily consumable format.

- The relationship between *V. nudiflora* and the vernonia supplement is rooted in the historical consumption of vernonia as a medicinal herb. While vernonia provides a modest amount of rosmarinic acid and other active ingredients when used in infusions or other traditional preparations, the NDI offers a standardized dose, making it easier for consumers to achieve therapeutic levels for health benefits.

- This evolution from traditional use to modern supplementation reflects a broader trend of integrating historical knowledge into contemporary health practices, bridging cultural heritage with scientific innovation.

3.1.2 Describe identity information verifying the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

- New Dietary Ingredient (NDI): A concentrated hydroethanolic extract (mother tincture) of the leaves of *V. nudiflora*, standardized to contain a specific percentage of rosmarinic acid.

  The following identity information demonstrates how the NDI—a hydroalcoholic tincture of vernonia leaves used as a diluted dietary supplement—is directly connected to the historically consumed vernonia plant, while also providing evidence of its traditional use, active compounds, and regulatory compliance.

- Identity Information:

  a. Botanical Identification:
  - Scientific Name: *V. nudiflora*
  - Common Names: Vernonia, Alecrim de Campo.

  b. Historical Use:

  Documented use in South American traditional medicine, mainly in the prevention and treatment of respiratory diseases and flu (Braga et al, 2007; Castro et al, 2024; Leitão et al, 2014) (EXHIBITS 22-24).

  c. Active Compounds:

  The plant contains sesquiterpene lactones, norisoprenoids, sesquiterpenoids, pentacyclic triterpenes, flavonoids, fatty acid derivatives, and caffeic acid esters such as rosmarinic acid, the later used as a marker.

  d. Supplement Composition:

  A dilution of the NDI in the following ratio: 24 g of *V. nudiflora* tincture (NDI) are diluted with purified water to a final volume of 240 ml (8 ounces).

  e. Regulatory Documentation:

  Safety studies in compliance with FDA regulations for NDIs, including evidence of safety and history of consumption.

3.1.3 Historical conditions of the use and cumulative exposure estimate for the historically consumed material

Historical Conditions of Use:

- As described in Sections 3.1.1 and 3.1.2, in traditional South American medicine, vernonia tea and tincture have been used for their health benefits, at least since the arrival of

European colonization in the 14th century. Traditionally, it is mostly prescribed for ailments such as flu and respiratory diseases.

Cumulative Exposure Estimate:

- N/A

3.1.4 Adverse events associated with historically consumed material

- According to a report by the Uruguayan Chamber of Phytomedicines, Natural Products, and Related Products, the rural population of Uruguay has traditionally used this plant as an infusion with no recorded adverse effects. Additionally, there have been no reports of toxicity in grazing animals (Ortas, 2023) (EXHIBIT 25).

- The Toxicology Laboratory under the Ministry of Livestock, Agriculture, and Fisheries of Uruguay—an official institution with responsibilities similar to those of the USDA in the USA—also certifies that there are no records of health issues in the country caused by the consumption of *V nudiflora*. (Collazo, 2024) (EXHIBIT 26).

- In Argentina, *V. nudiflora* is not included in the negative list of plant drugs (Provision 1788/2000 by ANMAT) that are excluded as constituents of phytotherapeutic medicines due to evidence of toxicity. (Anonymous, 2024) (EXHIBIT 27)

3.1.5 Alternative rationale for reasonable expectation of safety based on history of use

N/A

3.2. Other evidence of safety

3.2.1 Safety study type

Acute Systemic Toxicity via Oral Gavage in CD-1 Mice, Repeat Dose

3.2.2 Safety study title, if any

Hydroethanolic extract of Vernonanthura nudiflora - Lot Number 008

3.2.3 Citation for the safety study (either public or non-public), if any

Beraki, S (2023) (EXHIBIT 28)

3.2.4 Identity information verifying the relationship between the test article and the NDI or the dietary supplement

The test article was "Hydroethanolic extract of *Vernonanthura nudiflora* (NDI)". The test article was prepared per Sponsor instructions. The test article is a diluted plan extraction in liquid form and was tested as received. Physiological saline was used as a control.

3.2.5 Route of administration, serving size, frequency of use, interval between servings, and duration of use of the test article.

The test article was administered orally for a period of 14 days. Each animal was administered with 0.1 ml (approximately 5 ml/kg as per average mice weight) of the test article or control. The doses were adjusted based on last obtained body weight. The doses were rounded to the nearest 0.1 ml. Frequency of use, intervals between servings, and duration of use are described in the following section.

3.2.6 Study design and safety metrics

The study design is presented in Table 1. A total of twenty albino mice (ten male and ten female; Five males and five females for test article and five males and five females for corresponding control) were used. Animals were randomly assigned to test article or control group. Each animal was administered with 0.1 ml (approximately 5 ml/kg as per average mice weight) of the test article or control according to Table 1. The doses were adjusted based on last obtained body weight. The doses were rounded to the nearest 0.1 ml.

Table 1. Study Design

Group	Sex	Number of Animals (n)		Route of Administration	Dose volume	Observations	Body Weights	Gross Necropsy
		Test	Control					
1	M	5	5	Oral Gavage	0.1 ml	Immediately; 4 hours ± 15 minutes after dosing; daily	Prior to Dose 1, Dose 7, and Dose 14	Yes
2	F	5	5	Oral Gavage	0.1 ml	Immediately; 4 hours ± 15 minutes after dosing; daily	Prior to Dose 1, Dose 7, and Dose 14	Yes

3.2.7 Discussion of toxicity and conclusions

All test article and control group animals (male and female) survived the test period. None of the test group animals exhibited any biological reactivity at any of the specified time points during the fourteen-day observation period. All animals from the test group and control group (male and female) gained weight at the end of the test period. No

abnormalities were noted at gross necropsy. Conclusion: This test was conducted according to ISO 10993-11. All animals appeared healthy during the course of the study and gained weight at the end of the test. No biologically significant differences were noted between the test and control animals. No acute systemic toxicity was observed in tested animals. The test article met the requirements for the Acute Systemic Toxicity test using conditions listed in this report.

A 14-day repeat-dose oral toxicity study was conducted in mice to assess the preliminary safety of the dietary supplement. Although classified as an acute study, the once-daily administration over a two-week period of a dose of at least 30x the dose proposed for the dietary supplement provided relevant insight into the tolerability of the ingredient under conditions approximating intended daily use in humans. No mortality, clinical signs of toxicity, or adverse behavioral effects were observed during the dosing period, and there was no evidence of cumulative or delayed toxicity. While the study duration is shorter than the 90-day repeated-dose studies typically used to assess subchronic toxicity, the absence of adverse effects supports a reasonable expectation of safety. These data, taken in context with the proposed conditions of use and the traditional use of the plant, contribute to the overall safety profile of the ingredient and support its safety in the proposed usage. The repeat-dose design offers evidence that the ingredient is well tolerated with daily oral exposure. The absence of adverse effects at the tested dose supports a reasonable initial expectation of safety.

3.2.8 Alternative rationale for reasonable expectation of safety based on other evidence of safety

N/A

4 Basis For Concluding That the New Dietary Ingredient Will Reasonably Be Expected To Be Safe For Use in the Dietary Supplement (Required)

(You must either provide the information requested in Subsections 4.1 to 4.6, when applicable, or explain in Subsection 4.7 your alternative rationale for concluding, based on the totality of the scientific evidence, that the NDI will reasonably be expected to be safe under its proposed conditions of use in the dietary supplement.)

4.1 Determination of the No-Observed-Adverse-Effect-Level (NOAEL) or Lowest-Observed Adverse Effect Level (LOAEL)

N/A

4.2 Determination of safety factor

N/A

4.3 Determination of the Acceptable Daily Intake (ADI)

N/A

4.4 Determination of Estimated Daily Intake (EDI) and the EDI/ADI Ratio

N/A

4.5 Determination of margin of safety

N/A

4.6 Safety narrative and conclusion

N/A

4.7 Alternative basis for reasonable expectation of safety

Conclusions of the information presented in the present document:

- Scientific literature notes that *V. nudiflora* tea, decoction, and tincture have been used in South American traditional medicine since at least the 14th century, primarily for flu and respiratory illnesses, with no reported toxicity during this period.

- A report from the "Uruguayan Chamber of Phytomedicines, Natural Products, and Related Products" states that the rural population of Uruguay has been using this plant as an infusion without any documented adverse effects from this practice. Additionally, there have been no reports of toxicity in grazing animals.

- The Toxicology Laboratory, part of Uruguay's Ministry of Livestock, Agriculture, and Fisheries—an official institution with functions like the USDA in the USA—further confirms that there is no documented health issues in the country associated with the consumption of *V. nudiflora*.

- In Argentina, *V. nudiflora* is not included on the negative list of plant drugs (Provision 1788/2000 of ANMAT) that are not allowed as constituents of Phytotherapeutic Medicines due to evidence of toxicity.

- In an acute toxicity study of the NDI, the hydroethanolic extract of *V. nudiflora* (70/30 ethanol/water ratio) given orally in repeated doses by gastric tube (at a dose of over 30 times higher than the recommended dose), was tested in male and female albino rats (n = 10, active group) and compared to a control group (n = 10). In both groups, half were female rodents, and the other half were male rodents. Both the animals in the active group and those in the control group (males and females) survived the test period (14 days), with no toxic effects observed after macroscopic necropsy, and no biologically significant differences were found in both groups of animals. Thus, the extract complied with ISO 10993-11.

Considering the comprehensive scientific evidence presented, along with the fact that the proposed dietary supplement is a diluted form of the mother tincture (NDI) traditionally used in medicine—and that this NDI has been evaluated in an acute toxicity assay at a dose at least 30 times higher than that used traditionally—it can be concluded that the supplement is expected to be safe under the proposed manufacturing and usage conditions.

5 Reference List (Required)

List of attached documents:

- Alonso J. Vernonanthura nudiflora MONOGRAPH (2024) (EXHIBIT 01)
- Internal Field Report (EXHIBIT 02)

- Voucher of botanical source material (2025) (EXHIBIT 03)

- COA for rosmarinic acid in mother tincture (EXHIBIT 04)
- COA for rosmarinic acid in mother tincture (translated) (EXHIBIT 05)
- Tincture manufacture (Technical Report) (EXHIBIT 06)
- Kosher certificate for solvent manufacturer (EXHIBIT 07)
- Solvent certificate (EXHIBIT 08)
- Solvent certificate (translated) (EXHIBIT 09)
- Plant material handling (EXHIBIT 10)
- Plant material handling (translated) (EXHIBIT 11)
- Phytosanitary certificate (EXHIBIT 12)
- Phytosanitary certificate (translated) (EXHIBIT 13)
- Microbiological and heavy metals analysis for mother tincture (EXHIBIT 14)
- COA rosmarinic acid (EXHIBIT 15)
- COA rosmarinic acid (lab write and calculations) (EXHIBIT 16)
- Label (EXHIBIT 17)
- COA for rosmarinic acid in finished product (EXHIBIT 18)
- Heavy metals in finished product (EXHIBIT 19)
- Microbiology and accelerated stability in finished product (EXHIBIT 20)
- Anonymous. Projeto Flora do Brasil 2020. v393.147. Instituto de Pesquisas Jardim Botanico do Rio de Janeiro. Dataset/Checklist. doi:10.15468/1mtkaw (2020). (EXHIBIT 21). Flora e Funga do Brasil - Lista Oficial (Not available in PDF).
- Braga F, Bouzada M, Fabri R, de O Matos M, Moreira F, Scio E, Coimbra E. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. J Ethnopharmacol. 2007; 111(2):396-402. (EXHIBIT 22). Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil - ScienceDirect (Not available in PDF).
- Castro M, Monge M, Soares P, Rivera V, Dematteis M, Semir J (in memoriam) *Vernonanthura in* Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro. Available

at: <https://floradobrasil.jbrj.gov.br/FB105098>. Accessed on: 25 Dec. 2024. (EXHIBIT 23)

- Leitão F, Leitão S. G, Fonseca-Kruel V, Silva I, Martins K. Medicinal plants traded in the open-air markets in the State of Rio de Janeiro, Brazil: An overview on their botanical diversity and toxicological potential. Revista Brasileira de Farmacognosia 2014; 24(2), 225-247. (EXHIBIT 24)

- Ortas J. Dictamen sobre Vernonanthura nudiflora. Cámara Uruguaya de Fitomedicamentos, Productos Naturales y Afines. Montevideo, Uruguay. 15 de noviembre de 2023. (EXHIBIT 25)

- Collazo S. Dictamen sobre Vernonanthura nudiflora. Ministerio de Ganadería, Agricultura y Pesca de Uruguay. División de Laboratorios Veterinarios. 9 de abril de 2024. (EXHIBIT 26)

- Anonymous. ANMAT (Administración Nacional de Medicamentos, Alimentos y Tecnología Médica de Argentina). Disposición 1788/2000. Especialidades Medicinales. Medicamentos Fitoterápicos. .https://www.argentina.gob.ar/normativa/nacional/disposici%C3%B3n-1788-2000-62629/texto (Translation of the document provided as EXHIBIT 27)

- Beraki S. Acute Systemic Toxicity via Oral gavage in CD-1 Mice, Repeat Dose. Hydroethanolic extract of Vernonanthura nudiflora. Lot Number: 008. Pacific Biolabs. USA. 2023. Pag. 2-37. June 30 (2023). (EXHIBIT 28)

6 Comments

(You have the option to provide any additional information about the NDI or the dietary supplement that you believe will assist FDA in processing your notification.)