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June 29, 2009

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Dear Dr. Martin:

On behalf of Natreon, Inc. of New Brunswick, NJ, we are submitting for FDA review a GRAS notification for *Emblica Offinalis* Extract. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

Robert S. McQuate, Ph.D.
CEO & Co-Founder
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Enclosure: GRAS Notification – *Emblica officinalis* Extract (in triplicate)

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GRAS ASSESSMENT

***EMBLICA OFFICINALIS* EXTRACT - CAPROS®**
Food Usage Conditions for General Recognition of Safety

for

NATREON, INC.
New Brunswick, NJ

Evaluation by GRAS ASSOCIATES, LLC

Robert S. McQuate, Ph.D.
Richard C. Kraska, Ph.D., DABT
Wayne R. Bidlack, Ph.D.

June 29, 2009

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

CAPROS®, a purified and standardized extract from the ripened fruit of *Emblica officinalis* or amla, as described more fully below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by experts qualified by scientific training and experience; it is based primarily on common use in foods prior to 1958; and the evaluation accurately reflects the conditions of the intended uses of CAPROS® in foods.

Signed:

June 29, 2009

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

Date

B. Name & Address of Notifier

Natreon, Inc.
2D Janine Place
New Brunswick, NJ 08901

As the notifier, Natreon, Inc. accepts responsibility for the GRAS determination that has been made for CAPROS® and as described in the subject notification; consequently, CAPROS®, which meets the conditions described herein, is exempt from pre-market approval requirements for food ingredients.

C. Common Name & Identity of the Notified Substance

CAPROS® is the purified and standardized extract from the ripened fruit of *Emblica officinalis* (or amla, *Phyllanthus emblica*, or Indian gooseberry); the fruit from which the notified substance is derived is also known by several other names as described in Section II.B.

D. Conditions of Intended Use in Food

The standardized extract preparation from amla is intended to be added as a nutrient supplement into selected food categories at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect. Natreon anticipates that the per serving levels of CAPROS® will be 100 - 150 mg.

[1] See Federal Register 62 April 17, 1997, 18937; or http://www.cfsan.fda.gov/~lrd/fr970417.html.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, the standardized amla extract amla has been determined to be GRAS primarily on the basis of common use in foods prior to 1958 as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. DESCRIPTION OF *EMBLICA OFFICINALIS* EXTRACT

A. Background

Ayurveda, the traditional Indian health care system, is alleged to be the oldest medical system in the world (Scartezzini; Speroni, 2000). Selected references note the use of ayurvedic formulations to the 4th century BC (Sandasivan, 2006) and others date back to 2000 BC (Ojha, 2003).

Ojha provides useful background information on Ayurvedic practices (Ojha, 2003). Rasayanas, or vitalizers, are considered to be a specialized component within Ayurveda with the intent that rasayanas help prolong life, prevent aging and diseases, and eliminate or minimize degenerative processes. In short, rasayanas facilitate one's living a long and productive life. Rasayanas are also thought to counter adverse effects of xenobiotics within one's system as well as countering adverse effects of certain foods such as fats and oils (Chaudhuri, 2005).

Ayurveda recognizes the nutritional elements derived from foods, and rasayanas help optimize the availability of "essential nutrients" in the body. Rasayanas are thought to regulate metabolic processes, and this involves dietary, drug, and behavioral modalities. In so doing, rasayanas enhance and preserve vigor while both preventing and alleviating diseases.

Table 1 summarizes the expected health advantages of rasayana therapy. Ayurveda relies extensively on the use of medicinal plants to impart well-being to those seeking its benefits. Most rasayana formulations contain *Emblica officinalis* or amla.[2]

Table 1. Expected Health Advantages of Rasayana Therapy

Promotes longevity and prevents maladies of old age
Helps to attain a state of enhanced well-being, such as improving memory and intellect
Hastens recovery from disease

Emblica officinalis and extracts from the ripened fruit, leaves, stem, roots, flowers and seeds of *Emblica officinalis* have been consumed as part of Ayurvedic practices in India and throughout the Indian subcontinent and south Asia for about 3000-4000 years (see Section III.A).

Emblica officinalis has long been and remains enormously popular in India, and the extract is consumed as a nutraceutical in various foods; two rasayana products, chyawanprash and triphala, contain amla as the predominant ingredient (Dharmananda, 2006b). Not only is *Emblica officinalis* broadly consumed in India, but it has been noted to be used in over 20 countries of the world for health benefits (Ghosal, et al, 2002). It has been reported that over 50% of the total sales of ayurvedic products in India is chyawanprash and that the advantages to be derived from consumption of this product are commonly recognized among the Indian population. Virtually all producers of Ayurvedic-type formulations in India include chyawanprash among their offerings (Dharmananda, 2006a). Furthermore, individuals over the

[2] The names, "Emblica," "*Emblica officinalis*," "*E. officinalis*," "amla," Indian gooseberry, and *Phyllanthus emblica* will be used interchangeably in this evaluation. This material is also known by other names as seen in Section II.B.

age of 40 in India are said to consume 1 to 3 teaspoons of chyawanprash (or 10 g to 30 g) per day directly, as a bread spread, or mixed in warm milk (Dharmananda, 2006a; Anonymous, 2006a).

Emblica officinalis is a small- to medium-sized tree with greenish-gray or red bark, growing to a height of about 5.5 m. It produces a small, fleshy fruit weighing about 20 – 25 grams each. The species is native to India and grows in Pakistan, Uzbekistan, Sri Lanka, Bangladesh, southeast Asia, China, and Malaysia (Scartezzini andSperoni, 2000; Bhattacharya, et al., 1999). Amla is not palatable for direct consumption because of its high acidity and astringent taste. Because of its excellent nutritive content, it is incorporated into other food products (Pathak, 2002); amla is used extensively in pickles, chutneys, jams, jellies, preserves, baked in tarts, and used in vinegar and juices (Parmar and Kaushal, 1982, Anonymous, 2006a; Pathak, 2002).

The importance of amla is well recognized for both economic considerations and the health-imparting characteristics already noted. Furthermore, its widespread popularity across the warmer climates of Asia has resulted in *E. officinalis* being cultivated as an orchard crop in India, especially in the warmer, semiarid coastal locations in India, despite its propensity to grow wild. With orchard cultivation, about 200 trees can be accommodated per acre, and trees bear fruit for up to 65 to 70 years. India exports 5,000 to 10,000 kg of fruit per month to Singapore, Malaysia, and Europe (Pathak, 2002). A great deal of investigation has gone into understanding the optimal growing conditions, handling, storage, processing, and packaging conditions to obtain maximum value (Pathak, 2002).

The older literature extolled the health advantages of amla by citing the particularly high vitamin C content, noting that only one other natural botanical, Barbados cherry, had a higher vitamin C content on a per weight basis (Parmar and Kaushal, 1982; Pathak, 2002). It was not until 1996 when it was reported by Ghosal that amla was composed extensively of polyphenols and tannins (Pathak, 2002; Ghosal, 1996). The antioxidant capacity of amla is well documented and has been demonstrated to be due to the presence of a mixture of low molecular weight, hydrolysable tannins derived from gallic and ellagic acids (Pathak, 2002; Bhattacharya, et al., 1999). Also, see Section II.C. In fact, the antioxidant fractions of this fruit, with their ability to scavenge free radicals, are more stable than L-ascorbic acid and some of its derivatives (Ghosal, 2000).

The reported uses of *Emblica officinalis* are many and varied and are broadly cited in numerous references. Therapeutic value is claimed, as well as preventative and restorative health aspects (Ojha, 2003). Many reported health advantages are summarized in Table 2. As a rasayana, there is particular focus on the role that amla plays in alleviating disorders of aging and nutritional deficits (Dharmananda, 2006a). It imparts a rejuvenating quality to the body, and it repairs wear and tear due to aging and diseases. Amla is reported to replenish the body's vitality and to enhance longevity.

It is particularly noteworthy that amla is reported as an essential component in rasayanas such as triphala and chyawanprash to assist the body attain a state of improved nutrition that prevents aging and enhances longevity.

Clearly, amla has been and remains a product of substantial interest within India and south Asia.

The historical usage of amla and the present day reliance on this extract are reinforced by actions of the Indian government. Through the India Planning Commission, the Government of India underscored its

Table 2. Claimed Health Advantages Ascribed to Fruit of *Emblica Officinalis* Separately or as a Substantial Component of Chyawanprash or Triphala

Serve as anti-aging agent (a), (g)	Treatment for disorders of digestive system (a), (c), (k), (l),
Promote strength & vigor; rejuvenator (a)	Effective antiseptic (a)
Treatment of jaundice (a)	Palliative for snake bites & scorpion stings (a)
Serve as energy booster (b)	Effective antioxidant (c), (i), (j)
Inhibits tumor growth (c)	Cytoprotective properties (k)
Serves to alleviate cough (d), (f), (l)	Helps hair growth (e), (l), & (m)
Exhibits adaptogenic properties (k)	Improves skin complexion (e)
Promotes healing (d), (g)	Treatment for metabolic disorders---anemia, gout, & diabetes (c), (l)
Enhances fertility & increase sexual vigor (e), (f)	Treat urinary disorders (f)
Treatment for several bleeding disorders (l)	Antibacterial & antiviral agent (g), (j)
Immune system enhancer (e), (i), (j)	Improves liver function (c), (h),
Cognition and memory enhancer (b), (k)	Treatment for pulmonary and renal tuberculosis (f)
Treatment for asthma (d), (l)	Laxative/relieves constipation (g)
Treatment for bronchitis (a)	Improves digestion, assimilation & metabolism (e), (f), (h)
Reduces blood pressure (h)	Improves circulation & reduces serum cholesterol (h)

(a) Parmar and Kaushal, 1982; (b) Jayarajan, 2006; (c) Ghosal, et al., 2002; (d) Dharmanada, 2006a; e) Sadasivan, 2006; (f) Ojha, 2003; (g) Anonymous, 2006b; (h) Anonymous, 2006c; (i) Dabur, 2006; (j) Scartezzini & Speroni, 2000; (k) Bhattacharya, et al., 1999; (l) Dharmananda, 2006b; (m) Pathak, 2002.

wish to continue the utilization of health-imparting plants to support, in particular, the well-being of people in the rural areas of India. The demand for amla was reported for 2004-2005 to be 41,783 tons, which represents a 22.5% annual growth rate. This places amla as the number one medicinal plant in India based on demand calculations (Government of India, 2003; Brinkmann, 2003).

The Government of India seeks sustainable, managed growth and harvesting of medicinal plants. A Task Force was established to achieve this and other goals, and amla was explicitly targeted as one of the plants of interest (Government of India, 2000). This same Task Force reported that five major Indian pharmacies[3] required 115.6 tons per year of amla to supply their markets alone.

In 2003, India's National Medicinal Plants Board listed its 32 most important medical plants, and amla has been estimated to experience the highest projected tonnage with a 9.1% growth rate for 2004-2005. Further underscoring the interest placed by the Government of India[4] in greater utilization of amla is the extensive, detailed support and educational assistance available to those wishing to cultivate the shrub (Government of India, 2003; Brinkmann, 2003).

Not only has *Emblica officinalis* had a sustained and substantial history of consumption but it also remains as a highly valued article of commerce today within India and related geographies.

[3] Sandu Brothers, Natural Remedies (Bangalore), Ajanta Pharma, Shree Dhotpapeswar (Mumbai), and Gufic Healthcare (Mumbai).

[4] Along with the continuing interests in the health implications tied to the availability of amla, it is also recognized that the Government of India is interested in the economic advantages associated with its cultivation and exportation.

B. Chemical Name & Common or Usual Name

The material that is the subject of this GRAS notification is the standardized water extract of *Emblica officinalis* which is also commonly referred to as *Phyllanthus emblica* or amla. The fruit of *Phyllanthus emblica Linn* has the botanical name *Emblica officinalis Gaertn.* Emblica is a member of the family Euphorbiaceae. It has a number of synonyms, depending of the geographic location where found and used.

English names include: Emblica, myrobalan-tree, and Indian gooseberry.

Indian names with regional associates include: amalkamu and uririkai (Andhra Pradesh); amlaki and amluki (Assam); amla and amlaki (Bengal); amali and ambala (Gurjat); amla and aonla (Himachal Pradesh); amla, aonla, and onilika (Hindi); amalaka and nelli (Karnatka); nelli (Kerala); alathanda, khondona, and anola (Orissa); aonla (Punjab); adiphala, dhatri, amalaka, shriphala, and vrittophala (Sanskrit); nelli (Tamilnadu) (Parmar and Kaushal, 1982), and embelgi (Arab) (Scartezzini and Speroni, 2000).

The Indian Council of Agricultural Research also includes aawallaa, aamalaki, lellikai, nilllika, aamvala, and usareekai as additional synonyms (Pathak, 2002)

The water extract of the *E. officinalis* fruit that is subsequently purified in accordance with procedures developed by Natreon (see Section II.E) has the trade name of CAPROS®.

C. Chemical Composition

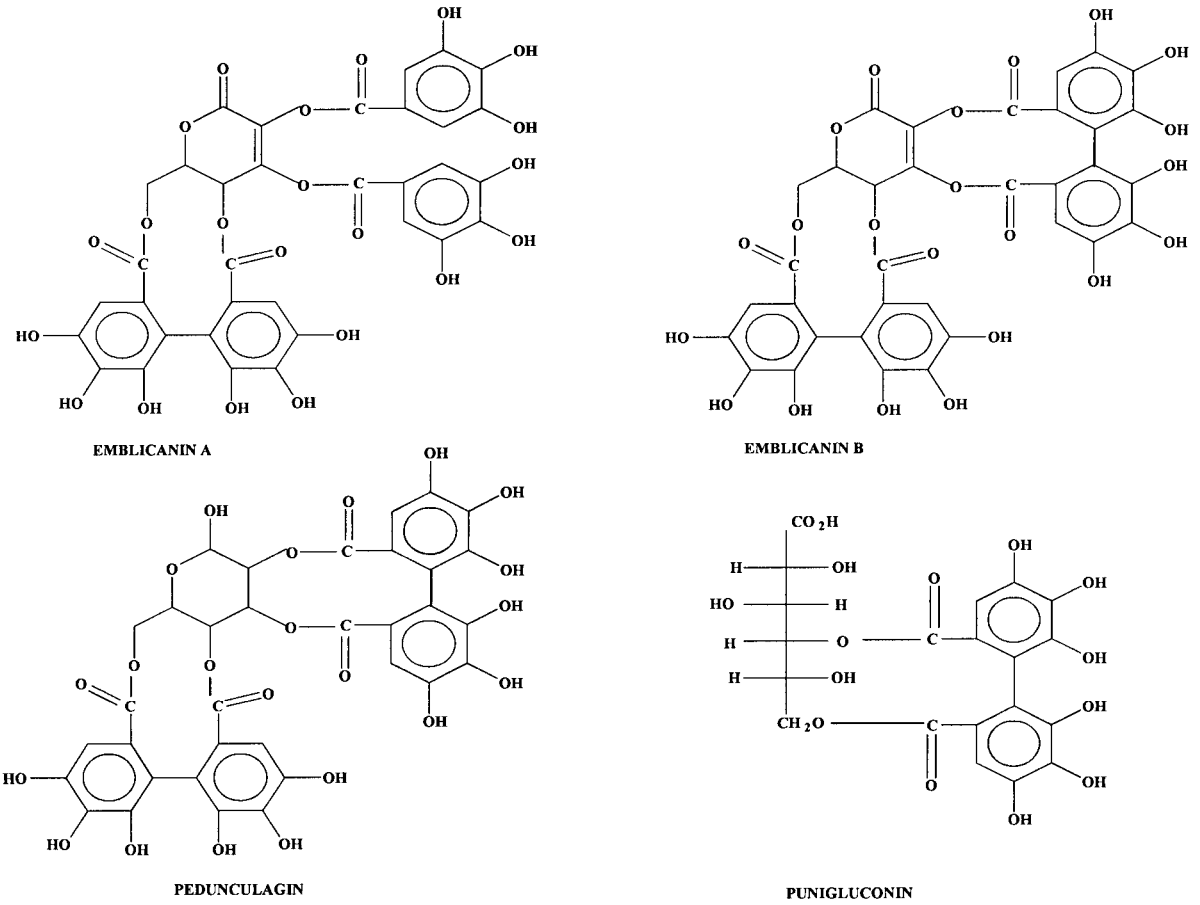
The extensive history of consumption of amla and the associated health-imparting character and associated physical well-being has prompted substantial interest in the chemical characterization of the fruit and its extract (Bhattacharya, et al., 1999; Pathak, 2002). According to Bhattacharya, et al., research performed during the second and third quarters of the 1900s yielded the conclusion that the biological and therapeutic effects of amla fruits, including the well-recognized antioxidant clinical effects, were a consequence of the high vitamin C content. It was also thought that the presence of hydrolysable tannins---with the gallic and ellagic acids---inhibited the degradation/oxidation of the vitamin C (Bhattacharya, et al, 1999).

This long-standing belief was determined to be incorrect with the published findings in 1996 by Ghosal and coworkers who undertook a comprehensive chromatographic and spectroscopic analysis with clever chemical transformations of the chemical constituents (Ghosal, et al., 1996). Neither free nor conjugated vitamin C was detected. At that time, it was established that the profound antioxidant activity of amla and its extract was due to the presence of a mixture of low molecular weight hydrolysable tannins that are derived from gallic and ellagic acids (Chaudhuri, 2005; Dharmananda, 2006b; Ghosal, et al., 2002).

The chemical structures are shown in Figure 1. The major bioactive constituents[5] and relative amounts are summarized in Table 3.

[5] The chemical names for these tannins are: emblicanin A = (2,3-di-o-galloyl-4,6-(S)-hexahydrodiphenoyl-2-keto-glucono-δ-lactone); emblicanin B = (2,3,4,6-bis-(S)-hexahydroxydiphenoyl-2-keto- glucono-δ-lactone); punigluconin = (2,3-di-o-galloyl-4,6-(S)-hexahydroxydiphenoylgluconic acid); and pedunulagin = (2,3,4,6-bis-(S)-hexahydroxydiphenoyl-D-glucose) (Scartezzini & Speroni, 2000).

Figure 1. Chemical Structures of Principal Bioactive Constituents of CAPROS®[a]



[a] From Chaudhuri, 2005.

Proximate analyses of fresh amla fruit pulp and its extract yield the following composition (Dharmananda, 2006b):

- Moisture 81 - 84%
- Protein 0.5 - 0.7%
- Fat 0.1 - 0.5%
- Minerals 0.7 - 2.4%
- Carbohydrate (including fiber) 14.0 - 14.3%
- Miscellaneous other compounds 0.5 - 0.7%

Table 3. Bioactive Constituents in Representative Sample of CAPROS®[a]

	Compounds	Relative Amounts in CAPROS® (%)
Primary bioactive Constituents:	emblicanin A	24 - 29
	emblicanin B	17 - 21
	punigluconin	6 - 8
	pedunculagin	11 - 14
Secondary bioactive Constituents:		
	rutin	0.1 - 10
	other galloellagitannoids	10 - 30

[a] From Ghosal, 2000 and unpublished results.

D. Extract Stability

Indian Herbs Research & Supply Co. Ltd. (1999) was engaged to ascertain the stability of CAPROS®, a standardized antioxidant extract of *Emblica officinalis*. Several identical samples of the test materials were packaged and held at ambient temperatures, and test samples were removed at six months intervals from 0 through 24 months to assess the physico-chemical characteristics, microbial status, and the free-radical scavenging capacities which served as a marker for the chemical potency of CAPROS®. Of particular interest from a chemical standpoint was the level of small tannoids, which serve as the constituents in CAPROS® for antioxidant activity.

High performance thin layer chromatography (HPTLC) was the method of choice in measuring the levels of the small tannoids, with UV-visible spectrophotometric detection at 260 nm. The test method was supplied as part of the stability testing results.

The method employed in assessing antioxidant capacity of the test samples utilized diphenylpicryl-hydrazyl (DPPH) as originally published in 1988.[6]

Examination of the data over the 24 months reveals the following observations:

- The color of the samples gradually changed from an initial off-white to yellow to yellow at 12 months which changed to yellowish brown at 18 months.
- The pH of a 1% solution was unchanged at 2.92-2.94.
- The percentage of small tannoids exhibited a net decrease from 69% to 64% over 24 months, whereas the gallic acid content increased from 0.66% to 1.894% over that same timeframe.
- No microbiological changes were noted.

[6] See Kato, K., Terao, N., Shimomoto, N., Hirata, M., (1988), J. Med. Chem., 31, 793-98.

- The free radical scavenging capacity of the samples was found to be essentially unchanged over the duration of the testing.

It was concluded from the stability testing that CAPROS® was stable for up to 24 months. It was acknowledged that the gallic acid level increased over time, but the biological activity as determined from the DPPH testing for radical scavenging capacity was unchanged over the 24 months of testing.

Appendix A contains a copy of the stability testing study report and supporting data, along with expanded stability testing under more severe processing conditions.

E. Method of Preparation of CAPROS®

The process whereby CAPROS® is prepared for commercial purposes to yield a standardized extract of *E. officinalis* is based primarily on the information detailed in the U.S. Patent # 6,124,248.[7] This patent was issued September 26, 2000, to S. Ghosal (Ghosal, 2000). The summary below is derived from the cited US patent.

As noted in Section II.C., the extract in question consists primarily of an enriched mixture of natural antioxidants of low molecular weight, hydrolysable tannoids. The resulting amorphous powder has patent protection for nutritional, cosmetic, and pharmaceutical uses.

The particular process that yields CAPROS® (at 4.2% by weight of the native fruit) involves harvesting the fruit from the *Emblica officinalis* tree and preparing a finely pulped sample that is subsequently extracted with a very dilute aqueous solution that contains selected salts. The salts are critical to the process since they impede microbiological growth while also preventing the degradation of the desired antioxidant constituents by hydrolytic enzymes (glycosidases) that are naturally present in the fresh amla fruit.

More specifically, the dilute aqueous solutions contain salts at levels ranging from 0.1 to 5%, and the specific salts that perform well were identified as sodium chloride, potassium chloride, calcium chloride, and magnesium chloride. Alternatively, the extracting solutions can be buffered with citrate or phosphate. The extraction process occurs with controlled heating to 70 ± 5°C which facilitates the destruction of the indigenous hydrolytic enzymes. Following the extraction itself, the resulting content must be filtered prior to being held under refrigeration (at ~10°C) for three days. The final processing step is the spray drying or vacuum drying to yield the amorphous powder.

The resulting material was analyzed to establish its chemical composition. The mixture is enriched in low to medium molecular weight tannoids with emblicanin-A and emblicanin-B as the more potent antioxidants. Punigluconin, pedunculagin, rutin, and gallo-ellagitannoids make up the balance of CAPROS®.

[7] While the patent describes the extraction process as occurring with water or aqueous alcohol, the actual production process used by Natreon relies solely on water extraction without the application of alcohol. Other US patents issued to S. Ghosal that address extraction and processing with additional applications of CAPROS® can be consulted. They are identified by the following US patent numbers: 6,235,721; 6,290,996; 6,362,167; and 6,649,150.

F. Finished Product Specifications for CAPROS®

The powdered extract from *E. officinalis* complies with the finished product specifications, which are found in Table 4, and were developed by Indian Herbs Research & Supply Co. Ltd. (1999).

Table 4. Specifications for CAPROS®[a]

Constituent Assays[b]	
Total low molecular weight hydrolysable tannins	≥ 60 %
Gallic acid	≤ 4 %
Heavy Metals	
Lead (Pb)	≤ 2 ppm
Arsenic (As)	≤ 1 ppm
Mercury (Hg)	≤ 1 ppm
Sulfated Ash	≤ 6 %
Water (Karl Fischer)	≤ 6 %
Microbials	
Total aerobic place count	≤ 5,000 cfu/g; no pathogens
Candida albicans	absent in 1 g
Escherichia coli	absent in 1 g
Pseudomonas aeruginosa	absent in 1 g
Salmonella	absent in 10 g
Staphylococcus aureous	absent in 1 g
Yeast & mold	≤ 1000 cfu/g

[a] From Certificate of Analysis in Appendix B, Natreon, Inc., 2001; Dickerhof, 2001.
[b] Analyses determined by HPLC and HPTLC; see Natreon, Inc., 2001 and Appendix B.

Appendix B contains Certificates of Analysis for three production batches of CAPROS® that were produced in accordance with the Method of Preparation as summarized in Section II.E.

G. Intended Dietary Use

1. Intended Food Categories in Which CAPROS® Will Be Used. FDA has defined several food categories in 21 CFR 170.3(n), and those listed below consist of the designated food categories in which Natreon intends to add CAPROS®, along with the anticipated use levels and numbers of daily servings. This information was derived from USDA food consumption survey data for both mean daily consumption levels and 90th percentile consumption levels; review of these consumption reports enables estimates of the dietary exposure to CAPROS® to be made.

Table 5. Proposed Food Categories & Use Levels for CAPROS®[a]

Food Category	Daily Amount Consumed (g)	Daily # Servings Consumed[b]	Use Levels Per Serving (mg)	Daily Total CAPROS® Dietary Exposure (mg)
Mean Consumption Level – All Individuals				
beverages, nonalcoholic[c]	423	1.2	100 - 150	120 - 180
processed fruits/fruit juices[d]	373	1.5	100 - 150	150 - 225
			TOTAL:	**270 – 405**
90th Percentile Consumption – All Individuals				
beverages, nonalcoholic[c]	648	1.8	100 - 150	180 - 270
processed fruits/fruit juices[d]	620	2.5	100 - 150	250 - 375
			TOTAL:	**430 – 645**

[a] Date extracted from USDA Food Consumption Survey Data (USDA, 1996).
[b] Serving size for nonalcoholic beverages is 12 fluid ounces or 360 g; serving size for processed fruits/fruit juices is 8 fluid ounces or 248 g.
[c] As defined in 21 CFR 170.3(n)(3).
[d] As defined in 21 CFR 170.3(n)(35).

An overestimate of dietary exposure for CAPROS® has been derived from the USDA food consumption survey information by considering males in the age group of 20 – 39 years who were reported to consume the largest amounts of nonalcoholic beverages and processed fruits/fruit juices of any of the US population segments (USDA, 1996). Furthermore, by considering the 90th percentile consumer within this population segment, we see that 2.3 servings of nonalcoholic beverages and 3.8 servings of processed fruits/fruit juices were consumed on a daily basis. When incorporating the maximum CAPROS® use level of 150 mg/serving into the designated food categories, the maximum total daily consumption level of CAPROS® was calculated to be 915 mg. This figure likely constitutes an overestimate of the daily amount of CAPROS® that would be consumed.

As a point of comparison, a single piece of the amla fruit, weighing 20 - 25 grams, contains 840 – 1,050 mg of CAPROS® since the documented yield of CAPROS® from the patented process is 4.2% (Ghosal, 2000).

2. Intended Technical Effect to Be Achieved in Foods. CAPROS®, when added into the foods listed in Section III.G.1, is to function as a **nutrient supplement** as defined by FDA in 21 CFR 170.3(o)(20). It is intended that the addition of CAPROS® into food categories described above would reduce stress and foster enhanced health.

III. EVIDENCE OF *EMBLICA OFFICINALIS* AND CAPROS® SAFETY

A. History of Use of *Emblica Officinalis*

According to FDA regulations,[8] GRAS determinations can be based on common use in food prior to January 1, 1958, and FDA acknowledges that common use in food prior to January 1, 1958, can serve as the basis of a GRAS determination if such "use occurred exclusively or primarily outside of the United States." However, such use outside of the US "shall be documented by published or other information and shall be corroborated by information from a second, independent source that confirms the history and circumstances of use of the substance. The information used to document and to corroborate the history and circumstances of use of the substance must be generally available; that is, it must be widely available in the country in which the history of use has occurred and readily available to interested qualified experts in this country."[9]

Table 2 lists a multitude of health advantages ascribed to the ingestion of *Emblica officinalis* as an Ayurvedic treatment for varied and broad applications. These perceived benefits provided sustained motivation over generations to consume *Emblica officinalis*. The use of *Emblica officinalis* as the predominant ingredient in popular rasayanas is reported to date to ancient times as both a home remedy used extensively throughout India and as prescribed by Ayurvedic practitioners. Rasayanas, especially *Emblica*-containing chyawanprash, have been consumed for thousands of years. Table 6a lists several references to ancient writings reported by Ojha where *Emblica* as consumed in chyawanprash was acknowledged for its health benefits.[10] When investigating its history of use, one encounters entertaining myths on the origins of chyawanprash. Among the myths, it was reported that chyawanprash was allegedly developed to rejuvenate an aging ancient sage, Chyawan Maharishi, upon his marriage to a young wife (Ojha, 2003, Anonymous, 2006a; Jajarajan, 2006). This and other myths notwithstanding, it has been established that an early reference to chyawanprash appeared in "Charaka Samhita around 2000 BC in the first chapter of Chikitsasthana called Abhayamalakiya Rasayana Pada" (Ojha, 2003).

The reports dating to antiquity as reported by Ojha on the consumption of amla for well-being were supported by numerous other references including those listed in Table 6b. Still other references to the historical uses of amla were encountered but could not be substantiated (see footnote 12 for details).

Dharmananda cited the principal Ayurvedic text, Charaka Samhita (100 AD) in the section "The Place of Chikitsa" as the location describing both triphala and chyawanprash as invaluable rasayanas in the Indian culture (Dharmananda, 2006a). Charaka Samhita contains detailed compositional information on chyawanprash which was described as a "fruit jam." Of the "active" components that make up chyawanprash, about 46% by weight is amla with over three dozen other "spicy ingredients" blended with amla. When considering the other dominant ingredients, such as honey, that comprise the finished product, the amla percentage totals 15%. Even with recipe variations over time and across geographic regions, amla was always determined to be the principal ingredient. According to Dharmananda, this conclusion was also noted in India Materia Medica.

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[8] See 21 CFR 170.30(a).

[9] See 21 CFR 170.30(c)(2).

[10] Although references are made to the health advantages and benefits associated with the consumption of *Emblica*, such references are provided to demonstrate the substantial history of use and the reason for the consumption of *Emblica* over time. The Expert Panel recognizes that the safety assessments for food additives and GRAS substances do not take into consideration the benefits to be derived from the consumption of the subject food ingredient.

Dharmananda further described the use of amla as a dominant herbal component in rasayanas such as chyawanprash and triphala by quoting from Charaka Samhita as follows:

> "They have healing virtues and are (used in sacred and ceremonial occasions). They restore the faults to their normal course. They are light (as food). They enkindle digestive fire, and are good (digestive aids). They promote longevity and induce nourishment. They deserve every praise (for the virtues they possess). They prevent the effects of age more than any other drug. They alleviate all varieties of ailments, and impart strength to the intellect and the senses..." (Dharmananda, 2006b).

Other standard Indian health and medical references include listings of amla. The Ayurvedic Pharmacopoeia of India lists amla when consumed either as fresh fruit pulp or as dried fruit pulp for multipurpose applications including treatment of anemia, jaundice, hemorrhage, acidity, and burning sensations, while further acknowledging its function as an immunomodulator and rasayana (Ayurvedic Pharmacopoeia, 2001). Amla is also mentioned in several Ayurvedic formulations such as in the Ayurvedic Formulary of India (Ayurvedic Formulary, 1981).

In their review of seven plants from India that have use in traditional medicines, Scartezzini and Speroni identify *Emblica officinalis* as a prominent plant of special distinction due to its strong antioxidant activity. The authors note that rasayanas, such as those containing *Emblica officinalis*, are used in drug therapies, but they are also included in the practice of Ayurveda as a rejuvenating recipe as part of a dietary regimen to promote well-being. *Emblica officinalis* is considered to be the most common ayurvedic plant (Scartezzini and Speroni, 2000).

Table 6. Historical References for the Consumption of *Emblica officinalis*[a]

a. Historical Citations Contained in Ojha, 2003

Charaka Samhita in the chapter of Chikitsasthana	2000 BC
Navanitaka	200 AD
Ashtanga Samgraha	600 AD
Ashtanga Hridaya	700 AD
Chakradatta	1050 AD
Chikisakalika under the heading of Shosha Chikitsa	900 AD
Sharngadhara Samhita in chapter Awaleha of Madhyama Khanda	1300 AD
Brihat Yoga Tarangini	1600 AD
Bhaishajya Ratnavali in chapter Rajayakshma	1700 AD

b. References Recognizing Longstanding Uses and Health Benefits of *Emblica officinalis*

Dhanvantri Nighantu (Ojha & Mishra, 1985)
Kaiyadeva-Nighantu (Kaiyadeva, 1979)
Medicinal Plants of India Volume 1 (Satyavati, et al., 1976)
Medicinal and Aromatic Plants of West Bengal (Pakrashi & Mukhopadhyay, 2001)
Raja Nighantu (Tripathi, 1982)
Indian Materia Medica (Pandey & Chunekar, 1986)
Database on Medicinal Plants Used in Ayurveda Volume 3 (Sharma, et al., 2001)
Botanical Safety Handbook (McGuffin, et al., 1997)

[a] English translations of Dhanvantri Nighantu, Kaiyadeva-Nighantu, Raja Nighantu, and *Indian Materia Medica* can be found in Appendix C.

While the evidence of substantial human consumption of amla in India and the Indian subcontinent is extensive, Scartezzini and Speroni also note that these plants were imported into western countries at the end of the 18th century. Dumont de Courset, a French botanist, grew about ten species at the Paris botanical garden, and it was reported that "fruits of this plant are available in Europe in any grocery as old medicament they were once used as laxative." Some interesting disagreement was expressed in the mid-1700s about the effects of consumption of the *Emblica* fruit. Rumpf G. Everhard proclaimed that eating the dried or candied fruit was effective against dysentery and against cephalea (Scartezzini and Speroni, 2000).

In 1919, Chick, et al. published their findings that the consumption of dried fruits of *Phyllanthus emblica* by Indian troops was effective in both preventing and curing scurvy (Chick, et al., 1919). The authors stated that the native population in India had long consumed the fruit as an antiscorbutic, and that this food was commonly bought and sold at the native bazaars. There was further reference to an 1837 Indian medical journal[11] whereby an outbreak of scurvy at Nassirabad, Rajputana in 1833-1834 was effectively treated with the dried fruits of *Phyllanthus Emblica.* The publication also reported that the minimum daily dose of the dry fruit needed to be effective was 14 grams.

Additional historical documentation of the consumption of amla, or Indian Gooseberry, was published in 1944 (Srinivasan, 1944). The antioxidant properties of the fruit were observed and had been thought to be the consequence of a protected form of vitamin C that was not itself subject to oxidation. At that time, the Food Department of the Government of India prepared the Indian Gooseberry to supply the vitamin C requirements for the Indian Fighting Forces to treat scurvy.

Several scientific publications that address various aspects of interest regarding amla acknowledge its long history of consumption while some specifically reference the listing of amla in Charaka Samhita (Ghosal, et al., 2002; Bhattacharya, et al., 1999; Mishra, et al., 1983; Singh and Sharma, 1971; Jacob, et al., 1988).

Parmar and Kaushal reference *Emblica officinalis* as one of the oldest Indian fruits, stating that the health-imparting properties were cited in old Ayurvedic texts, including Charaka Samhita and Sushrut Samhita (Parmar and Kaushal, 1982). A more detailed report on the growing locations and conditions of amla was offered with citations to documentation produced in 1905, 1906, and 1933. Several authors were cited that mentioned the health benefits of *Emblica officinalis* in publications from 1892, 1935, 1959, and others post-1958. In particular, sources cited by Parmer and Kaushal include several articles that date to the 1890s and early 1900s.[12]

[11] MacNab, (1837), Quart. Journ. Calcutta Medical and Physical Society, vol 1, no page number cited.

[12] The following sources were encountered but could not be retrieved for evaluation: Chopra, R. N., Chopra, I. C., Handa, K. L. and Kapur, L. D., (1958), "Chopra's Indigenous Drugs of India," U. N. Dhar & Sons Pvt. Ltd, Calcutta; Duthie, J. F., (1905), "Flora of the Upper Gangetic Plain," Vols I, II & III, Bishan Singh Madendra Pal Singh, Dehradun; Burkill, I. H., (1955), "A Dictionary of the Economic Products of Malay Peninsula. I. The Crown Agent for the Colonies," London; Kirtikar, K. R. and Basu, B. D., (1935), "Indian Medicinal Plants," Vols I, II, III, & IV, Bishan Singh Madendra Pal Singh, Dehradun; Brandis, D., (1906), "Indian Trees," Bishan Singh Mahendra Pal Singh, Dehradun; Watt, G., (1889), "A Dictionary of the Economic Products of India," I., Cosmo Publications, Delhi; Watt, G. (1889a), "A Dictionary of the Economic Products of India," II., Cosmo Publications, Delhi; Watt, G., (1890), "A Dictionary of the Economic Products of India," III., Cosmo Publications, Delhi; Watt, G., (1890a), "A Dictionary of the Economic Products of India," IV., Cosmo Publications, Delhi; Watt, G., (1891), "A Dictionary of the Economic Products of India," V., Cosmo Publications, Delhi; Watt, G., (1892), "A Dictionary of the Economic Products of India," VI., Cosmo Publications, Delhi; Watt, G., (1893), "A Dictionary of the Economic Products of India," VI. (Part II), Cosmo Publications, Delhi; Watt, G., (1893a), "A Dictionary of the Economic Products of India," VI. (Part IV), Cosmo Publications, Delhi; and Watt, G., (1908), "The Commercial Products of India," Today and Tomorrow's Publishers, New Delhi.

It is instructive to note that Shah and co-workers observed that assessing clinical efficacy for ayurvedic medicinals commonly requires material administration over longer time periods---citing literature on such Ayurvedic practices. This implies that historical exposures to amla occurred in a more extended time basis and did not occur with brief exposures (Shah, et al., 1997).

A well-established Indian firm, Dabur India, Ltd., which was founded in 1884, supplies the marketplace with chyawanprash and has done so for many years. On its website, Dabur documents its sale of chyawanprash prior to 1958 in noting that it modified its packaging of its popular chyawanprash product to expand the availability of this ancient restorative product (Dabur, 2006).

Milando is a distributor that supplies Dabur Chyawanprash to European markets. As reported previously, it was documented that Europeans consumed amla in the 1700s, and we see that it remains an item of commerce presently.

In noting the widespread popularity of chyawanprash in India, Dharmananda reported that over 50% of the total sales of Ayurvedic formulations in India are chyawanprash, thus extending age-old consumption practices into modern day India. Dharmananda maintains that Indians on a daily basis consume 1 – 3 teaspoons (or 10 – 30 grams) of the finished food that contains about 15% amla; this amount is equivalent to the daily per person consumption of amla of 1,500 to 4,500 mg (Dharmananda, 2006b).

With very specific information supporting the claims of consumption of *Emblica officinalis* for numerous health benefits, there remains no doubt of the extensive use of amla and the complex mixtures comprising amla and its antioxidant extract by large numbers of Indians for centuries, and the consumption of *Emblica officinalis* has carried forward to modern times not only in India but in Japan, Europe and the US.

The extensive history of use of the *Emblica officinalis* dating back thousands of years, particularly within India, has established a near reverence for the use of this material for a broad scope of applications as summarized in Table 2. Over the course of the past century, and particularly so during the past 25 years, some *in vitro*, animal and human studies have been undertaken to better document the health benefits associated with the consumption of *Emblica officinalis* and its antioxidant-rich extract. Interestingly, scholars who are familiar with the ancient Ayurvedic medical practices acknowledge that the scientific investigations into *Emblica officinalis,* chyawanprash, and other similar foods and formulations falling within Ayurveda do not parallel the more usual course of scientific inquiry since a substantial body of human usage experiences provides the foundation of acceptance (Ojha, 2003, p 135).

As noted previously, the GRAS evaluation of the subject amla extract is based on common use in foods prior to 1958. However, numerous scientific studies, including both human and animal testing, have been conducted on amla and its extract that corroborate the GRAS determination from the common use considerations. The accumulated corroborating information has been compiled in Appendix D.

IV. ASSESSMENT OF COMPOSITE INFORMATION FOR GRAS STATUS

A. FDA Safety Standard and Common Use in Foods GRAS Criteria

The FDA definition of "safe" or "safety" as it applies to food ingredients is "reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[13] It is FDA's operational definition of food ingredient safety that serves as the framework against which this evaluation is provided.

As noted in Section III.A., FDA permits GRAS determinations to be made based on common use in food prior to January 1, 1958. This approach does not require the quantity or quality of scientific procedures required for approval of a food additive.[14] What do we know about the historical uses of amla and its extracts? In fact, the evidence of historical consumption of *Emblica officinalis* as a food containing the subject antioxidant constituents is compelling when considering the numbers of public references to consumption of *E. officinalis* in India and the Indian subcontinent over thousands of years. Such evidence is strengthened by the detailed public references to amla consumption in Europe in the 1700s, consumption by the Indian military for scurvy prevention and cure in the 1800s, and further scientific investigations in the early- to mid-1900s. This accumulated historical usage documentation is compatible with the "reasonable certainty of no harm" standard cited earlier.

It can be concluded from the composite history of use information as discussed in detail in Section III.A that there has been a substantial history of consumption of *Emblica officinalis* and its constituents without reported adverse effects by a significant number of consumers that spans several generations.

B. Dietary Consumption Levels of Amla and Amla Extract

Quantitation of human exposure from historical considerations is challenging. We know that the typical individual piece of amla fruit which weighs 20 - 25 grams will contain about 840 – 1,050 mg of the ingredients in CAPROS®, but we do not know the daily consumption equivalent of amla since we have not been able to document that amla is consumed as the raw fruit, apparently as a consequence of the high acidity and astringent taste (See Section II.A). Therefore, some reliance has been placed on scientific procedures as contained in Appendix D to corroborate the common use in food considerations as allowed by proposed 21 CFR 170.36.

What do we learn about the safety of amla and its antioxidant fraction extract from the animal and *in vitro* studies? The LD_{50} that exceeds 5,000 mg/kg bw demonstrates the absence of acute toxicity, and the negative Ames test supports the lack of mutagenic potential for CAPROS®. Of the remaining animal studies cited, only the unpublished subchronic rat and rabbit study test results (see Appendix G) are reliable in a quantitative context, revealing the absence of adverse effects at the 100 mg/kg bw/day and 500 mg/kg bw/day levels. Some decreases in GOT, BUN and creatinine levels were noted, and they are believed to be non-specific changes. Furthermore, a recent clinical study rules out adverse effects on kidneys, even in uremic patients (Chen, et al., 2009). However, assuming that a 100-fold safety factor would apply in extrapolating from subchronic animal exposure to chronic human experience, the

[13] See 21 CFR 170.3(i).
[14] See 21 CFR 170.30(c)(1).

subchronic test results would support a human daily consumption level of CAPROS® of 70 – 350 mg for a 70 kg adult.

Examining the human studies that have been summarized in Table 7 (as found in Appendix D) reveals human daily exposures to *Emblica officinalis* extending to 2100 mg. The results from the human testing suggest the absence of toxicity, even at the highest reported exposures. The published studies illustrate a cluster of safe daily usage levels around 450 - 500 mg. The unpublished 6-week clinical trial with CAPROS®, which was tested at 500 mg/kg bw/day and at 1000 mg/kg bw/day, corroborates this range as being safe; the unpublished subchronic rat and rabbit studies on CAPROS® when allowing for the 100-fold safety factor, is compatible with the determination that a daily exposure of 400 mg of CAPROS® is safe.

Dharmananda's representations that the contemporary Indian population consumes amla-containing chyawanprash---and the calculated amount of antioxidant extract ingested---would be just under 200 mg without reported adverse effects just from this single amla-containing food. This representation addresses the topic of chronic human exposure more directly than any of the clinical test results identified.

C. High Consumer Safety Assessment

It is necessary when assessing safe consumption levels to ensure that the individuals who consume disproportionately larger than average amounts in their diets are not placed at risk. In light of the composite information cited with common use in foods considerations which are complemented by scientific studies, CAPROS® incorporation as an ingredient in selected foods in the US food supply would enable the typical consumer to safely ingest 400 mg on a daily basis. This quantity of CAPROS® is less than half of the amount found in a single piece of amla fruit.

As seen in Table 5, the consumer at the 90th percentile level of consumption would be expected to consume 430 - 645 mg of CAPROS® per day. Does this larger daily exposure provide an adequate margin of safety for the 90th percentile consumer? When considering the published human exposure studies cited in Appendix D and summarized in Table 7 (as found within Appendix D), we see that the amla antioxidant extract has been safely consumed at daily levels of 1000 - 1500 mg, and some individuals consumed 2100 mg per day for two months without adverse effects. Consequently, consumption by high level US consumers at nearly 650 mg per day is likely to be well tolerated.

Interestingly, by considering males in the age group of 20 – 39 who consume the largest numbers of servings of the nonalcoholic beverages and processed fruits/fruit juices of any population segment, and by further considering the 90th percentile consumers within this population segment, the Expert Panel calculated a daily dietary exposure to CAPROS® of 915 mg, an amount roughly equal to that amount found in a single piece of amla fruit. Even this overestimate falls within the range of 1000 – 1500 mg of the amla extract that has been safety consumed by humans.

D. Common Knowledge Considerations

In considering common knowledge criteria for GRAS determinations, FDA notes that

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."[15]

[15] See 21 CFR 170.30(a).

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following elements:

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that consensus (but not unanimity) exists among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

The safety evaluation of CAPROS® and *E. officinalis* as addressed in Sections III and IV relied in large measure on considerations pertaining to common use in foods prior to 1958, most of which have been published in the open literature or are available on the Internet. Furthermore, most of the supporting scientific procedures content found in Appendix D has been published in the scientific literature. Unpublished corroborating information, including the Natreon-sponsored 6-week clinical trial and the 90-day subchronic animal testing studies on CAPROS®, has also been a factor in the conclusions drawn.

The remaining aspect of the common knowledge requirement for GRAS conclusions is the need to demonstrate consensus that the safety of the proposed food use would be generally recognized. The knowledge of the health benefits linked to the consumption of *Emblica officinalis* has truly been established, recognized, and documented over thousands of years with the inclusion of information in various Ayurvedic texts and Indian handbooks and compendia. Particularly over the course of the last century, a more scientific approach has been taken with the dissemination of information on the safety of consumption of amla and its extracts. Ghosal, et al. and Scartezzini and Speroni have both written review articles that reinforce the view that amla consumption, with the component antioxidant fraction, is not only safe but is beneficial (Ghosal, et al., 2002; Scartezzini and Speroni, 2000).

Of particular note is the inclusion of *Emblica officinalis* in the American Herbal Products Association's compilation of safe botanicals (McGuffin, et., 1997). In describing the safety assessment procedures, botanical and herbal safety determinations followed the guidelines established by WHO and their Programme on Traditional Medicines. The following served as the guiding principle:

> **"...if the product has been traditionally used without demonstrated harm no specific restrictive regulatory action should be undertaken unless new evidence demands a revised risk-benefit assessment."**

Their safety evaluations are conducted with an understanding of the intended use; the botanicals and herbs can be whole, cut, powdered, and the safety assessment further extends to the crude extracts, as long as no chemicals are added.

Four different classifications are available with the Botanical Safety Handbook, and *Emblica officinalis* received the most benign classification in that it "can be safety consumed when used appropriately" (McGuffin, et al., 1997).

The Government of India through its actions to support and expand the production of amla and products derived therefrom for health and well-being of its citizens contribute to the general consensus of safety from ingestion (Government of India, 2003; Government of India, 2000).

Finally, the 2003 book authored by Ojha, Chyawanprash from Vedic to Genomic Era, summarizes a substantial volume of information on chyawanprash with amla as the principal constituent, reportedly at a level of 90% by weight. In short, many have been aware of the availability and consumption of amla for long periods of time with both passive and active acceptance of the safe food uses of amla.

E. Summary of Amla Extract Safety Assessment

In summary, ample evidence is available to demonstrate that the proposed consumption levels of amla and CAPROS® are safe. The preponderance of the information including, but not limited to, the following serves as the basis for the GRAS determination:

- Well-documented historical use in India and south Asia without adverse effects;
- Present day popularity and consumption by significant numbers of consumers world-wide, particularly in India and south Asia; and
- Safety corroboration from various clinical studies, animal testing, and *in vitro* testing on amla extract and CAPROS®.

This composite information and well-documented history of safe uses of *Emblica officinalis* support a GRAS conclusion with the anticipated adult mean human exposure of 400 mg per day. This figure reasonably reflects historical food uses of the subject material to conform to the "reasonable certainty of no harm" standard referenced previously. In addition, the supporting information fulfills the common knowledge requirement for general recognition of safety.

V. CONCLUSIONS[16]

The cumulative historical usage information compiled on *Emblica officinalis* and reviewed in concert with corroborating scientific studies on *Emblica officinalis* and the extract of the antioxidant fraction, CAPROS®, provides the basis for the conclusion that a daily CAPROS® exposure of 400 mg as a nutrient supplement with proposed food usage as summarized in Table 5 is generally recognized as safe.

This independent GRAS determination is based extensively on the common use in foods basis in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use. It is further understood that this GRAS conclusion applies to CAPROS® as long as it is produced in accordance with good manufacturing practices and meets appropriate food grade specifications.

CAPROS® is Generally Recognized As Safe when consumed as a nutrient supplement in designated food categories when: (1) CAPROS® is produced in accordance with FDA's Good Manufacturing Practices requirements; (2) it meets appropriate food grade specifications such as has been designated in Table 4; and (3) adult human exposure from the designated food categories occurs at an exposure level of up to 400 mg per day.

Robert S. McQuate, Ph.D. June 29, 2009

Richard C. Kraska, Ph.D., DABT June 29, 2009

Wayne R. Bidlack, Ph.D. June 29, 2009

[16] The credentials for the individuals serving on the Expert Panel can be found in Appendix I, where the educational and professional backgrounds for Richard C. Kraska, Ph.D., DABT, Dr. Wayne R. Bidlack, and Robert S. McQuate, Ph.D. are summarized. Each has extensive technical background in the evaluation of food ingredient safety. Drs. Kraska and McQuate each worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Bidlack is Professor of Food Science and former Dean of the College of Agriculture at California State Polytechnic University, Pomona. He has worked extensively in food safety matters over the years and frequently serves as a consultant to the food industry. Dr. McQuate served as Chair of the Panel.

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APPENDIX A

Stability Studies - CAPROS®

INDIAN HERBS

INDIAN HERBS RESEARCH & SUPPLY Co. LTD.
P.B. No. 5, Sharda Nagar, Saharanpur (U.P.) 247 001

STABILITY STUDY OF CAPROS™

Study No.: SS-1 Page 1 of 14

Protocol Prepared by . _____ Date · January 2, 1999

Protocol Approved by . _____ Date . January 2, 1999

Study conducted at:

Indian Herbs Research & Supply Co. Ltd.
P B No. 5, Sharda Nagar, Saharanpur - 247 001, U.P , India

Test Substance Identification:

Capros™ (Standardized Extract of *Emblica officinalis*)
Lot No.: **309**
Date of Manufacturing **December 1998**
Manufacturer · Indian Herbs Research & Supply Co. Ltd., Saharanpur - 247 001 U.P. India

Date Sample Received for the Study : 4th January 99, 1st June 99, 6th December 99, 2nd June 00, 7th December 2000, 5th April 2001

Dates of Test 7th January 99, 2nd June 1999, 9th December 99, 4th June 2000, 7th December 2000, 10th April 2001

Study Completion Date : 10th April 2001

Note Book No.: 1 **Page No.** 38, 103, 163 &
Note Book No.: 2 **Page No.** 33, 96

Nature's Way to Health
Phone : 725044, 725045, 725046, 725459. Cable · 'INDIANHERB'. Fax No. : (0132) 726288. E-Mail : ihsre@vsnl.com
FACTORY : Darra Shivpuri, Saharanpur, Phone : 721784
U.P S.T. No. : SR-0229952 dt. 01-04-1991. C.S.T No.: SR-5133183 dt. 10-04-1991

INDIAN HERBS

INDIAN HERBS RESEARCH & SUPPLY Co. LTD.
P.B. No. 5, Sharda Nagar, Saharanpur (U.P.) 247 001

1. OBJECTIVE

To study the stability of Capros™ by keeping the samples at ambient temperature and to analyse them completely at the frequency of 0, 6, 12, 18 and 24 months.

2. EXPERIMENTAL

The stability of Capros™ was studied by keeping the samples at ambient temperature. The samples were packed in aluminium sachet (5 gm) which were packed in a carton. Sufficient number of these cartons were kept for the study. The samples were withdrawn at 6 months interval upto 2 yrs. and analysed for its physico-chemical properties and DPPH radical scavenging activity. Capros™, Lot No. 309, Mfg.date - December 1998 was randomly used for this study. The results are provided in Table-1 and subsequent HPTLC finger prints have been provided in Fig.1 to 5. The HPTLC test method used is attached to this report.

3. CONCLUSION

HPTLC finger printing analysis of Capros™ analysed at different period of time does not show any significant difference indicating the overall stability of Capros™. Table-1 also indicates that Capros™ is stable in given experimental condition, however Gallic acid content are increasing with time but this does not affect the biological activity as evidenced by DPPH radical scavenging test. Hence it is evident from the present study that Capros™ kept at ambient temperature is stable upto 2 yrs

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INDIAN HERBS

INDIAN HERBS RESEARCH & SUPPLY Co. LTD.
P.B. No. 5, Sharda Nagar, Saharanpur (U.P.) 247 001

Table -1 Results of Capros™ kept at ambient temperature for different time duration

S. No	PARAMETERS	Initial	After 6 months	After 12 months	After 18 months	After 24 months
1	Appearance	Fine hygroscopic powder	Fine hygroscopic powder	Fine hygroscopic powder	Fine hygroscopic powder	Fine hygroscopic* powder
2	Colour	Off-white to yellowish	Off-white to yellowish	Yellowish	Yellowish brown	Yellowish brown
3	Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
4	Taste	Sour	Sour	Sour	Sour	Sour
5.	*p*H of 1% solution	2 93	2.92	2.92	2.93	2.94
6.	% of Small tannoids	69 183	70 236	68 607	66 411	64 089
7.	% of Gallic acid	0.66	0 84	1.20	1 52	1 84
8	DPPH radical scavanging activity (IC_{50} value)	15 µg	13 µg	14 µg	15 µg	16 µg
9	**Microbiological tests****					
	Total aerobic plate count					3×10^3 CFU/g
	Escherichia Coli					Absent
	Salmonella sp.					Absent

* Light caking tendency was observed (Improved packing may obviate this deficiency)

** **Note** : These tests were done in April, 2001.

Nature's Way to Health

Phone · 725044, 725045, 725046, 725459. Cable : 'INDIANHERB'. Fax No. . (0132) 726288. E-Mail : ihsre@vsnl.com
FACTORY : Darra Shivpuri, Saharanpur, Phone : 721784

U.P.S.T. No. : SR-0229952 dt 01-04-1991, C.S.T. No.: SR-5133183 dt. 10-04-1991

Figure-1

C A M A G TLC Evaluation Software
****
INDIAN HERBS RESEARCH & SUPPLY CO.LTD.
SHARDA NAGAR SAHARANPUR-247001 (U.P)

TLC/HPTLC-Integration (CATS V4.05, S/N:0202A016 / Sc3 V1.14, S/N:041123)

HPTLC analysis of Capros Batch.no. 309 mfg. date Dec.98.(Initial).

Calibr.Table	Calibration table, created : INDIAN HERBS(R&D)
	File name : CAPRO_1 7/JAN/99 13:58:06
Scan	User name while measuring : INDIAN HERBS(R&D)
	File name : CAPRO_1 7/JAN/99 13:58:42
Integration	User while integrating : INDIAN HERBS(R&D)
	File name : CAPRO_1 7/JAN/99 13:30:52

Analytical and chromatographic conditions:

Analysis	:analysis of Capros (Initial).
Plate material	:Silica gel 60 F254
Solvent	.Ethyl acetate: A.A.:F.A.:Water 100:11:11:27
Application mode	:Camag linomat IV
Development mode	:Camag twin trough chamber

Scanner settings:

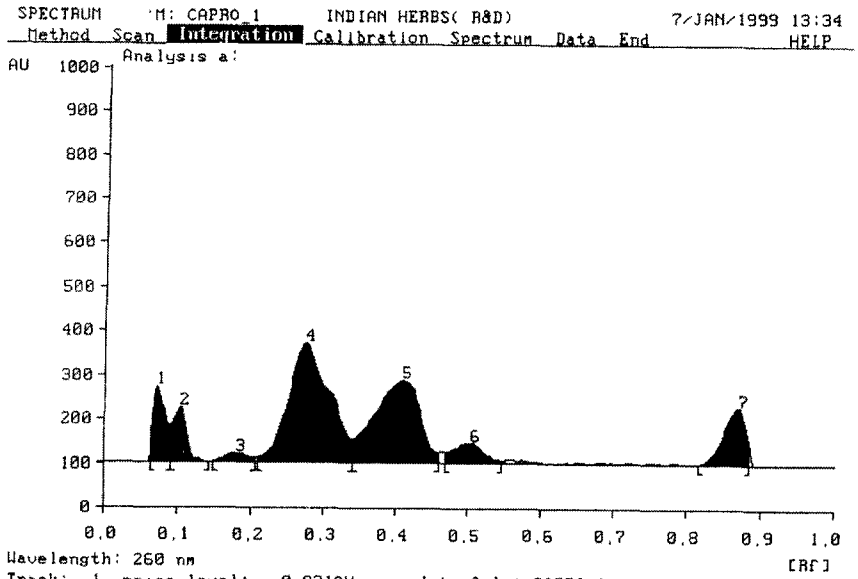
Plate size (width x height)...	20 x 20 cm
Application position Y	: 10.0 mm
Position of solvent front	: 70 0 mm
Scan start position Y	: 10.0 mm
Scan end position Y	: 70.0 mm
Scan start position X	: 31.8 mm
Distance between tracks X	: 0 0 mm
Number of tracks	: 1
Lamp..	Deuterium
Monochromator bandwidth...	20 nm
Wavelength	: 260 nm
Slit dimension .	5.0 x 0.45 mm
Data step resolution...	100 µm
Display scaling	: 1000 AU
Measurement mode...	Absorption / Reflection..
Scanning speed	: 20 mm/s
Optical filter	. Second order
Zeroing mode	: Automatic
Y-positon for 0 adjustment	: 10.0mm, Track: 1
Quick scan from	: 10.0mm to 70.0mm, all tracks
Offset	: 10%
Sensitivity	: Automatic (40)
High voltage of PM	: 435 V

Track optimization...

Track optimization :	OFF
Optimization mode...	7 tracks x 0.4 mm
Peak threshold, height :	100 AU
Peak threshold, slope :	5

User name : INDIAN HERBS(R&D) //JAN/1999 ID:7CF01070D1E3307 page 1
Signature : Supervisor:

Figure-1 Study No.SS-1



Track 1, Analysis a:

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.06	23.6	0.07	172.5	18.11	0.09	85.5	2017.4	7.42
2	0.09	85.5	0.10	126.2	13.25	0.14	0.0	1621.6	5.96
3	0.15	3.1	0.18	21.9	2.29	0.21	11.4	505.9	1.86
4	0.21	12.9	0.28	271.4	28.48	0.34	53.8	10704.1	39.34
5	0.34	53.8	0.41	186.5	19.57	0.46	25.1	8339.9	30.65
6	0.47	27.0	0.51	45.9	4.82	0.55	7.3	1364.9	5.02
7	0.82	1.1	0.87	128.5	13.48	0.89	49.4	2652.6	9.75

Total height = 952.9 total area = 27206.5

User name : INDIAN HERBS(R&D) 7/JAN/1999 ID:7CF01070D21140C page 1
Signature : Supervisor:

Figure-2 Study No.SS-1

C A M A G TLC Evaluation Software
****
INDIAN HERBS RESEARCH & SUPPLY CO.LTD.
SHARDA NAGAR SAHARANPUR-247001 (U.P)

TLC/HPTLC-Integration (CATS V4.05, S/N:0202A016 / Sc3 V1.14, S/N:041123)

HPTLC analysis of Capros Batch.no. 309 mfg. date Dec.98.(After six months)

Calibr.Table	Calibration table, created : INDIAN HERBS(R&D)
	File name : CAPRO_2 2/JUN/99 14:35:10
Scan	User name while measuring : INDIAN HERBS(R&D)
	File name : CAPRO_2 2/JUN/99 14:35:44
Integration	User while integrating : INDIAN HERBS(R&D)
	File name : CAPRO_2 2/JUN/99 13:37:42

Analytical and chromatographic conditions:

Analysis	:analysis of Capros (After six months).
Plate material	:Silica gel 60 F254
Solvent	:Ethyl acetate: A.A.:F.A.:Water 100.11:11:27
Application mode	:Camag linomat IV
Development mode	:Camag twin trough chamber

Scanner settings:

Plate size (width x height).	: 20 x 20 cm
Application position Y	: 10.0 mm
Position of solvent front	: 70 0 mm
Scan start position Y	: 10.0 mm
Scan end position Y	: 70 0 mm
Scan start position X	: 41.8 mm
Distance between tracks X	: 0 0 mm
Number of tracks	: 1
Lamp...	Deuterium
Monochromator bandwidth...	20 nm
Wavelength	: 260 nm
Slit dimension...	6.0 x 0 45 mm
Data step resolution...	100 µm
Display scaling	: 1000 AU
Measurement mode...	Absorption / Reflection...
Scanning speed	: 20 mm/s
Optical filter	: Second order
Zeroing mode	: Automatic
Y-positon for 0 adjustment	: 10.0mm, Track: 1
Quick scan from	: 10.0mm to 70.0mm, all tracks
Offset	: 10%
Sensitivity	: Automatic (23)
High voltage of PM	: 432 V

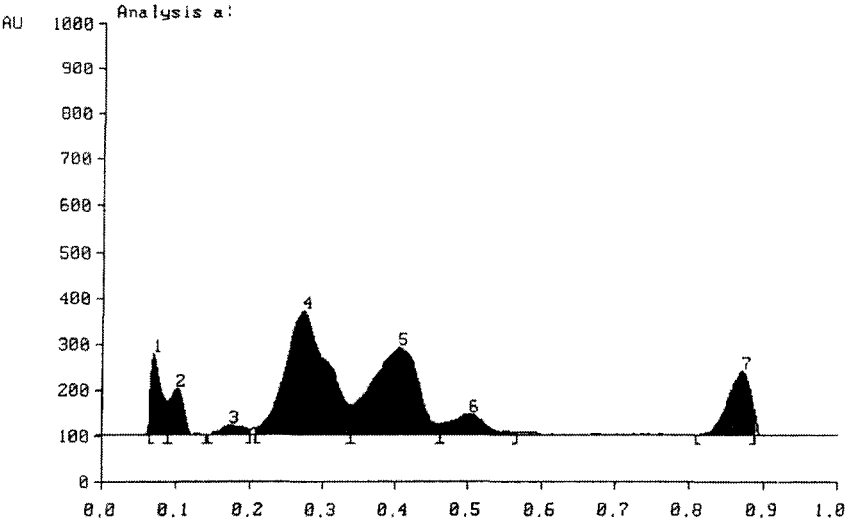
Track optimization...

Track optimization :	OFF
Optimization mode...	7 tracks x 0.4 mm
Peak threshold, height :	100 AU
Peak threshold, slope :	5

User name : INDIAN HERBS(R&D) 2/JUN/1999 ID:7CF06020D252/2D page 1
Signature : Supervisor:

Figure-2 Study No. SS-1

SPECTRUM :M: CAPRO_2 INDIAN HERBS(R&D) 2/JUN/1999 13:37
Method Scan Integration Calibration Spectrum Data End HELP



Wavelength: 260 nm
Track: 1, noise level: 0.839AU, raw data file: CAPRO_2
U4.05 S/N:0202A016 CAMAG SOFTWARE (c) 1998 SCANNER 3: 041123

Track 1, Analysis a:

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.07	79.6	0.07	175.1	18.60	0.09	74.5	1725.5	6.26
2	0.09	74.5	0.10	101.4	10.77	0.14	0.0	1320.0	4.79
3	0.14	0.0	0.18	20.3	2.16	0.20	12.3	476.7	1.73
4	0.21	15.3	0.27	269.8	28.66	0.34	64.1	10918.7	39.62
5	0.34	64.1	0.41	189.1	20.08	0.46	22.8	8567.1	31.09
6	0.46	22.8	0.50	45.9	4.88	0.57	7.2	1544.0	5.60
7	0.81	0.4	0.87	139.8	14.85	0.89	50.0	3003.2	10.90

Total height = 941.4 total area = 27555.3

User name : INDIAN HERBS(R&D) 2/JUN/1999 ID:7CF06020D250241 page 1
Signature : Supervisor:

Figure-3 Study No.SS-1

C A M A G TLC Evaluation Software
****
INDIAN HERBS RESEARCH & SUPPLY CO.LTD.
SHARDA NAGAR SAHARANPUR-247001 (U.P)

TLC/HPTLC-Integration (CATS V4.05, S/N:0202A016 / Sc3 V1.14, S/N:041123)

HPTLC analysis of Capros Batch.no. 309 mfg. date Dec.98.(After one year)

Calibr.Table	Calibration table, created : INDIAN HERBS(R&D)
	File name : CAPRO_3 9/DEC/99 14:47:00
Scan	User name while measuring : INDIAN HERBS(R&D)
	File name : CAPRO_3 9/DEC/99 14:47:38
Integration	User while integrating : INDIAN HERBS(R&D)
	File name : CAPRO_3 9/DEC/99 13:42:18

Analytical and chromatographic conditions:

Analysis	:analysis of Capros (After one year).
Plate material	:Silica gel 60 F254
Solvent	:Ethyl acetate: A.A.:F A.:Water 100:11:11:27
Application mode	:Camag linomat IV
Development mode	:Camag twin trough chamber

Scanner settings:

Plate size (width x height)...	20 x 20 cm
Application position Y	: 10.0 mm
Position of solvent front	: 70.0 mm
Scan start position Y	: 10.0 mm
Scan end position Y	: 70.0 mm
Scan start position X	: 51.8 mm
Distance between tracks X	: 0.0 mm
Number of tracks	: 1
Lamp...	Deuterium
Monochromator bandwidth..	20 nm
Wavelength	: 260 nm
Slit dimension...	6.0 x 0.45 mm
Data step resolution...	100 µm
Display scaling	: 1000 AU
Measurement mode...	Absorption / Reflection...
Scanning speed	: 20 mm/s
Optical filter	: Second order
Zeroing mode	: Automatic
Y-positon for 0 adjustment	: 10.0mm, Track: 1
Quick scan from	: 10.0mm to 70.0mm, all tracks
Offset	: 10%
Sensitivity	: Automatic (23)
High voltage of PM	: 432 V

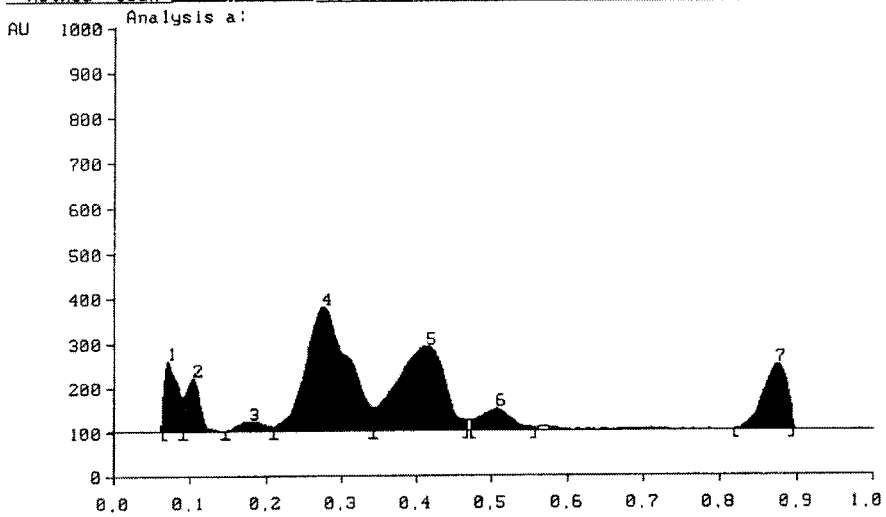
Track optimization...

Track optimization :	OFF
Optimization mode...	7 tracks x 0.4 mm
Peak threshold, height :	100 AU
Peak threshold, slope :	5

User name : INDIAN HERBS(R&D) 9/DEC/1999 ID:7CF0C090D2A1062 page 1
Signature : Supervisor:

Figure-3 Study No.SS-1

SPECTRUM :M: CAPRO_3 INDIAN HERBS(R&D) 9/DEC/1999 13 41
Method Scan Integration Calibration Spectrum Data End HELP



Wavelength: 260 nm
Track: 1, noise level: 0.931AU, raw data file: CAPRO_3
V4.05 S/N:0202A016 CAMAG SOFTWARE (c) 1998 SCANNER 3: 041123

Track 1, Analysis a:

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.07	64.2	0.07	158.0	16.41	0.09	79.4	1853.4	6.60
2	0.09	79.4	0.11	119.6	12.43	0.15	0.4	1537.3	5.48
3	0.15	0.4	0.18	22.2	2.31	0.21	10.0	553.4	1.97
4	0.21	10.0	0.28	277.7	28.85	0.34	52.7	10959.9	39.06
5	0.34	52.7	0.42	188.7	19.60	0.47	24.2	8463.5	30.16
6	0.47	24.6	0.51	49.3	5.12	0.56	7.4	1413.5	5.04
7	0.82	1 5	0.88	147.0	15.27	0.90	14.9	3279.0	11.69

Total height = 962.4 total area = 28060.0

User name : INDIAN HERBS(R&D) 9/DEC/1999 ID:7CF0C090D292625 page 1
Signature : Supervisor:

Figure-4 Study No.SS-1

C A M A G TLC Evaluation Software
****
INDIAN HERBS RESEARCH & SUPPLY CO.LTD.
SHARDA NAGAR SAHARANPUR-247001 (U.P)

TLC/HPTLC-Integration (CATS V4.05, S/N:0202A016 / Sc3 V1.14, S/N:041123)

HPTLC analysis of Capros Batch.no. 309 mfg. date Dec.98.(After one year & six months).

Calibr.Table	Calibration table, created : INDIAN HERBS(R&D)
	File name : CAPRO_4 4/JUN/00 15:07:02
Scan	User name while measuring : INDIAN HERBS(R&D)
	File name : CAPRO_4 4/JUN/00 15:07:44
Integration	User while integrating : INDIAN HERBS(R&D)
	File name : CAPRO_4 4/JUN/00 15:33:10

Analytical and chromatographic conditions:

Analysis	:analysis of Capros (After one year & six months).
Plate material	:Silica gel 60 F254
Solvent	:Ethyl acetate: A.A.:F.A.:Water 100:11.11:27
Application mode	:Camag linomat IV
Development mode	:Camag twin trough chamber

Scanner settings:

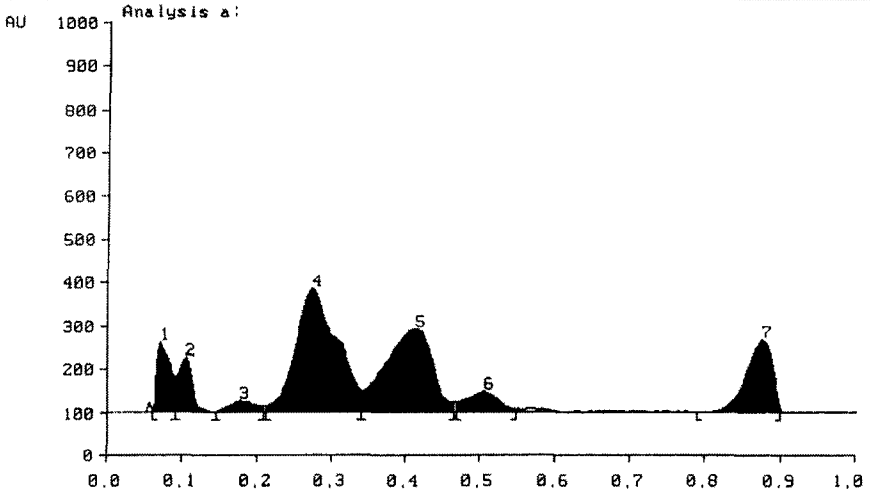
Plate size (width x height)..	20 x 20 cm
Application position Y	: 10.0 mm
Position of solvent front	: 70.0 mm
Scan start position Y	: 10.0 mm
Scan end position Y	: 70.0 mm
Scan start position X	: 61.7 mm
Distance between tracks X	: 0.0 mm
Number of tracks	: 1
Lamp...	Deuterium
Monochromator bandwidth...	20 nm
Wavelength	: 260 nm
Slit dimension...	6.0 x 0.45 mm
Data step resolution...	100 µm
Display scaling	: 1000 AU
Measurement mode...	Absorption / Reflection..
Scanning speed	: 20 mm/s
Optical filter	: Second order
Zeroing mode	: Automatic
Y-position for 0 adjustment	: 10.0mm, Track: 1
Quick scan from	: 10.0mm to 70.0mm, all tracks
Offset	: 10%
Sensitivity	: Automatic (23)
High voltage of PM	: 432 V

Track optimization...

Track optimization :	OFF
Optimization mode...	7 tracks x 0.4 mm
Peak threshold, height :	100 AU
Peak threshold, slope :	5

Figure-4

SPECTRUM :M: CAPRO_4 INDIAN HERBS(R&D) 4/JUN/2000 15 30
Method Scan Integration Calibration Spectrum Data End HELP



Wavelength: 260 nm
Track: 1, noise level: 0.778AU, raw data file: CAPRO_4
V4.05 S/N:0202A016 CAMAG SOFTWARE (c) 1998 SCANNER 3: 041123

Track 1, Analysis a:

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0.06	5.5	0.07	160.0	16.03	0.09	80.3	1988.2	6.81
2	0.09	80.3	0.11	125.7	12.59	0.15	0.2	1608.3	5.51
3	0.15	0.2	0.18	26.1	2.61	0.21	13 8	642 5	2.20
4	0.21	15.9	0.28	283.8	28.42	0.34	48.6	11053.0	37.84
5	0.34	48.6	0.42	190.2	19.05	0.47	23.7	8458.1	28.96
6	0.47	24.7	0.51	46.7	4.68	0.55	7.3	1399.9	4 79
7	0.79	0.3	0.88	166.0	16.63	0.90	13 1	4061.3	13.90

total height = 998.5 total area = 29211.3

Figure-5 ,Study No.SS-1

C A M A G TLC Evaluation Software
****
INDIAN HERBS RESEARCH & SUPPLY CO.LTD.
SHARDA NAGAR SAHARANPUR-247001 (U.P)

TLC/HPTLC-Integration (CATS V4.05, S/N:0202A016 / Sc3 V1.14, S/N:041123)

HPTLC analysis of Capros Batch.no. 309 mfg. date Dec.98.(after two year)

Calibr.Table	Calibration table, created . INDIAN HERBS(R&D)
	File name : CAPRO_5 7/DEC/00 15:15:24
Scan	User name while measuring : INDIAN HERBS(R&D)
	File name : CAPRO_5 7/DEC/00 15:16:02
Integration	User while integrating . INDIAN HERBS(R&D)
	File name : CAPRO_5 7/DEC/00 15:40:46

Analytical and chromatographic conditions:

Analysis	:analysis of Capros (after two year)
Plate material	:Silica gel 60 F254
Solvent	:Ethyl acetate: A.A.:F.A.:Water 100:11:11:27
Application mode	:Camag linomat IV
Development mode	:Camag twin trough chamber

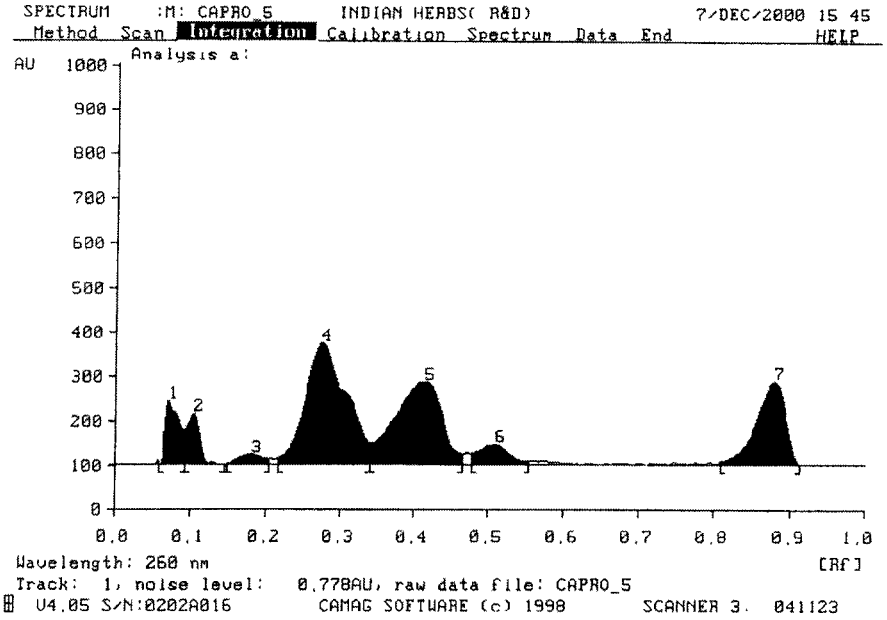
Scanner settings:

Plate size (width x height). .	20 x 20 cm
Application position Y	: 10.0 mm
Position of solvent front	: 70.0 mm
Scan start position Y	: 10.0 mm
Scan end position Y	: 70.0 mm
Scan start position X	: 33.7 mm
Distance between tracks X	: 0.0 mm
Number of tracks	: 1
Lamp...	Deuterium
Monochromator bandwidth...	20 nm
Wavelength	: 260 nm
Slit dimension...	6.0 x 0.45 mm
Data step resolution...	100 µm
Display scaling	: 1000 AU
Measurement mode...	Absorption / Reflection...
Scanning speed	: 20 mm/s
Optical filter	: Second order
Zeroing mode	: Automatic
Y-positon for 0 adjustment	: 10.0mm, Track: 1
Quick scan from	: 10.0mm to 70.0mm, all tracks
Offset	: 10%
Sensitivity	: Automatic (28)
High voltage of PM	: 432 V

Track optimization...

Track optimization :	OFF
Optimization mode...	7 tracks x 0.4 mm
Peak threshold, height :	100 AU
Peak threshold, slope :	5

Figure-5



Track 1, Analysis a:

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	0 06	3.8	0.07	142.7	14.64	0.09	81 3	1905.6	6 57
2	0 09	81 3	0.11	115.0	11.80	0 15	0.0	1361.8	4.69
3	0.15	1.4	0.18	23.9	2.46	0.21	13.2	530.1	1.83
4	0.22	16.2	0.28	274.1	28.14	0.34	49.8	10498.5	36.18
5	0.34	49 8	0.42	184.8	18.97	0.47	24.8	8289.5	28.57
6	0.48	25.5	0.51	47.2	4.84	0.56	8.2	1342.0	4.63
7	0 81	6.5	0.88	186.6	19.16	0.91	0.0	5088.0	17.54

Total height = 974.3 total area = 29015.5

SIGNATURES

CAPROS™

This study meets the requirements of the Good Laboratory Practice standards as per Indian regulations.

Date : 10th April 2001

Name : SUDHAKAR AGRAWAL

Laboratory Director

We the undersigned declare that the methods, results and the data contained in this report faithfully reflect the procedures used and raw data collected during the study

Date : 10th April 2001

Name SATISH CHAUHAN

Laboratory Incharge

Date : 10th April 2001

Name : ANIRUDH KUMAR

Laboratory Technician

leena/ncapros-1

Nature's Way to Health

Phone : 725044, 725045, 725046, 725459. Cable . 'INDIANHERB'. Fax No. : (0132) 726288. E-Mail : ihsre@vsnl com
FACTORY : Darra Shivpuri, Saharanpur, Phone : 721784

U.P.S.T. No. : SR-0220952 dt. 01-04-1991 C.S.T. No. : SR-5133183 dt. 10-04-1991

INDIAN HERBS

INDIAN HERBS RESEARCH & SUPPLY Co. LTD.
P.B. No. 5, Sharda Nagar, Saharanpur (U.P.) 247 001

Confidential

Test method # CAPROS™ 100A

QUANTIFICATION OF SMALL TANNOIDS IN CAPROS™

Scope of the Method

This is a high performance thin layer chromatographic (HPTLC) test method used for quantifying the active fraction in CAPROS™.

Method

1. **Sample Preparation**: Weigh accurately 100 mg of CAPROS™ in a flask and dissolve it in 25 ml of distilled water. Shake well for about 5 minutes, and then add additional 25 ml of distilled water. It was shaken for about 10 minutes in a sonicator bath Filter to remove any suspended particles. Discard initial 2-3 ml and collect 5 ml in a flask. Use 5 µl of test samples for HPTLC analysis.

Sample Concentration	- 100 mg / 50 ml
Sample Applied	- 5 µl
Standard Used	- Gallic Acid
Concentration of Standard	- 0.1 mg / ml
Quantity of Standard Applied	- 1, 2, 5, 10 µl

2 **Adsorbent**: Silica gel 60 F 254 TLC plate, E.Merck, #5554

3. **Sample Applicator**: Camag Linomat IV

4 **Solvent System**: Ethyl Acetate/Formic Acid/Acetic Acid/Water (~100/11/11/27, upper layer)

Preparation of Solvent System: Measure 33.5 ml Ethyl Acetate in a 50 ml measuring cylinder and add to this, 3 7 ml Formic Acid and 3.7 ml Acetic Acid and make up the volume to 50 ml using distilled water (9.1 ml). Transfer it into separating funnel and mix gently. Allow it stand for about 1 hour. Discard the water-phase (lower layer) and use the upper layer as the solvent system.

5. **Development Chamber**: 10x10 cm or 20x10 cm twin trough chamber Place ordinary filter paper in the development chamber for saturation. Keep the chamber closed with the lid

6. **Scanner**: Camag's TLC Scanner III with CATS 4.05 software.

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U.P.S.T. No. : SR-0229952 dt. 01-04-1991, C.S.T. No.. SR-5133183 dt. 10-04-1991

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INDIAN HERBS RESEARCH & SUPPLY Co. LTD.
P.B. No. 5, Sharda Nagar, Saharanpur (U.P.) 247 001

7 **Application Procedure**: Apply separately 5 µl of test solution and standard solutions (1, 2, 5, 10 µl) of Gallic Acid on to a TLC plate. Develop the TLC plate in the above solvent system and allow it to run up to about 80 mm. Remove the TLC plate form the chamber and allow it to air dry or use hair drier. Scan the plate at 260 nm in absorbency mode.

Quantification of Small Tannoids

Calculate the percentage of small tannoids using the following formula in test sample

Calculation:

$$\% \text{ Small Tannoids in CAPROS}^{TM} = \frac{\text{Water Soluble Extractive Value} \times \text{Sum of \% of peak area between first \& last peak}}{100}$$

Determination of Water Soluble Extractive Value

Weigh accurately 1.0 gm CAPROS™ in 100 ml flask and extract with water (25 ml x 4) over a steam water bath. Filter the extract using a laboratory grade filter paper and collect clear filtrate and dry it in an oven at 100-110°C to constant weight. Calculate the water-soluble extractive value as follows.

$$\% \text{ Water Soluble Extractive Value} = \frac{\text{Weight of Residue in GM} \times 100}{\text{Weight of Material Taken in GM}}$$

Quantification of Gallic Acid

The content of Gallic Acid can be determined by using a linear regression equation obtained from the calibration graph plotted between concentration and the area of standard Gallic Acid

Note . The method provides reproducible results. Occasionally, the base line peak may be divided into two peaks, which does not interfere with the final results.

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U P S T No : SR-0229952 dt. 01-04-1991, C.S.T. No.: SR-5133183 dt. 10-04-1991

Stability study of Capros® after Pasteurization and Retort treatment

Study No. NAT/CHEM/08/08

DATE: July' 2008

Conducted at

Research and Development Center

Natreon Inc. (India)

CL 18A, Salt Lake City, Sector II

Kolkata 700 091

Project Staff Signatures

General Manager **Dr. Runa Ghosh**

.....

Date:.....

Investigators **Dr. Dipankar Banerjee**

.....

Date:.....

Dr. Sauryya Bhattacharyya

.....

Date:.....

Co-Investigator **Dr. Debashis Pal**

.....

Date:.....

Project Associates **Mr. Partha Ganguly**

.....

Date:.....

Mr. Amartya K Gupta

.....

Date:.....

Miss Piali Nath

.....

Date:.....

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Preface

General

Study conducted at: **Natreon Inc.**
CL18A, Sec-II, Salt Lake City
Kolkata 700 091, INDIA

Project No.: NAT/CHEM/08/07

Test item: **Capros® [Lot CA0806104]**

Title **Stability study of Capros® after Pasteurization and Retort treatment**

Project Staffs

Investigators **Dr. Dipankar Banerjee**
Dr. Sauryya Bhattacharyya

Co-Investigator **Dr. Debashis Pal**

Project associates **Mr. Partha Ganguly**
Mr. Amartya K Gupta
Miss Piali Nath

Schedule

Initiation of study	July, 2008
Completion of study	July, 2008
Final report	July, 2008

Quality Assurance

The study was conducted in accordance with good laboratory practice guidelines and analytical results were obtained from instruments conforming good laboratory practice softwares.

Amendment Procedures

This protocol can be amended at the discretion of the General Manager. Changes or revisions of this project protocol will be documented stating reasons, signed by the investigators.

Deviation from project protocol

Nil

Archiving

The following records are stored in the scientific archives Natreon, Inc., Kolkata according to GLP regulations:

i) Copy of the project protocol

ii) All raw data generated during study period

iii) A signed copy of final report

iv) Record of samples

Objective

The objective of the present study was to establish the stable nature of Capros® after going through processes like pasteurization and Retort treatment.

Materials and Methods

1. Characterization of test item

Name	**Capros® [CA0806104]**
Colour	Yellow
Physical state	solid
Storage	stored *in vacuo* at room temperature

2. Pasteurization of Capros®

Capros® was dissolved in Milipore water to prepare stock solutions of 1 and 10 mg/ml. The solutions were heated at 65°C for 30 minutes in a closed vessel (Pasteurization). HPLC-PDA analyses as well as determination of antioxidant profile of the samples before and after treatment were done.

3. Capros® undergoing retort (autoclave) treatment

Capros® was dissolved in Milipore water to prepare stock solutions of 1 and 10 mg/ml. The solutions were autoclaved for 15, 30 and 60 minutes separately at 15 psi pressure (≈ 120°C). HPLC-PDA analyses as well as determination of antioxidant profile of the samples before and after treatment were done.

4. Conditions of HPLC of Capros®

Column – NovaPak RP C_{18} [150 x 3.9 mm, 4 μm] (Waters)

Mobile phase – 0.1 M Sodium acetate (pH 3.9)

Flow rate – *0.6 ml/min (isocratic mode)*

Detection – PDA 280 nm

5. *In vitro* antioxidant assays

5.1. ABTS radical cation decolorisation assay

The assay was performed according to the method of Pellegrini *et al.* (2003). Briefly, neutralization of the radical and reduction of color at 734 nm by the samples was measured and calculated with respect to the control. The results were expressed as IC_{50} of the samples.

5.2. DPPH radical decolorisation assay

The assay was performed according to the method of Gulcin *et al.* (2004). Briefly, neutralization of the radical and reduction of color at 517 nm by the samples was measured and calculated with respect to the control. The results were expressed as IC_{50} of the samples.

Results

Pasteurization process: The HPLC profile of Capros® remained nearly unchanged after going through pasteurization process in both the concentrations (Fig. 1). This was also substantiated by the similar IC_{50} values of Capros® in *in vitro* antioxidant assays before and after treatment (Fig.2).

Retort process: The chromatographic profile changed to a certain extent after being subjected to retort process, in both the concentrations, as evident from HPLC analyses (Fig. 3). However, in spite of change in HPLC chromatogram the antioxidant profiles remained nearly same, after 15 or 30 minutes retort treatment, and deteriorated slightly after 60 minutes of treatment (Fig. 4). During the retort treatment, pedunculagin and punigluconin of Capros® was destroyed and a new peak appeared (t_R ~2.8 min). However, the nature of the LMwHT viz., the Emblicanins remained unchanged but their proportions varied. This indicated that the Emblicanins may play a crucial role in maintaining the antioxidant capacity of Capros®.

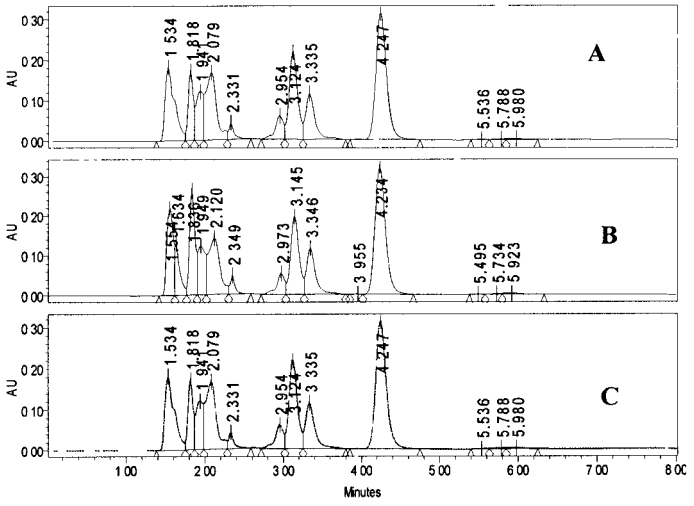

Figure 1 – Comparative HPLC profile of Capros® after pasteurization [A=Capros®; B=Capros® (1 mg/ml) after pasteurization; C= Capros® (10 mg/ml) after pasteurization]

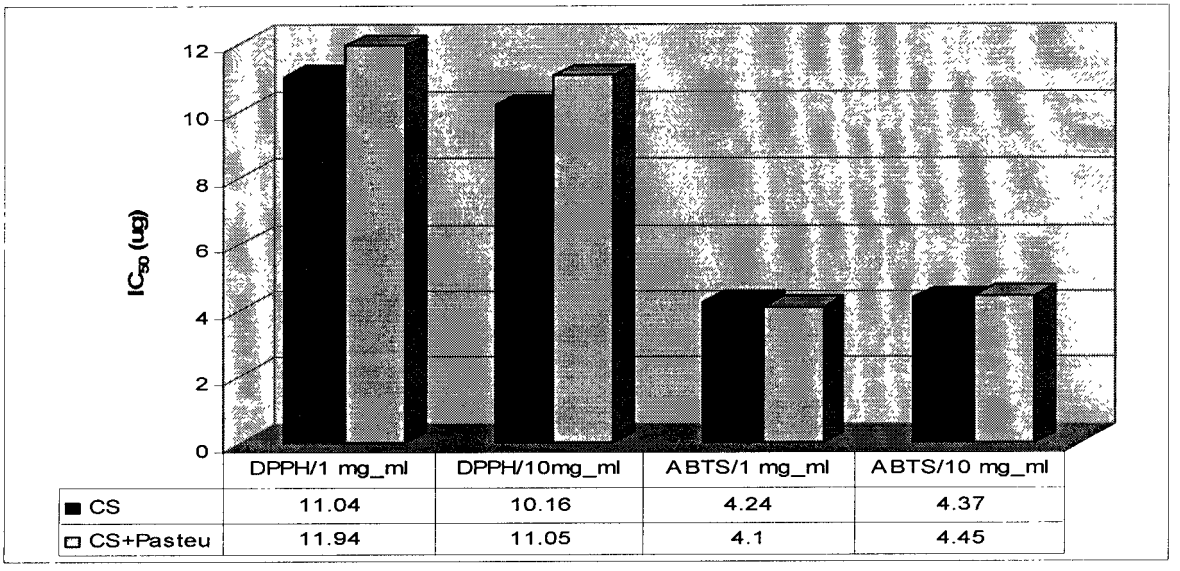

Figure 2 – Change in the antioxidant profile of Capros® after pasteurization [CS = Capros®; Pasteu = pasteurization at 65°C for 30 minutes]

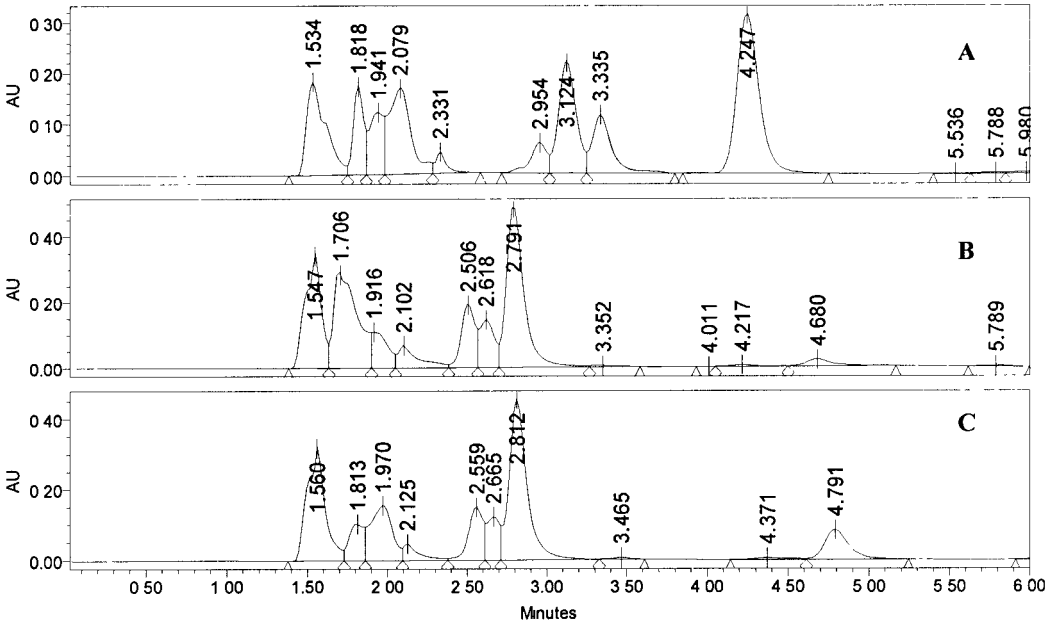

Figure 3 – Comparative HPLC profile of Capros® after retort treatment for 60 minutes [A=Capros®; B=Capros® (1 mg/ml) after retort treatment; C= Capros® (10 mg/ml) after retort treatment]

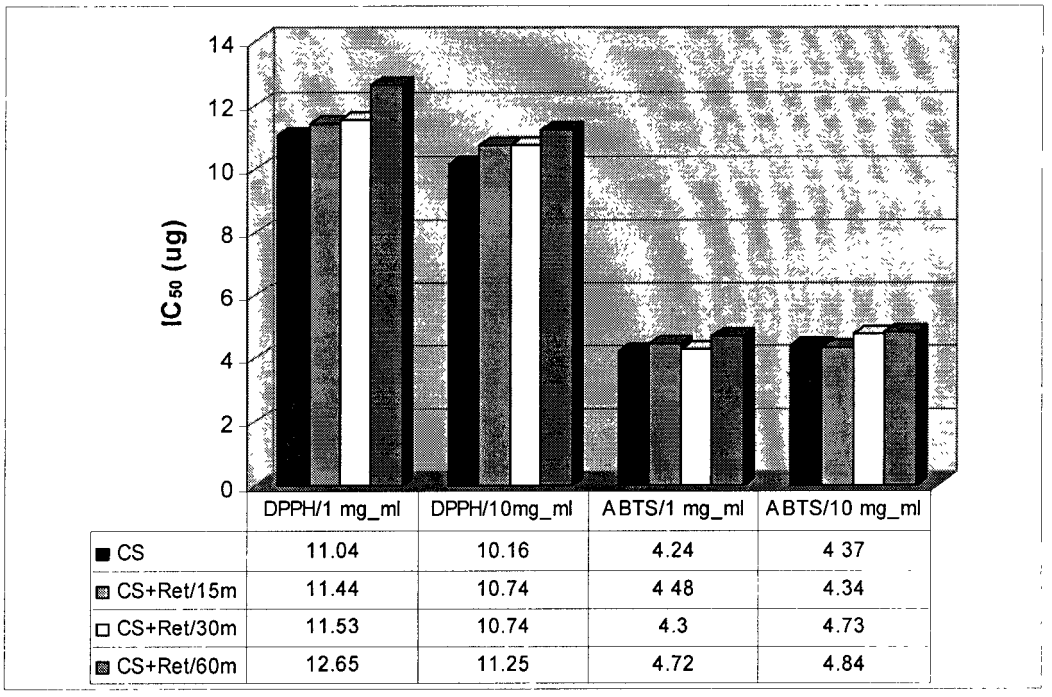

Figure 4 – Change in the antioxidant profile of Capros® after retort treatment [CS = Capros®; Ret = retort treatment]

Conclusion

Chromatographic profile of Capros® remained unchanged after pasteurization whereas the profile changed to a certain extent after retort process at both higher and lower concentrations. After retort treatment, peaks of pedunculagin and punigluconin disappeared, a new peak at t_R ~2.8 minute appeared. The peaks of Emblicanins remained unchanged though their proportions varied. It was also observed that the antioxidant profile remained nearly unchanged after pasteurization and negligibly changed after retort treatment. The generated peak at t_R ~2.8 minute needs to be investigated further as it appeared to contribute a part of the antioxidant activity. Thus, the present study indicated that after pasteurization and retort process the activity of Capros, in terms of its antioxidant activity was not significantly altered. It could be well utilized in such nutritional industries where high temperature or pressure is needed for preparative purpose.

References

- **Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M. and Brighenti, F. 2003. *J. Nutr.*, 133, 2812-2819.**
- **Gulcin, I., Kufrevioglu, O.I., Oktay, M. and Buyukokuroglu, M.E. 2004. *J. Ethnopharmacol.*, 90(2-3), 205-215**

APPENDIX B

Natreon Certificates of Analysis

Certificate of Analysis

CAPROS®: A standardized extract of *Phyllanthus emblica* fruits

Batch No.:	CA0806098
Date of Manufacturing:	June 2008
Date of Testing:	July 2008

Product Description

Appearance:	Free-flowing powder
Color:	Yellow
Odor:	Characteristic
Taste:	Astringent
Water-soluble extractive value:	≥ 80% w/w (Found 96.90%)

Analytical Profile	Specifications	Results
Identity	Conformance	Complies
Assay (by HPLC/HPTLC)		
Low molecular-weight hydrolysable tannins	≥ 60% (w/w)	61.00%
Water (Karl Fischer)	≤ 6% (w/w)	3.25%
Sulfated ash	≤ 6% (w/w)	5.80%
Heavy Metals		
Lead (Pb)	≤ 0.0002 %	0.000050 %
Arsenic (As)	≤ 0.0001 %	0.000043 %
Mercury (Hg)	≤ 0.0001 %	0.000052 %
Microbiological profile		
Aerobic bacteria (no pathogen)	≤ 5000 CFU/g	100 CFU / g
Yeast and mold	≤ 1000 CFU/g	50 CFU / g
Candida albicans	absent in 1 g	nil
Escheria coli	absent in 1 g	nil
Pseudomonas aeruginosa	absent in 1 g	nil
Salmonella	absent in 10 g	nil
Staphylococcus aureous	absent in 1 g	nil

Storage:	Store in original, sealed containers at +15° to +25 °C, avoid light
Stability:	3 years

Quality Assurance

Certificate of Analysis

CAPROS®: A standardized extract of *Phyllanthus emblica* fruits

Batch No.:	CA08060100
Date of Manufacturing:	June 2008
Date of Testing:	July 2008

Product Description

Appearance:	Free-flowing powder
Color:	Yellow
Odor:	Characteristic
Taste:	Astringent
Water-soluble extractive value:	≥ 80% w/w (Found 96.50%)

Analytical Profile	Specifications	Results
Identity	Conformance	Complies
Assay (by HPLC/HPTLC)		
Low molecular-weight hydrolysable tannins	≥ 60% (w/w)	61.00%
Water (Karl Fischer)	≤ 6% (w/w)	3.75%
Sulfated ash	≤ 6% (w/w)	5.86%
Heavy Metals		
Lead (Pb)	≤ 0.0002 %	0.000058 %
Arsenic (As)	≤ 0.0001 %	0.000010 %
Mercury (Hg)	≤ 0.0001 %	0.000019 %
Microbiological profile		
Aerobic bacteria (no pathogen)	≤ 5000 CFU/g	≤10 CFU / g
Yeast and mold	≤ 1000 CFU/g	≤10 CFU / g
Candida albicans	absent in 1 g	nil
Escheria coli	absent in 1 g	nil
Pseudomonas aeruginosa	absent in 1 g	nil
Salmonella	absent in 10 g	nil
Staphylococcus aureous	absent in 1 g	nil

Storage: Store in original, sealed containers at +15° to +25 °C, avoid light
Stability: 3 years

Quality Assurance

Certificate of Analysis

CAPROS®: A standardized extract of *Phyllanthus emblica* fruits

Batch No.:	CA08060104
Date of Manufacturing:	June 2008
Date of Testing:	July 2008

Product Description

Appearance:	Free-flowing powder
Color:	Yellow
Odor:	Characteristic
Taste:	Astringent
Water-soluble extractive value:	≥ 80% w/w (Found 96.10%)

Analytical Profile	Specifications	Results
Identity	Conformance	Complies
Assay (by HPLC/HPTLC)		
Low molecular-weight hydrolysable tannins	≥ 60% (w/w)	62.00%
Water (Karl Fischer)	≤ 6% (w/w)	3.70%
Sulfated ash	≤ 6% (w/w)	5.81%
Heavy Metals		
Lead (Pb)	≤ 0.0002 %	0.000047 %
Arsenic (As)	≤ 0.0001 %	0.000005 %
Mercury (Hg)	≤ 0.0001 %	0.000009 %
Microbiological profile		
Aerobic bacteria (no pathogen)	≤ 5000 CFU/g	≤10 CFU / g
Yeast and mold	≤ 1000 CFU/g	≤10 CFU / g
Candida albicans	absent in 1 g	nil
Escheria coli	absent in 1 g	nil
Pseudomonas aeruginosa	absent in 1 g	nil
Salmonella	absent in 10 g	nil
Staphylococcus aureous	absent in 1 g	nil

Storage: Store in original, sealed containers at +15° to +25 °C, avoid light
Stability: 3 years

Quality Assurance

APPENDIX C

English Translations of Selected Indian References

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APPENDIX D

Scientific Studies & Assessment Addressing Safety of CAPROS® & Amla

D-1. Animal and *in vitro* Testing on CAPROS® and Amla

As reported by Ojha, the safe history of use of amla as a food for health advantage and as a component in rasayanas, in blends, and as extracts for both preventive and curative purposes has diminished the perceived need for detailed scientific inquiry to establish the safety of amla in more recent times. Even so, there has been interest in better understanding what health consequences are available from amla and how it functions as it does. These interests have prompted *in vitro* studies and animal studies to be performed principally to demonstrate efficacy and possible mechanisms of action.

Safety testing has been performed on the antioxidant-rich extract of the fruit of *Emblica officinalis*[17]--- CAPROS®--- and this includes mutagenicity testing, acute testing (LD_{50}), and subchronic testing, to bolster the safety findings gathered from the long history of use of *Emblica officinalis* as a fresh or dried fruit.

CAPROS® Ames Test. Utesch conducted a genotoxicity study on the fruit extract of *Emblica officinalis* using *Salmonella typhimurium* and *Escherichia coli* strains (Utesch, 2001). It was concluded that the material tested was not mutagenic. See Appendix E.

CAPROS® Acute Toxicity. Product Safety Labs performed acute oral toxicity testing on CAPROS® and reported the LD_{50} of >5,000 mg/kg (Product Safety Labs, 2000). Ten healthy rats, 5 male and 5 female rats, were tested with observations being made over 14 days. All animals survived, gained weight and appeared to be healthy with no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for the animals when necropsied after the 14-day observation period. See Appendix F.

CAPROS® Subchronic Toxicity. A 90-day feeding study using CAPROS® was undertaking using both rodent (rat) and non-rodent (rabbit) species (Muruganandam and Das, 2000). Two test groups per species were studied with dose levels of 100 mg/kg bw/day and 500 mg/kg bw/day. The food and water intake was measured, as was the total body weight and organ weights. Hemopoietic, hepatic, and renal systems were monitored. Decreases in glutamic oxaloacetic transaminase (GOT), blood urea nitrogen (BUN), and creatinine levels were seen at some doses at 45 or 90 days after treatment. Decreases in these parameters are believed to be non-specific and are not indicative of any adverse effect. The contract laboratory at which this study was conducted indicated that the absolute levels of these parameters measured are within historical values seen for these laboratory animals at the facility. It was concluded that CAPROS® use was safe without any toxicity effects detected under the conditions of the test. See Appendix G.

Amla Protective Effects. Studies with amla extract, fresh amla fruit, and the fresh juice from amla revealed various protective effects without observed adverse effects as reported by several investigators.

Amla extract at levels of 20 mg/kg bw/day and at 40 mg/kg bw/day when administered over 20 days to streptozotocin-induced diabetic rats were effective in relieving oxidative stress (Rao, et al, 2005). Administering the same material at the same dosages was also observed to be effective in treating hypercholesterolemia in cholesterol-fed rats and in reducing the effects of atherosclerosis (Kim, et al, 2005).

[17] Ghosal has demonstrated that the antioxidant-rich fraction that is extracted from amla via his patented process to yield CAPROS® is no less than 4.2% by weight of the native fresh fruit (Ghosal, 2000).

A 16-week feeding study with rabbits using pulp from fresh amla fruit at a level of 1g/kg bw/day revealed that the animals grew well without adverse effects, and the amla successfully reduced cholesterol levels (Thakur and Mandal, 1984). Lipid-lowering effects were also noted by Mathur, et al. (Mathur, et al., 1996) upon administering fresh amla fruit juice at a level of 5 mL/kg per day to cholesterol-fed rabbits over a period of 60 - 120 days. Plaque regression was observed, and serum and tissue lipid levels were decreased, suggesting that amla fruit may be an effective hypolipidemic agent.

Nandi, et al. investigated the effects of the amla fruit extract on reducing cytotoxicity in mice following the administration of a known carcinogen (Nandi, et al., 1997). The dried amla fruit extract of unknown composition and quantity was administered over 28 days following the administration of the carcinogen. The subject material was found to protect against cancer and counteracted the cytotoxic effects by inhibiting nitrosamine formation. Jose, et al. also reported that aqueous extracts of amla and chyawanprash (precise compositions uncertain) were established to be cytotoxic to certain cancer cells in culture above a threshold level, and the extract reduced ascites and solid tumors in mice when orally administered at a level of 1250 mg/kg bw/day. The animal lifespan was extended by 20-61% (Jose, et al., 2001).

Other protective effects without noted adverse effects associated with amla consumption were observed in rats and mice. Jose and Kuttan reported that amla extract of unknown composition when administered to rats at a level of 500 mg/kg bw/day inhibited the liver damage induced by CCl_4. Various biochemical liver changes produced by the CCl_4 were reversed or prevented (Jose and Kuttan, 2000). In a similar study, Roy, et al. reported that 685 mg/kg bw/day of a crude extract of *E. officinalis* when administered for 7 days to mice counteracted the hepatotoxic and renotoxic effects of lead nitrate and aluminum sulfate (Roy, et al., 1991).

In his book devoted to the comprehensive understanding of chyawanprash and key aspects that impart health and well-being to those consuming this rasayana, Ojha summarizes the literature findings of additional animal testing (Ojha, 2003, Chapter IV). He points out that antistress and immunomodulator properties have been linked with chyawanprash with its high *E. officinalis* content. The ability to alleviate toxic manifestations of anticancer drugs and other chemicals was cited, and both the antioxidant character and anti-aging properties were recognized.

Except for the CAPROS® rat and rabbit studies summarized above, the precise compositions of the materials administered to the animals is either not provided or cannot be determined from the information supplied.

D-2. Toxicology of Chemical Constituents

A toxline search was conducted on the five chemical substances specifically identified in Table 3. No information was found on emblicanin A, emblicanin B, or punigluconin.

While some information was located on pedunculagin, no traditional toxicology studies were found. One study reported that pedunculagin is a potent inhibitor of the enzyme DNA topoisomerase II and, thereby, may exhibit some antitumor activity (Kashiwada, et al., 1993). In another study, pedunculagin was found to inhibit the mutagenic activity of Trp-P-2 and N-OH Trp-P-2 (Okuda, et al., 1985).

Much more information is available on rutin. Rutin is a glycoside of the flavanoid, quercetin, which is present in many fruits and vegetables, as well as tea leaves. Quercetin is a substance that is present in the diet at levels up to 200 mg per day. Rutin is hydrolyzed to quercetin in the gastrointestinal tract by

various enzymes. There have been many promising studies on the anti-carcinogenic activity on quercetin and rutin (Yang et al., 2000).

D-3. Human Experience & Testing

As was the case with the Animal and *in Vitro* Testing discussed above, there has been much interest in establishing the efficacy of *Emblica officinalis* antioxidant extracts regarding human uses. Here, too, one encounters references to human testing of varying durations and dosages.

Table 7 contains a compilation of human study results following the administration of *Emblica officinalis* in the form of the dried fruit, the raw fruit or pulp, or as an antioxidant-rich extract. In some cases, it is unclear as to the actual compositions that were tested. With test materials identified as chyawanprash, the dosage was first converted to amla content (which comprises 90% by weight of chyawanprash), which is subsequently converted to the antioxidant extract equivalent by using the 4.2% conversion reported by Ghosal (Ghosal, 2000) as the actual yield due to the extraction process. The true percentage of the extract found in the native fruit could be slightly larger, so the estimated daily exposures reported for some entries in Table 7 could be slightly understated.

Table 7. Summary of Selected Human Testing Studies with *Emblica officinalis* and Its Extract

Reference	No. of Subjects	Est. Daily Exposure EO Extract (mg)	Duration	Comments
Pharma Intel, 2002	24 24	500 mg as CAPROS® 1,000 mg as CAPROS®	6 w 6 w	Exhibited reduced total cholesterol & LDL, but not dose-related; no serious adverse effects, but minor effects---weakness, irregular bowels, backache---reported by 3 subjects.
Yadav, et al, 2003	25 m	1512[a] mg as EO extract	2 m	Genoprotective effects observed.
Manjunatha, 2001	10 m	567[b] mg as EO extract	8 w	Effective in lowering blood sugar & improves blood lipid profile but statistically weak; no adverse effects noted.
Paranjpe, et al., 2000	22	7.6[c] mg as EO extract	28 d	Blend of materials imparted significant benefit in treatment of piles.
Chawla, et al, 1982	38	378[d] mg as EO extract	28 d	Effective in treating dyspepsia; loose stools & vomiting reported by 3 patients.
Jacob, et al., 1988	35 m	2100 mg as EO extract[e]	28 d	Effective in reducing cholesterol in normal & hyperlipidemic men, considering both LDL & VLDL; health benefit reversed after discontinuing administration of test material.
Mishra, et al., 1983	14	336 mg as EO extract[f]	60 d	Effective treatment for diabetes with no adverse effects.
Singh & Sharma, 1971	20	378 mg as EO extract[g]	7 d	Effective in treating gastric disorders; no adverse effects noted.
Chen et al,, 2009	17	450 mg in tablets	4 m	Reduced oxidative stress in uremic patients with no adverse effects noted.

[a] 40 g of chyawanprash, which was noted to be 90% amla, were administered; assuming that 4.2% amla is the antioxidant extract as reported by Ghosal (Ghosal, 2000), the calculated amount of EO extract is 1512 mg.
[b] 15 g of chyawanprash were administered; assuming that it is 90% amla as reported by Ojha (Ojha, 2003), of which 4.2% is EO extract as represented above, the calculated exposure to EO extract was 567 mg.
[c] 180 mg of amla powder were administered; this amount is equivalent to 7.6 mg of EO extract by using the above calculation.
[d] 9 g amla were administered, which corresponds to 378 mg EO extract.
[e] Based on administration of 50 g of amla.
[f] Based on administration of 8 g of amla.
[g] Based on administration of 9 g of amla.

The testing with *E. officinalis*, its antioxidant fraction extract, or chyawanprash focused on the beneficial health aspects that would be derived from its consumption. While safety was not the defined objective of these investigations, only minor adverse effects were reported. The majority of studies showed no adverse effects. In no case were the numbers of subjects very large, and the duration of testing in every case was less than 4 months and more commonly extended 4 to 8 weeks. The dosages considered were determined based on the probable *E. officinalis* extract equivalent which serves as a more direct comparator with CAPROS®. Furthermore, no dose-related effects emerged from these investigations.

The maximum daily exposure from Table 7 was found to be 2100 mg while dosages in the range of 300 to nearly 600 mg were more common. Dharmananda has reported that it is common for Indians to consume on a daily basis 10 - 30 g of chyanwanprash which he maintains consists of 15% amla when mixed with honey to yield a food with jam-like character (Dharmananda, 2006b). This chronic exposure to amla translates to a daily ingestion of 63 – 189 mg of the antioxidant extract of amla.

Dr. Tuhin Kanti Biswas from the J. B. Roy State Ayurvedic Medical College & Hospital in Kolkata, India, has supplied a testimonial (see Appendix H) without supporting information that proclaims that *Emblica officinalis* has been consumed by a variety of patients without adverse effects at daily levels of 3 to12.5 g. Noting that the antioxidant extract fraction constitutes 4.2% of the fresh fruit based on the Ghosal patented process, one estimates the level of antioxidant extract equivalent to be 126 to 525 mg per day.

The 1919 publication by Chick, et al, reports that daily consumption of 14 g of the dried fruit of *E. officinalis* was effective in preventing and curing scurvy (Chick, et al., 1919). The estimated content of the antioxidant extract contained in 14 g of the dried fruit is conservatively calculated to be 588 mg; this would be the amount if the fresh fruit were consumed. With the drying process, the amount of the antioxidant extract would surely be higher.

In Chapter V of his book on Chyanwanprash, Ojha summarizes clinical test results for a number of studies that address efficacy with various health conditions including adjuvant treatment of tuberculosis, the physiological metrics of inhibiting the aging process, radioprotective effects during chemotherapy, protective effects for smokers, immunomodulation, and treatment for depression (Ojha, 2003). Over half of the studies cited were unpublished, and the summaries in some cases omitted key study parameters that might otherwise allow some safety information to be extracted. The patient populations were a bit larger than those noted in Table 7, but duration of treatment was not noted in one case, while in other cases it was unclear as to the actual composition of test material and what the actual dosage was. Depending on the assumptions that one were to make, it appears that some of the clinical trials involved chyawanprash dosages that could possibly translate to antioxidant extract equivalents of 700, 900, 1700, and 1900 mg per day. Of those studies that noted test duration, most lasted from 4 to 13 weeks, while one had a component that lasted 12 months. The information reported was intriguing due to the possibly higher dosages and larger subject populations, but the summaries were generally inadequate in some important capacity and could not be used with confidence to augment the other human studies cited.

A number of companies today offer products that contain amla as the primary constituent or as a major component in a blend. These supplements provide amla as a powder (or as part of formulated products such as chyawanprash or triphala) at daily amla doses of up to 1600 mg (Anonymous, 2006b and see www.iherb.com).

Sabinsa Corporation recently announced that it had completed an independent GRAS determination for Saberry™, its amla extract, and Sabinsa recommends a daily use level of 200 – 1000 mg for its extract (see www.saberry.net/nutraceutical).

Taiyo Kagaku Co., Ltd. produces SunAmla, an amla-containing product, for Japanese markets while continuing to research the health effects of amla.

A casual search of "amla" on the Internet reveals a number of other firms that are serving as distributors of amla-containing dietary supplements that are available in the US. Himalayan Herbal Healthcare is a dominant Indian firm that sells amla-containing products. Metagenics carries a handful of products that contain amla, and other companies such as Tattva's Herbs, Vadik Herbs, Indian Herb Care, and Banyan Botanicals offer an array of supplements with amla.

APPENDIX E

Emblica Genotoxicity Study Report – Ames Test

MERCK

Art. 130165 – T14997 **Bacterial mutagenicity assay**

Toxicology

Study Report

Genotoxicity

Art. 130165 (Emblica ™) - Bacterial Mutagenicity Assay, Salmonella typhimurium and Escherichia coli

Author: D. Utesch

Report for: Merck KGaA
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Test facility: Merck KGaA
Pharma Ethicals
Preclinical R & D
Institute of Toxicology
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Date of approval: December 11, 2001

MERCK

Signature page

We hereby declare that the work described in this report was performed under our supervision and that the report provides a true and accurate record of the results obtained

Study Director	Dr. D. Utesch	Dec 11, 2001 Date
Director, Institute of Toxicology	Dr P J Kramer	Dec 12, 2001 Date

This document has been approved electronically. Additionally, a print-out of the document has been signed by the editing author and archived. A paper copy of the signature sheet is available on request

Responsible Personnel

Study Director	Dr. D. Utesch
Deputy Study Director	Dr. T. Broschard

Study Schedule

Date of initiation of study:	May 29, 2001
Start of experimental phase:	September 04, 2001
End of experimental phase.	September 20, 2001
Date of study completion:	December 11, 2001

Statement of compliance with GLP

The study was conducted in compliance with the most recent edition of the OECD Principles of Good Laboratory Practice and with the principles of Good Laboratory Practice according to Annex 1 German Chemicals Act and the Principles of Good Laboratory Practice of the EU. The GLP standards applied include also the requirements of Japanese, US and other major non-European guidelines.

No unforeseen circumstances were observed which might have affected the validity of the data and the quality or integrity of the study.

		December 11, 2001
Study Director	Dr. D. Utesch	Date

This document has been approved electronically. Additionally, a print-out of the document has been signed by the editing author and archived. A paper copy of the signature sheet is available on request

QAU statement

The Quality Assurance Unit (QAU) hereby declares that the study was inspected on the following dates and the findings of each audit were reported to the study director and the management.

The results reported in this study were checked on the basis of our current QAU-SOPs. This statement confirms that the final report reflects the raw data.

Date of inspection	Type of inspection	Date of report to study director and management
May 29, 2001	Study Protocol	May 31, 2001
July 04, 2001	Study Performance	July 10, 2001
September 24, 2001	Study Performance	September 24, 2001
October 19, 2001	Study Report	December 11, 2001

December 11, 2001

M.J. Sendler

Date

This document has been approved electronically. Additionally, a print-out of the document has been signed by the editing author and archived. A paper copy of the signature sheet is available on request.

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1. Summary

The investigations for mutagenic potential were performed using Salmonella typhimurium tester strains TA 98, TA 100, TA 102, TA 1535 and TA 1537, and Escherichia coli WP2 uvrA. The plate incorporation test with and without addition of liver S9 mix from Aroclor 1254-pretreated rats was used. Two independent experimental series were performed. In the two series with S9 mix, 10 % and 30 % S9 in the S9 mix were used in the 1st and 2nd series, respectively.

Art. 130165 was dissolved in water (aqua bidest) and tested at concentrations ranging from 5.00 to 5000 µg/plate. Precipitation of the test material on the agar plates and toxicity to the bacteria was not observed.

Daunomycin, N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine and cumene hydroperoxide served as strain specific positive control compounds in the absence of S9 mix 2-Aminoanthracene and benzo[a]pyrene were used for testing the bacteria and the activity of the S9 mix. Each treatment with the substances used as positive controls led to a clear increase in revertant colonies, thus, showing the expected reversion properties of all strains and good metabolic activity of the S9 mix used.

With and without addition of S9 mix as the external metabolizing system, Art. 130165 was not mutagenic under the experimental conditions described.

2. Introduction

The present study was conducted to investigate the test material for mutagenic potential in a bacterial reverse gene mutation assay.

The applied in vitro technique, described by Ames et al. (1975), Green and Muriel (1976), Maron and Ames (1983) and Utesch et al. (1987), is an internationally accepted standard procedure. The study was designed to comply with the following guidelines: ICH Guidelines, EEC Commission Directive, the OECD Guidelines, and the Requirements of the Labor Ministry of Japan - Notification dated June 13, 1979 - and the Notification No. 1-24 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare of Japan, dated September 11, 1989 (cf. References).

3. Materials and methods

3.1 Test material

Name of test material:	Art. 130165 (Emblica ™)
Batch No.:	4003
CAS No.:	90028-28-7
Indication:	cosmetic skin care
Description:	*Phyllanthus emblica* fruit extract
Supplier:	Merck KGaA, Darmstadt, CHN, COS
Analytical Report:	Merck KGaA, Darmstadt, G AL, Dr. Schewitz
Date of release:	July 12, 2001
Released until:	July 31, 2003

Solvent: Aqua bidest. (H_2O)

The solutions were prepared directly before use.

3.2 Negative and positive controls

The negative control consisted of the solvent used for the test material (c.f. 3.1). Untreated negative controls were not tested.

The following strain specific positive control compounds were used:

Name	Abbreviation	Supplier	Solvent
Daunomycin	DAUN	Fluka 30450	H_2O
N-Ethyl-N'-nitro-N-nitrosoguanidine	ENNG	Aldrich E4 150-5	DMSO
9-Aminoacridine	9-AA	Sigma A-7295	Ethanol
Cumene hydroperoxide	CUM	Sigma C-0524	DMSO
2-Aminoanthracene	2-AA	Sigma A-1381	DMSO
Benzo[a]pyrene	B(a)p	Fluka 12780	DMSO

3.3 Solvents in general

Preferentially distilled water or dimethyl sulfoxide (DMSO), alternatively acetone or ethanol, are used as solvents. Analysis of the historical data of the laboratory and experience of other research groups (Maron et al. 1981) showed that the amounts of the selected solvents used have no influence on the number of spontaneous revertants of any strain. For this reason only the respective solvent control was used as the negative control in this study.

Since on the one hand organic solvents may have diverse effects on e.g. gene regulation and, on the other hand, high amounts of water (added as the solvent) will dilute the top agar, usually the maximum amount of solvent is limited to 100 µl per plate for water and 10 µl per plate for DMSO, ethanol, acetone or other organic solvents. Only the highest test material concentration may be plated with either 316 µl water or 31.6 µl organic solvent.

3.4 Strains tested

3.4.1 Salmonella typhimurium strains

Salmonella typhimurium TA 98, TA 100, TA 102, TA 1535, and TA 1537.

Origin of Salmonella strains:
The Salmonella strains TA 98, TA 100, and TA 1535 were obtained from B. N. Ames, University of Berkeley, California, USA, on August 15, 1985. Salmonella typhimurium TA 102 was obtained from B. Diener, Institute of Toxicology, University of Mainz, Germany, on June 24, 1992. Salmonella strain TA 1537 was obtained from J. Hengstler, Institute of Toxicology, University of Mainz, Germany, on December 8, 2000.

Genotype of Salmonella strains:
TA 98 (his D 3052, uvrB, rfa + R-factor)
TA 100 (his G 46, uvrB, rfa + R-factor)
TA 102 (his G 428, rfa + R-factor)
TA 1535 (his G 46, uvrB, rfa)
TA 1537 (his C 3076, uvrB, rfa)

All these strains contain mutations in the histidine operon, thus imposing a requirement for histidine in the growth medium.

His G 46 is a substitution mutation which is reverted to prototrophy by a large variety of mutagens inducing base pair substitutions.

His D 3052, in contrast, is a frameshift mutation which is reverted by e.g. a deletion of two base pairs -CG-/-GC-. It is easily reverted by aromatic amines and their derivatives.

His C 3076 is a frameshift mutation as well. It appears to have a run of C's at the site of mutation. It is well reverted by carcinogens such as 9-aminoacridine.

His G 428 is an ochre mutation, -TAA-/ATT-, in the his G gene which is located on the extrachromosomal multicopy plasmid pAQ1. It is reverted by mutagens such as formaldehyde, glyoxal, various hydroperoxides, bleomycin, phenylhydrazine, streptonigrin and cross-linking agents such as psoralens and mitomycin C

In addition to the mutation in the histidine operon, TA 98, TA 100, TA 1535, and TA 1537 contain two further mutations that greatly increase their sensitivity to mutagens. The deletions uvrB and rfa cause a loss of the excision repair and a partial loss of the lipopolysaccharide barrier which makes them more permeable to chemical agents and completely non-pathogenic. The tester strain TA 102 contains the deletion rfa but lacks the deletion uvrB.

Differences between TA 1535 and their corresponding strain TA 100 are due to the plasmid pKM 101 (R-factor). The presence of this R-factor in TA 100 causes much greater sensitivity to a number of carcinogens and, additionally, resistance to ampicillin In addition to the R-factor plasmid, pKM 101, the tester strain TA 102 contains also the multicopy plasmid, pAQ1, which carries the his G 428 mutation and a tetracycline resistance gene.

3.4.2 Escherichia coli WP2 uvrA

Origin of the Escherichia coli strain:
Escherichia coli WP2 uvrA was distributed by the National Collections of Industrial & Marine Bacteria Ltd., Aberdeen, Scotland (NCIMB 11188 from Aug. 18, 1977) and obtained from H. Träger, Knoll AG, Ludwigshafen, Germany, on December 23, 1994.

Genotype of Escherichia coli strain.
This strain carries a defect in one of the genes for tryptophan biosynthesis, imposing a requirement for tryptophan in the growth medium. Strain WP2 uvrA not only possesses the tryptophan mutation, but also lacks excision repair (uvrA), so that it is more readily mutated by treatment with UV, for instance.

Tryptophan-independent revertants may arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed This second possibility may occur in several different ways so that the system seems capable of detecting all types of mutagens which substitute one base for another.

The tryptophan reversion system does not detect agents causing frameshift mutations

3.4 3 Storage conditions

The tester strains obtained were cultured overnight in nutrient broth. A large batch of samples taken from these cultures was frozen. For freezing 0.8 ml of the broth culture and 0.07 ml DMSO were mixed in sterile screw-cap vials which were then transferred into liquid nitrogen. One of these samples of each strain was permanently stored as the master copy, while the others (= frozen permanents) were used in the individual experiments.

3.4.4 Preparation of bacteria suspension

Fresh suspensions of the tester strains were prepared for each day's experiments. For each strain, 100 ml Standard I Nutrient Broth (MERCK, Article-No. 7882) were inoculated by 500 µl of a frozen permanent culture and incubated overnight in a + 37°C shaking incubator. For the R-factor strains, ampicillin was added to the nutrient broth (25 µg/ml). S. typhimurium TA 102 was incubated overnight in the presence of 200 µg tetracycline per 100 ml nutrient broth.

Cell density of the overnight cultures was checked by measuring the optical density of the cell suspensions and corrected if necessary. The suspensions were then maintained at ice-bath temperature until use

3.5 Exogenous metabolizing system (S9 mix)

3.5.1 Preparation of liver homogenate (S9)

S9 is routinely prepared in our laboratory following the proposals of Ames et al. (1975). Male Wistar, HSdCpb:Wu rats from Harlan Winkelmann, Borchen, Germany, aged 6-8 weeks, were given a single intraperitoneal injection of Aroclor 1254 (500 mg/kg body weight) diluted in Miglyol 812 oil (MERCK, Article-No. 275707). The animals received drinking water and a standard diet ad libitum. About 16 hours before sacrifice, the feed was removed. Five to seven days after application of Aroclor, the rats were sacrificed and the livers collected in ice-cooled, sterilized beakers containing 0.15 M KCl. The livers were homogenized in a sterile glass potter homogenizer containing 3 ml of 0.15 M KCl per gram of liver wet-weight. The homogenate was spun at 9000 x g for 10 minutes at about +4°C and the supernatant fluid was decanted and transferred into sterilized and precooled plastic tubes. The S9 was then frozen in liquid nitrogen and stored at -196°C.

The batch of the liver homogenate fraction (S9) used in this study was prepared at the Institute of Toxicology of MERCK KGaA, Darmstadt, in June, 2001. The acceptance criterion (cf. 3.5.2) was entirely met.

3.5.2 Composition and characterization of S9 mix

The mixture of S9 plus the added cofactors is termed S9 mix.

Components	Quantity per ml S9 mix	
	1st Series	2nd Series
Liver homogenate (S9)	0.10 ml	0.30 ml
$MgCl_2$/KCl aqueous solution (0.4 M/1.64 M)	0.02 ml	0.02 ml
Glucose-6-phosphate, disodium salt	5 µmol	5 µmol
NADP, disodium salt	4 µmol	4 µmol
Sodium phosphate buffer (0.2 M, pH 7.4)	0.50 ml	0 50 ml
Distilled water	0.38 ml	0.18 ml

Every S9-batch is tested for its metabolic activity by the use of specific substrates, requiring different enzymes of the P450-isoenzyme family. The mutagenicity of 2-aminoanthracene, benzo[a]pyrene, and 3-methylcholanthrene is thus determined once for every S9-batch. Clear increases in the number of revertants (cf. 3.7 Interpretation of results) for S. typhimurium TA 98, TA 100, and TA 1537 with all positive controls and for TA 1535 with 2-aminoanthracene are used as an acceptance criterion for each S9-batch. In this study 2-aminoanthracene, and benzo[a]pyrene for TA 102, are used as the concurrent positive controls for the different strains in the presence of S9 mix.

3.6 Test description

3 6.1 Technique for testing

Salmonella typhimurium and Escherichia coli strains were tested in accordance with the plate incorporation method described by Ames et al. (1975) and the OECD and EEC guidelines for this test system. The methods were extensively harmonized to follow the requirements of the Labor Ministry of Japan - Notification dated June 13, 1979 and the Notification No. 1-24 of the Pharmaceutical Affairs bureau, Ministry of Health and Welfare, dated September 11, 1989.

Number of plates used·
3 parallel plates were used for each concentration step of the test material and the positive controls. Twice as many solvent control plates were used for each bacterial strain.

Type of plates used:
Commercially available Merckoplate Minimal-Glucose-Agar plates (MERCK, Article-No. 13496) were used for all strains except for S. typhimurium strain TA 102 For that strain, Minimal-Glucose plates were self-plated using DIFCO Agar (DIFCO 0140-01).

Components added to each plate.	Quantity
Bacteria suspension	0.1 ml
Test material (or negative / positive controls)	0 1ml (0.01ml)
Sodium phosphate buffer (pH = 7.4; 0.2 M) (in studies without metabolic activation)	0 5 ml
S9 mix (in studies with metabolic activation)	0.5 ml
Top agar solution (incl. L-histidine. 7.75 mg/L; D-biotin, 12.2 mg/L, L-tryptophan 10.2 mg/L.)	2.0 ml

Incubation of plates was performed at + 37 °C for 2 to days.

Counting of revertant colonies:
Revertant colonies were either scored using an Artek MiniCount colony counter or manually. The presence of a background lawn of non-revertant cells was checked for each plate. Tables of individual and mean values are generated by use of a validated, automated data processing program (COLONY V2.31, York Electronic Research, York, UK).

3.6.2 Test material concentrations

The test material concentrations used were selected according to the EEC and OECD guidelines for this test system and the requirements of the Labor Ministry of Japan.

If the test material is soluble in the solvent of choice it should be investigated in the first experimental series at seven concentrations, separated by half-log intervals (i.e. a factor of √10), ranging from 5 to 5000 µg per plate. In the second experimental series, usually 5 concentrations including at least 4 non-toxic concentrations should be tested. Signs of toxicity to the bacteria are a reduction in the number of spontaneous revertants or a clearing of background lawn of non-revertant bacteria. Precipitation of the test material, if it occurs, should not interfere with scoring of the colonies. Depending upon the precipitation characteristics of the test material and toxicity data ascertained in the first series, high concentrations may be omitted or lower concentrations may additionally be tested in the second experimental series.

On the basis of the criteria stated above, the following test material concentrations were used for Art. 130165:
1st series: 5.00, 15.8, 50.0, 158, 500, 1580 and 5000 µg per plate
2nd series: 50.0, 158, 500, 1580 and 5000 µg per plate

3.7 Interpretation of results

The following interpretation criteria were predetermined in the Study Protocol.

3.7.1 Acceptance of negative and positive controls

According to the publications of Levin et al. (1982) and Kier et al. (1986) and the historical controls of the laboratory, usually the following mean numbers of revertants are acceptable as negative (or solvent) controls for the bacterial strains used:

TA 98 :	15 - 60	TA 1535:	3 - 37
TA 100:	75 - 200	TA 1537:	4 - 31
TA 102:	200 - 450	WP2 uvrA:	10 - 70

The positive control compounds should induce a "clear increase" in the number of revertants (see below).

3.7.2 Criteria for negative and positive results

Definitions:
The assessment of test material-induced effects is dependent on the number of spontaneous revertants of each bacterial strain (solvent controls) and the increase in the number of revertants at the test material concentration which shows the highest number of colonies. The following criteria, based upon the historical controls of the laboratory and statistical considerations, are established:

Mean Number of Colonies (Solvent Control)	Maximal Mean Number of Colonies over the Actual Solvent Control (Test Material)	
≤ 10	≤ 9	≥ 30
≤ 30	≤ 19	≥ 40
≤ 80	≤ 29	≥ 80
≤ 200	≤ 49	≥ 120
≤ 500	≤ 79	≥ 200
Assessment:	"No Increase"	"Clear Increase"

All further results, ranging between "no" and "clear", are assessed as "weak increases"

Interpretations:
A test material is defined as non-mutagenic in this assay if
- "no" or "weak increases" occur in the first and second series of the main experiment. ("Weak increases" randomly occur due to experimental variation.)

A test material is defined as mutagenic in this assay if
- a dose-related (over at least two test material concentrations) increase in the number of revertants is induced, the maximal effect is a "clear increase", and the effects are reproduced at similar concentration levels in the same test system,
- "clear increases" occur at least at one test material concentration, higher concentrations show strong precipitation or cytotoxicity, and the effects are reproduced at the same concentration level in the same test system.

In all further cases, a third test series with the bacterial strain in question should be performed. If the criteria for a positive test result are not fulfilled in at least two out of the three series, the test material is defined as being non-mutagenic in this test system.

3.8 Archive statement

After termination of the study, the study protocol, the raw data and the final report are stored in the archives at the Institute of Toxicology of Merck KGaA.

4. Results

Art. 130165 (Emblica ™) was examined for mutagenic activity in two series of in vitro microbial assays employing Salmonella typhimurium TA 98, TA 100, TA 102, TA 1535 and TA 1537, and Escherichia coli WP2 uvrA as indicator organisms The test material was tested directly and after metabolic activation by liver S9 mix obtained from rats pretreated with Aroclor 1254. Precipitation of the test material on the agar plates and toxicity to the bacteria was not observed. The individual and summarized data are presented in the tables section.

The validity of the mutation assay can be assessed by the results obtained for the negative and positive controls. The negative control mutant frequencies were all in the regular range (cf. section 3.7.1). Slight deviations from those values, if they occur, are accepted if they appear in only one test series and, furthermore, all other parameters of that series are in the regular range. The strain specific positive control compounds, namely daunomycin, N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, and cumene hydroperoxide in the absence of S9 mix, yielded the expected mutant frequencies that were greatly in excess of the negative controls. The genotype of the tester strains used was thus confirmed. 2-Aminoanthracene and benzo[a]pyrene, which require metabolic activation, were strongly mutagenic. This indicates that the exogenous metabolizing system used in the present investigation (S9 mix) was functioning. Thus, the demands predetermined in the study protocol and in section 3 of this report have been met in total.

In both series of experiments, each performed with and without the addition of rat liver S9 mix as the external metabolizing system, Art. 130165 (Emblica ™) showed no increase in the number of revertants of any bacterial strain (cf. "Definitions" in section 3.7.2). According to the criteria for negative and positive results predetermined in the Study Protocol (cf. also "3.7.2 Criteria for negative and positive results" in this report) Art. 130165 (Emblica ™) was not mutagenic under the described experimental conditions.

5. References

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TABLE / Series No. 1

Art. 130165: Individual and Mean Number of Revertant Colonies "14997

Strain	Concentr. [µg/plate]	+/- S9-Mix †	Mean revertant colonies per plate	SD*	Individual revertant colonies per plate
TA 98	Solvent	-	19	3	18, 17, 21, 21, 14, 21
	5	-	19	2	21, 20, 17
	15.8	-	21	8	16, 30, 17
	50	-	21	3	25, 20, 19
	158	-	16	6	22, 16, 11
	500	-	17	4	13, 18, 20
	1580	-	15	7	14, 9, 22
	5000	-	24	3	24, 21, 27
	Solvent	+	25	5	34, 20, 22, 28, 21, 25
	5	+	30	5	36, 26, 29
	15.8	+	23	3	25, 20, 24
	50	+	24	7	32, 21, 18
	158	+	24	9	32, 15, 26
	500	+	27	10	26, 38, 18
	1580	+	28	7	35, 28, 21
	5000	+	24	3	26, 21, 26
TA 100	Solvent	-	123	19	92, 128, 111, 123, 143, 144
	5	-	128	8	128, 136, 121
	15.8	-	120	4	125, 117, 119
	50	-	144	10	133, 151, 148
	158	-	113	2	115, 112, 112
	500	-	126	14	125, 113, 140
	1580	-	114	16	96, 122, 125
	5000	-	127	13	112, 133, 136
	Solvent	+	142	8	151, 129, 143, 144, 136, 148
	5	+	152	29	178, 120, 157
	15.8	+	138	9	138, 146, 129
	50	+	151	12	148, 164, 141
	158	+	153	9	152, 145, 163
	500	+	148	18	168, 145, 132
	1580	+	131	10	120, 140, 134
	5000	+	152	6	158, 147, 152

† : Test material incubated in the presence (+) or absence (-) of S9-Mix;
* : Standard deviation.

Table continued on next page.

TABLE 1 / Series No.: 1

Art. 130165: Individual and Mean Number of Revertant Colonies T14997

Strain	Concentr [µg/plate]	+/- S9-Mix #	Mean revertant colonies per plate	SD*	Individual revertant colonies per plate
TA 102	Solvent	-	306	17	276, 300, 306, 307, 323, 323
	5	-	300	12	296, 314, 291
	15.8	-	277	8	280, 268, 282
	50	-	284	26	256, 289, 308
	158	-	274	19	267, 296, 260
	500	-	302	3	301, 305, 299
	1580	-	304	22	298, 329, 286
	5000	-	364	25	362, 340, 390
	Solvent	+	297	28	274, 314, 302, 262, 287, 340
	5	+	283	28	256, 281, 312
	15.8	+	298	3	299, 295, 300
	50	+	265	16	247, 277, 272
	158	+	301	34	282, 341, 281
	500	+	340	41	293, 360, 366
	1580	+	264	25	236, 271, 285
	5000	+	267	40	259, 270, 333
TA 1535	Solvent	-	16	3	13, 16, 19, 12, 18, 19
	5	-	15	4	18, 17, 11
	15.8	-	17	2	19, 16, 17
	50	-	14	1	15, 13, 13
	158	-	17	2	15, 18, 17
	500	-	19	4	20, 22, 14
	1580	-	19	9	27, 9, 20
	5000	-	18	2	16, 20, 18
	Solvent	+	22	4	16, 24, 29, 20, 24, 20
	5	+	17	4	13, 21, 18
	15.8	+	17	6	24, 14, 14
	50	+	19	2	19, 20, 17
	158	+	20	4	21, 23, 15
	500	+	19	10	12, 31, 14
	1580	+	19	3	19, 17, 22
	5000	+	20	4	18, 18, 25

\# : Test material incubated in the presence (+) or absence (-) of S9-Mix;
* : Standard deviation.

Table continued on next page.

TABLE 1 / Series No.: 1

Art. 130165: Individual and Mean Number of Revertant Colonies ™14997

Strain	Concentr [µg/plate]	+/- S9-Mix #	Mean revertant colonies per plate	SD*	Individual revertant colonies per plate
TA 1537	Solvent	-	7	3	9, 8, 10, 6, 5, 3
	5	-	9	3	8, 12, 6
	15.8	-	8	3	7, 6, 11
	50	-	7	4	11, 4, 5
	158	-	7	2	8, 8, 5
	500	-	7	1	6, 8, 6
	1580	-	6	1	5, 7, 6
	5000	-	6	3	5, 4, 9
	Solvent	+	10	3	14, 10, 5, 11, 10, 11
	5	+	11	6	9, 17, 6
	15.8	+	13	3	10, 16, 12
	50	+	10	3	7, 11, 13
	158	+	11	1	12, 11, 11
	500	+	12	3	14, 13, 9
	1580	+	12	1	12, 12, 12
	5000	+	10	2	10, 12, 8
WP2 uvrA	Solvent	-	33	3	34, 35, 31, 31, 38, 30
	5	-	35	7	42, 35, 29
	15.8	-	45	9	55, 39, 41
	50	-	36	7	30, 43, 35
	158	-	39	6	46, 36, 34
	500	-	29	12	41, 28, 18
	1580	-	31	2	33, 32, 29
	5000	-	19	2	19, 17, 21
	Solvent	+	36	6	43, 44, 33, 32, 28, 36
	5	+	31	3	33, 32, 27
	15.8	+	40	3	36, 41, 42
	50	+	35	5	30, 34, 40
	158	+	32	6	39, 27, 30
	500	+	43	3	45, 40, 43
	1580	+	29	4	24, 30, 32
	5000	+	21	3	23, 18, 21

\# : Test material incubated in the presence (+) or absence (-) of S9 Mix;
* : Standard deviation.

End of table

TABLE 1 / Series No.: 2

Art. 130165: Individual and Mean Number of Revertant Colonies T14097

Strain	Concentr. [µg/plate]	+/- S9-Mix #	Mean revertant colonies per plate	SD*	Individual revertant colonies per plate
TA 98	Solvent	-	20	6	22, 14, 27, 17, 14, 27
	50	-	13	1	13, 13, 14
	158	-	19	3	19, 22, 17
	500	-	17	6	17, 22, 11
	1580	-	23	4	20, 22, 27
	5000	-	18	2	19, 15, 19
	Solvent	+	32	6	31, 29, 43, 31, 3[illegible], 26
	50	+	33	8	41, 26, 33
	158	+	29	6	35, 24, 28
	500	+	35	11	33, 25, 46
	1580	+	29	5	34, 25, 28
	5000	+	26	7	19, 32, 26
TA 100	Solvent	-	131	9	128, 141, 124, 136, 117, 138
	50	-	141	18	130, 130, 162
	158	-	128	16	145, 126, 114
	500	-	126	3	128, 127, 123
	1580	-	131	7	123, 132, 137
	5000	-	144	21	156, 157, 120
	Solvent	+	175	13	188, 170, 183, 177, 183, 151
	50	+	177	16	163, 174, 194
	158	+	176	6	174, 171, 182
	500	+	183	13	172, 180, 197
	1580	+	149	5	146, 155, 147
	5000	+	154	19	166, 132, 164
TA 102	Solvent		309	39	297, 290, 340, 305, 364, 255
	50	-	325	19	303, 336, 337
	158	-	326	29	321, 357, 299
	500	-	292	13	306, 281, 289
	1580	-	325	41	337, 359, 280
	5000	-	350	22	374, 346, 331
	Solvent	+	264	51	274, 327, 255, 185, L, 277
	50	+	292	43	337, 288, 251
	158	+	300	-	L, L, 300
	500	+	289	144	L, 187, 390
	1580	+	375	8	383, 376, 367
	5000	+	302	33	L, 279, 325

\# : Test material incubated in the presence (+) or absence (-) of S9-Mix;
* : Standard deviation.

L : lost due to experimen. error

Table continued on next page.

TABLE 1 / Series No : 2

Art. 130165: Individual and Mean Number of Revertant Colonies T14997

Strain	Concentr. (µg/plate)	+/- S9 Mix #	Mean revertant colonies per plate	SD*	Individual revertant colonies per plate
TA 1535	Solvent	-	27	4	18, 27, 28, 29, 30, 29
	50	-	25	3	22, 28, 25
	158	-	36	3	38, 33, 38
	500	-	29	7	30, 22, 35
	1580	-	28	5	34, 24, 27
	5000	-	27	4	26, 31, 24
	Solvent	+	28	5	35, 24, 32, 27, 23, 25
	50	+	31	3	31, 29, 33
	158	+	31	2	29, 33, 32
	500	+	23	1	22, 24, 24
	1580	+	22	4	18, 25, 23
	5000	+	30	4	31, 33, 25
TA 1537	Solvent	-	12	3	8, 11, 16, 14, 11, 10
	50	-	14	1	14, 14, 13
	158	-	11	3	10, 14, 9
	500	-	16	4	15, 20, 12
	1580	-	19	11	11, 14, 31
	5000	-	16	2	14, 18, 15
	Solvent	+	12	3	10, 8, 15, 15, 9, 12
	50	+	16	3	14, 16, 19
	158	+	11	2	12, 9, 12
	500	+	15	1	15, 15, 16
	1580	+	12	0	12, 12, 12
	5000	+	14	1	14, 15, 13
WP2 uvrA	Solvent	-	38	5	34, 40, 34, 36, 38, 48
	50	-	39	10	42, 47, 28
	158	-	41	8	47, 32, 44
	500	-	37	9	47, 29, 36
	1580	-	40	16	53, 22, 44
	5000	-	21	5	19, 17, 27
	Solvent	+	47	8	51, 52, 40, 43, 39, 59
	50	+	53	12	41, 54, 64
	158	+	32	8	36, 37, 23
	500	+	47	7	42, 43, 55
	1580	+	40	3	37, 43, 39
	5000	+	21	4	25, 21, 17

\# : Test material incubated in the presence (+) or absence (-) of S9-Mix;
* : Standard deviation

End of table

TABLE 2 / Series No.: 1

Positive Controls. Individual and Mean Number of Revertant Colonies 114997

Strain	Positive Control	Concentr. [µg/plate]	+/- S9-Mix #	Mean revertant colonies per plate	SD*	Individual revertant colonies per plate
TA 98	DAUN	1	-	573	71	626, 600, 492
TA 100	ENNG	5	-	717	77	673, 672, 806
TA 102	CUM	150	-	887	168	711, 904, 1045
TA 1535	ENNG	10	-	622	45	673, 604, 589
TA 1537	9-AA	50	-	368	30	340, 364, 400
WP2 uvrA	ENNG	5	-	1470	52	1524, 1465, 1420
TA 98	2-AA	2	+	371	44	322, 384, 407
TA 100	2-AA	2	+	556	44	506, 591, 571
TA 102	B(a)p	10	+	1046	10	1034, 1051, 1053
TA 1535	2-AA	2	+	188	9	194, 192, 178
TA 1537	2-AA	5	+	272	30	287, 238, 292
WP2 uvrA	2-AA	10	+	306	24	328, 309, 281

\# Test material incubated in the presence (+) or absence (-) of S9-Mix;
* Standard deviation.

DAUN Daunomycin
CUM Cumene hydroperoxide
2-AA 2-Aminoanthracene
ENNG N-Ethyl-N'-nitro-N nitroso-guanidine
9-AA 9-Aminoacridine
B(a)p Benzo(a)pyrene

TABLE 2 / Series No : 2

Positive Controls: Individual and Mean Number of Revertant Colonies T14997

Strain	Positive Control	Concentr. [µg/plate]	+/- S9-Mix #	Mean revertant colonies per plate	SD*	Individual revertant colonies per plate
TA 98	DAUN	4	-	940	21	920, 938, 961
TA 100	ENNG	5	-	1242	31	1206, 1261, 1259
TA 102	CUM	150	-	1105	43	1129, 1055, 1130
TA 1535	ENNG	10	-	994	80	912, 1072, 998
TA 1537	9-AA	50	-	534	164	426, 457, 723
WP2 uvrA	ENNG	5	-	1465	54	1476, 1406, 1513
TA 98	2-AA	2	+	281	20	297, 259, 286
TA 100	2-AA	2	+	549	52	496, 600, 550
TA 102	B(a)p	10	+	1008	95	1095, 906, 1024
TA 1535	2-AA	2	+	176	20	178, 156, 195
TA 1537	2-AA	10	+	214	41	250, 223, 170
WP2 uvrA	2-AA	10	+	258	33	226, 257, 291

\# Test material incubated in the presence (+) or absence (-) of S9-Mix;
* Standard deviation.

DAUN Daunomycin
CUM Cumene hydroperoxide
2-AA 2-Aminoanthracene
ENNG N-Ethyl-N'-nitro-N-nitroso-guanidine
9-AA 9-Aminoacridine
B(a)p Benzo(a)pyrene

TABLE 3 / Series No.: 1

Art. 130165: Summary of the Mean Number of Revertant Colonies T14997

Test Material	Concentration [µg/plate]	+/- S9-Mix	Mean revertant colonies / plate		
			TA 98	TA 100	TA 102
Solvent control		-	19	123	306
Art. 130165	5	-	19	128	300
	15.8	-	21	120	277
	50	-	21	144	284
	158	-	16	113	274
	500	-	17	126	302
	1580	-	15	114	304
	5000	-	24	127	364
Solvent control		+	25	142	297
Art. 130165	5	+	30	152	283
	15.8	+	23	138	298
	50	+	24	151	265
	158	+	24	153	301
	500	+	27	148	340
	1580	•	28	131	264
	5000	+	24	152	287
Positive controls	Name		DAUN	ENNG	CUM
	Conc. [µg/plate]	-	4	5	150
	Revert./plate		573	717	887
	Name		2-AA	2-AA	B(a)p
	Conc. [µg/plate]	+	2	2	10
	Revert./plate		371	556	1046

Test Material	Concentration [µg/plate]	+/- S9-Mix	Mean revertant colonies / plate		
			TA 1535	TA 1537	WP2 uvrA
Solvent control		-	16	7	33
Art. 130165	5	-	15	9	35
	15.8	-	17	8	45
	50	-	14	7	36
	158	-	17	7	39
	500	-	19	7	29
	1580	-	19	6	31
	5000	-	18	6	19
Solvent control		+	22	10	36
Art. 130165	5	+	17	11	31
	15.8	+	17	13	40
	50	+	19	10	35
	158	+	20	11	32
	500	+	19	12	43
	1580	+	19	12	29
	5000	+	20	10	21
Positive controls	Name		ENNG	9-AA	ENNG
	Conc. [µg/plate]	-	10	50	5
	Revert./plate		622	368	1470
	Name		2-AA	2-AA	2-AA
	Conc. [µg/plate]	+	2	5	10
	Revert./plate		188	272	306

DAUN Daunomycin
CUM Cumene hydroperoxide
B(a)p Benzo(a)pyrene
ENNG N-Ethyl-N'-nitro-N-nitroso-guanidine
2-AA 2-Aminoanthracene
9-AA 9-Aminoacridine

TABLE 3 / Series No.: 2

Art. 130165: Summary of the Mean Number of Revertant Colonies T14997

Test Material	Concentration [µg/plate]	+/- S9-Mix	Mean revertant colonies / plate		
			TA 98	TA 100	TA 102
Solvent control		-	20	131	309
Art 130165	50	-	13	141	325
	158	-	19	128	326
	500	-	17	126	292
	1580	-	23	131	325
	5000	-	18	144	350
Solvent control		+	32	175	264
Art 130165	50	+	33	177	292
	158	+	29	176	300
	500	+	35	183	289
	1580	+	29	149	375
	5000	+	26	154	302
Positive controls	Name		DAUN	ENNG	CUM
	Conc. [µg/plate]	-	4	5	150
	Revert./plate		940	1242	1105
	Name		2-AA	2-AA	B(a)p
	Conc. [µg/plate]	+	2	2	10
	Revert./plate		281	549	1008

Test Material	Concentration [µg/plate]	+/- S9-Mix	Mean revertant colonies / plate		
			TA 1535	TA 1537	WP2 uvrA
Solvent control		-	27	12	38
Art. 130165	50	-	25	14	39
	158	-	36	11	41
	500	-	29	16	37
	1580	-	28	19	40
	5000	-	27	16	21
Solvent control		+	28	12	47
Art. 130165	50	+	31	16	53
	158	+	31	11	32
	500	+	23	15	47
	1580	+	22	12	40
	5000	+	30	14	21
Positive controls	Name		ENNG	9-AA	ENNG
	Conc. [µg/plate]	-	10	50	5
	Revert./plate		994	534	1465
	Name		2-AA	2-AA	2-AA
	Conc. [µg/plate]	+	2	10	10
	Revert./plate		176	214	258

DAUN Daunomycin
CUM Cumene hydroperoxide
B(a)p Benzo(a) pyrene
ENNG N-ethyl-N'-nitro-N-nitroso-guanidine
2-AA 2 Aminoanthracene
9 AA 9 Aminoacridine

APPENDIX F

Acute Oral Toxicity Study in Rats
CAPROS® LD_{50}

psl product safety labs
725 Cranbury Road • East Brunswick, New Jersey 08816-3206 • 732-254-9200 • 800-425-0002 • Fax-732-254-6736

ACUTE ORAL TOXICITY STUDY IN RATS - LIMIT TEST

PROTOCOL NO.:	P320
AGENCY:	FDA
STUDY NUMBER:	9484
SPONSOR:	RONA/EM INDUSTRIES, INC. 7 Skyline Drive Hawthorne, NY 10532
TEST SUBSTANCE IDENTIFICATION:	CAPROS Batch #4003
TEST SUBSTANCE DESCRIPTION:	Light brown powder
DATE RECEIVED:	July 31, 2000
PSL REFERENCE NO.:	E00731-5D
DATE OF PROTOCOL APPROVAL:	August 7, 2000
DATES OF TEST:	August 8-22, 2000
STUDY COMPLETION DATE:	September 11, 2000
NOTEBOOK NO.:	00-40: pages 157-174

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to CAPROS by the oral route.

2. SUMMARY

An acute oral toxicity test was conducted with rats to determine the potential for CAPROS to produce toxicity from a single dose via the oral route. Under the conditions of this study, the single dose acute oral LD_{50} of the test substance is greater than 5,000 mg/kg of bodyweight in male and female rats.

Five thousand milligrams of the test substance per kilogram of bodyweight was administered to ten healthy rats by oral gavage. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Bodyweights were recorded prior to administration and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

All animals survived, gained weight and appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. Gross necropsy findings at terminal sacrifice were unremarkable.

3. MATERIALS

A. Test Substance

The test substance identified as CAPROS, Batch #4003 was received on July 31, 2000 and was further identified with PSL Reference Number E00731-5D. The test substance was a light brown powder and was stored at room temperature. The sample was administered as a 40% w/w suspension in distilled water. Preliminary solubility testing conducted by PSL indicated that suspensions in excess of 40% (i.e. 50-90%) were too viscous to be administered properly. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the sponsor.

Characterization of the test substance provided to Product Safety Labs by the sponsor was:

Composition: Standardized plant extract (edible fruit extract)

pH: Not applicable

Solubility: Not provided

Stability: Test substance is expected to be stable for the duration of testing

Expiration Date: Not applicable

B. Animals

3.B.1 Number of Animals: 10

3.B.2 Sex: 5 male and 5 female

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino

3.B.4 Age/Bodyweight: Young adult (9-10 weeks)/males 207-229 grams and females 169-209 grams at experimental start

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on July 25, 2000

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room: Temperature Range: 17-25°C

4.A.3 Photoperiod: 12 hour light/dark cycle

4.A.4 Acclimation Period: 14 days

4.A.5 Food: Purina Rodent Chow #5012

4.A.6 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system

4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted at least once a year and the records are kept on file at Product Safety Labs. The dates of the most recent analyses are presented in Appendix A.

B. Identification:

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study 9484, constituted unique identification.

5. PROCEDURE

A. Selection of Animals

Prior to dosing, a group of animals was fasted for approximately 19 hours by removing feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Ten healthy rats (five male and five female) were selected for test.

B. Dose Calculations

Individual doses were calculated based on the initial bodyweights, taking into account the specific gravity (determined by PSL) and concentration of the test suspension.

C. Dosing

Each animal received 5,000 mg/kg of the test substance, administered using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. The test substance was administered as a 40% w/w suspension in distilled water. After administration, each animal was returned to its designated cage. Feed was replaced approximately three hours after dosing. The day of administration was considered Day zero of the study.

D. Bodyweights

Individual bodyweights of the animals were recorded prior to test substance administration (initial) and again on Days 7 and 14 (termination).

E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes at 1, 3 and 4 hours post-dosing and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma.

F. **Necropsy**

All rats were euthanized via CO_2 inhalation on Day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined

6. **STUDY CONDUCT**

This study was conducted at Product Safety Labs, 725 Cranbury Road, East Brunswick, NJ 08816, to comply with the good laboratory practices as defined in 21 CFR 58: U.S. FDA Good Laboratory Practice Standards and in accordance with Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food, Guidelines for Acute Oral LD_{50} Toxicity Studies.

7. **QUALITY ASSURANCE**

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

8. **DEVIATIONS FROM FINAL PROTOCOL**

None

9. ***RECORDS TO BE MAINTAINED***

A copy of this signed report, together with the protocol and all raw data generated at Product Safety Labs, is retained in the Product Safety Labs Archives.

10. **RESULTS**

Individual bodyweights and doses are presented in Table 1. Individual cage-side and necropsy observations are presented in Tables 2 and 3, respectively.

All animals survived, gained weight and appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

11. **CONCLUSION**

Under the conditions of this study, the single dose acute oral LD_{50} of CAPROS is greater than 5,000 mg/kg of bodyweight in male and female rats.

SIGNATURES

CAPROS

This study meets the requirements of the Good Laboratory Practice Standards as defined by 21 CFR 58: U.S. FDA with the following exception: The stability, characterization, identity and verification of the test substance concentration as received and tested were not evaluated at Product Safety Labs. These assessments are the responsibility of the study Sponsor.

Daniel J. Merkel, B.S.
Study Director

Date September 11, 2000

We the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Gary Wnorowski, B.A.
Laboratory Director

Date Sept 11, 2000

Sandeep S. Cheema, BVSc.
Principal Toxicology Technician

Date 9/11/00

TABLE 1: INDIVIDUAL BODYWEIGHTS AND DOSES

Animal No.	Sex	Bodyweight (g)			Dose[1]
		Initial	Day 7	Day 14	ml
7182	M	207	282	326	2.3
7183	M	229	286	315	2.6
7184	M	208	251	267	2.3
7185	M	218	266	294	2.4
7186	M	220	272	298	2.5
7187	F	169	202	220	1.9
7188	F	186	227	247	2.1
7189	F	209	243	263	2.3
7190	F	178	206	236	2.0
7191	F	178	213	234	2.0

[1] Administered as a 40% w/w suspension in distilled water. Specific Gravity - 1.113 g/ml.

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Findings	Day of Occurrence
MALES		
7182-7186	Active and healthy	0-14
FEMALES		
7187-7191	Active and healthy	0-14

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Tissue	Findings
MALES		
7182-7186	All tissues/organs	No gross abnormalities
FEMALES		
7187-7191	All tissues/organs	No gross abnormalities

APPENDIX A: FEED AND WATER ANALYSES

Animal feed analysis independently performed on December 10, 1999 for the presence of the following contaminants:

Chlorinated Pesticides in soils, plant tissue and water

Chlorinated Pesticides = ND

Aldrin	Dylox (Trichlorfon)
BHC-A (Alpha-Hexachlorocyclohexane)	Endosulfan I & II
BHC-B (Beta-Hexachlorocyclohexane)	Endsolfan Sulfate
BHC-D (Delta-Hexachlorocyclohexane)	Endrin
BHC-G (Lindane)	Endrin Aldehyde
Captan	Esfenvalerate
Chlordane	Fenvalerate
Chlorpyrifos-Methyl	Heptachlor
Chlorpyrifos (Dursban)	Heptachlor Epoxide
4,4 DDD	Mavrik (Tau-Fluvalinate)
4,4 DDE	Methoxychlor
4,4 DDT	Mirex
Dieldrin	Quintozene

Detection Limit = 0.005 ppm — Water = 0.03ppb

Detection limit may be higher due to matrix interferences — ND = None Detected

LABORATORY: FOOD PRODUCTS LABORATORY, INC.
12003 N.E. Ainsworth Circle
Suite 105
Portland, OR 97220

Water analysis performed as of November 19, 1999 for NJDEPE Safe Drinking Water Act parameters.

LABORATORIES:

NEW JERSEY LABORATORIES
NJDEPE LAB I.D. #15001
A.A. Labs Division
222 Easton Avenue
New Brunswick, NJ 08901

SILLIKER LABORATORIES
OF NEW JERSEY, INC.
400 South Avenue
Garwood, NJ 07027

Results of feed and water analysis for possible contaminants: Acceptable, none detected or within regulatory standards.

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit randomly selects intervals for QA inspections prior to study initiation. Records of the findings of these inspections are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

Inspections were made of:

DATE	PROCEDURE INSPECTED
8/8/00	Initial bodyweights
8/14/00	Day 6 in-life observations
8/31/00	Raw data
8/31/00	Draft report
9/8/00	Final report

Findings reported to: Study Director 8/31/00

Management 9/8/00

Frank Fielder, B.S.
Quality Assurance Supervisor

APPENDIX G

Sub-Chronic Toxicity Studies of CAPROS®

SUB-CHRONIC TOXICITY STUDIES OF CAPROS®

Progress Report (February, 2000 to May, 2000)

Project No.: IH/Res/02/2000

Experimental trial site

Indian Herbs Research & supply co. Ltd
Saharanpur, UP, India
Telephone No.: 91 0132 725044
Email: info@indianherbs.org

Project Staff Signatures

Principal-Investigator **Dr. A.V. Muruganandam**

.....
Date:.....

Co-Investigator **Dr. S. N. Das**

.....
Date:.....

Member of the Quality
Assurance Unit

Trial Monitor **Dr. Joydeep De**

.....
Date:.....

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Preface

General

Sponsor:	**Natreon Inc.** **CL18A, Sec-II, Salt lake City** **Kolkata 700 091, INDIA**
Study Monitor	**Dr. Biswajit Auddy** **Natreon Inc.**
Project No.:	***IH/Res/02/2000***
Test item:	**CAPROS®**
Title:	**Sub-chronic toxicity studies of CAPROS®**

Project Staff

Principal-Investigator	**Dr. A.V. Muruganandam**
Co-Investigator	**Dr. S. N. Das**
Quality Assurance Unit	**Dr. Joydeep De**

Schedule

Submission of project Protocol	January, 2000
Sanction of project Proposal	January, 2000
Receipt of test drug	February, 2000
Initiation of study	February, 2000
Proposed end of study	May, 2000
Final report	May, 2000

Quality Assurance

The study was conducted in accordance with Good Laboratory Practice (GLP) Regulations of the WHO (WHO Document, 1998). The "Principles of laboratory animal care" (NIH Publication # 85-23, 1985) were also followed in this study. The biochemical parameters were determined in State government certified laboratory.

The trial was assessed for compliance with the project proposal. The Quality assurance unit periodically inspected the study or the test facilities. A quality assurance statement will be included in the report.

Amendment Procedures

This protocol can be amended at the discretion of the study director and/or in consultation with the sponsor. Changes or revisions of this project protocol will be documented stating reasons, signed by the study director.

Deviation from project protocol

No deviation from the protocol was undertaken in the present study.

Archiving

The following records were stored in the scientific archives of, R & D centre, Indian Herbs Research & supply co. Ltd, Saharanpur, UP, India according to GLP regulations:

v) Copy of the project protocol
vi) All raw data generated during study period
vii) A signed copy of final report

Summary

CAPROS®, fruit extract of *Phyllanthus emblica* (synonymus with *Emblica officinalis)*, is a patented nutritional supplement ingredient (protected under US Patent 6,124,268 and other pending US and foreign patents) which contains optimized levels of bio-actives. Fruit extracts of *Phyllanthus emblica* have been used for thousands of years in India for the preventative, curative and health restorative properties.

Health benefits provided by CAPROS® are reduction of levels of lipids and glucose [1,2], prevention of platelet aggregation [3], providing gastroprotective effects [4], improving liver function [5], providing chemopreventive actions [6], cough suppressive actions [7], and improving hemoglobin levels, RBC levels [8, 9, 10, 11, 12].

A detailed sub-chronic toxicity study was carried out in rats and rabbits to evaluate toxicity, if any, of CAPROS®. Two doses were tested in the present study: CAPROS® was well tolerated in rats and rabbits when administered orally at the doses of 100 and 500 mg/kg for 90 days. No behavioral, haemopoietic, hepatic and renal dysfunction was observed in rats and rabbits. Hence it may be concluded that CAPROS® is safe without any chronic toxic effects.

Conclusion

CAPROS® was well tolerated in rats and rabbits when administered orally at the doses of 100 and 500 mg/kg for 90 days. An increase in the levels of hemoglobin and RBC was observed with CAPROS® treated groups, which is well within the physiological range of animals and in concomitant with the reported positive effects of Amla. No haemopoietic, hepatic and kidney dysfunction was observed in rats and rabbits. Hence it may be concluded that CAPROS® is safe without any chronic toxic effects.

Objective

To find out the toxic effect, if any, of CAPROS® in rats (rodents) and rabbits (non-rodents) on 90 days oral feeding.

Materials and Methods

Characterization of test item

The test item and information concerning the test item were provided by sponsor.

Name:	CAPROS®
Lot No.:	309; Mfg. dated December 1999
Expiry date:	November 2002

Purpose:	Anti-oxidant nutraceuticals
Colour:	Light yellow
Physical state:	Free flowing powder
Storage:	Stored in RT and protected from light and humidity

Treatment details

Animals: Albino rats (Sprague Dawley strain) and rabbits (New Zealand white rabbits) collected from the animal house, R&D Center of Indian Herbs, Saharanpur, were used in the study. The animals were housed in colony cages maintained at an ambient temperature of 25°C ± 2°C with 12 hours light and dark cycle. Rats and rabbits were divided into three groups each containing 6 rabbits and 20 rats of equal proportions of male and female in each group.

Drug treatment: CAPROS® was administered orally by dissolving in distilled water, using orogastric cannula, to rats and rabbits at the doses of 100 and 500 mg/kg daily, for 90 days. Control rats and rabbits received equal volume of vehicle (distilled water) only.

Parameter studied

During the experimental period following parameters were studied.

i) Periodic observation of rats and rabbits to detect any change in their behavior, nature of fecal matter, any kind of discharge from natural orifices and mortality.

ii) The quantity of feed and water intake was recorded on a periodic basis for each group.

iii) Body weight of rats and rabbits of different groups was recorded on day 30, 60 and 90.

iv) Blood was collected in heparinized test tubes on day 90 in case of rats by retro-orbital puncture and day 45 and 90 in case of rabbits from ear marginal veins. These tubes were centrifuged at 3000 rpm for 10 minutes for separation of plasma. Small quantity of blood was also taken in test tubes without anti-coagulant and was allowed to coagulate in slant position at 37°C to separate the serum.

v) A portion of whole blood was used for haematological study as per standard method

vi) Plasma biochemical parameters like Glucose, Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyuruvate Transaminase (GPT), Alkaline Phosphatase, Total Lipid, Total cholesterol, HDL cholesterol and Creatinine were determined by diagnostic kits of Span Diagnostics Ltd. Serum calcium concentrations were estimated using kits of Accurex Diagnostis Pvt. Ltd. Total protein and albumin were estimated by using kits of Reckon Diagnostic Pvt. Ltd.

vii) Rats and rabbits of control and experimental groups were sacrificed after putting them under ether anesthesia, then severing neck vessels for bleeding on 91st day of experiment. Thorough post-mortem examination was conducted to

detect any gross lesions in vital organs. The gross weights of Liver, Spleen, Kidneys, Heart, Lungs and Adrenal glands were recorded by electronic balance after removing extraneous tissues. Kidney, spleen and liver tissues were preserved in 10% formal saline for histo-morphological examinations.

Statistical analysis: Students "t" test was applied for the analysis of data.

Results

Gross effect of CAPROS® on rats and rabbits: It was observed that rats and rabbits of control, as well as experimental groups, did not show any change in their behavior. They were alert and active. No diarrhea or any discharge from natural orifices was detected during the period of experiment.

Effect of CAPROS® on food and water intake: It was observed that on administration of CAPROS® (100, 500 mg/kg), there was no adverse effect on food and water intake of rats and rabbits. A significant increase in the feed intake was observed with the rats receiving CAPROS® at the dose of 100 mg/kg and significant increase in the feed and water intake was observed in the rabbits receiving 500 and 100 mg/kg CAPROS®, respectively (Table-1 A, B, C & D).

Effect of CAPROS® on body weight gain: No significant difference in the body weight gain was observed in the CAPROS® treated rat and rabbit groups in comparison to respective control group (Table 2 A, B, C & D). No growth retardation effect was observed on administration of CAPROS®.

Effect of CAPROS® on haemopoietic system: The hematological examination revealed that mean hemoglobin concentration of CAPROS® treated rat and rabbits was found to be significantly higher than the control group (Table 3 A, B, C & D). There was significant increase in the red blood cell count of the CAPROS® treated rats and rabbits suggesting that CAPROS® exerts a positive effect on haemopoietic system of bone marrow. However, the increases in the level of Hemoglobin and RBC are well within the reported physiological range of the rats and rabbits. This finding is also in concomitant with the reported positive effect of Amla that it increases the Hemoglobin and RBC in individuals and being also recommended in anemic condition [8, 9, 10, 11, 12]. Amla being an Ayurvedic rasayan, it tries to maintain the homeostasis of the animals. No leukocytosis or leukopoenia was noticed in rats and rabbits of any group in the study. The differential white blood cell count also did not show any abnormality in their population percentage as compared to the control group. The mean value of platelet count in rabbits receiving 100 mg/kg was significantly higher in comparison to vehicle-treated rabbits, suggesting that CAPROS® may have some stimulatory effect on bone marrow (Table-3B).

Effect of CAPROS® on liver: Various biochemical estimations (GOT, GPT, Glucose, Cholesterol, HDL, Total protein and Albumin) of rats and rabbits of experimental groups revealed that hepatic function of experimental groups was well preserved

(Table 4 A, B, C & D). The glucose and cholesterol metabolism of experimental groups was not disturbed. The plasma GOT level of rabbits

treated with CAPROS® (500 mg/kg) was found to be significantly lower than the control rabbits suggesting that there was no leakage of this enzyme from hepatocytes due to CAPROS® administration (Table 4 C & D). The total protein and albumin values were not reduced in experimental rats compared to the control group which indicates that protein synthesis of liver was not disturbed by CAPROS®.

Effect of CAPROS® on kidney: Normal kidney function was observed in rats and rabbits administered with CAPROS® (100 and 500 mg/kg) as the blood urea nitrogen (BUN) and plasma creatinine levels of both the experimental groups did not show any rise in their values. On the other hand, a decrease in the BUN and creatinine levels was observed in rats and rabbits treated with CAPROS® at the dose 500 mg/kg (Table- 4 A, B & D). However, the decreased value lies within the physiological range (0.2-0.8 mg/dl) of the animals. Control animals had the creatinine levels at the upper side of the limit. Capros (500 mg/kg) decreased the value near to the reported mean value (0.5 mg/dl) of the animal.

Effect of CAPROS® on vital organ weights: CAPROS® administration up to 500 mg/kg for 90 days did not bring any gross changes in liver, kidney, lungs and spleen. There was no significant difference in organ weights of rats of experimental groups as compared to the control group (Table-5 A & B).

CONCLUSION

CAPROS® was well tolerated in rats and rabbits when administered orally at the doses of 100 and 500 mg/kg for 90 days. An increase in the RBC levels, Hemoglobin level and decrease in the creatinine level were observed with Capros treatment. However, these changes, are well within the physiological range of animal and are due to the positive effects of amla on health. No haemopoietic, hepatic and kidney dysfunction was observed in rats and rabbits on oral administration of Capros™. Hence it may be concluded that CAPROS® is safe without any chronic toxic effects. No histopathological studies were conducted as there were no toxic effects observed in the present study.

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Table-1A Daily feed (gm) and water (ml) intake of Male rats under oral CAPROS® administration for 90 days

Group/Treatment	16-30 Days	46-60 Days	76-90 Days
Control	9.82±0.192 23.6±0.592	18.72±0.606 41.14±1.45	20.02±0.841 39.2±1.396
CAPROS® 100 mg/kg	10.62*±1.53 23.40±0.388	21.46±0.729 39.20±1.10	20.46±0.755 39.24±1.26
CAPROS® 500 mg/kg	9.146*±0.127 21.84±0.348	18.52±0.704 40.94±1.258	18.37±0.467 36.52±0.854

(Values are Mean+SE, n=10). Superscript* indicates statistical significance in comparison to control group. * denote P<0.05)

Table-1B Daily feed (gm) and water (ml) intake of Female rats under oral CAPROS® administration for 90 days

Group/Treatment	16-30 Days	46-60 Days	76-90 Days
Control	9.14±0.274 22.42±0.597	15.72±0.723 40.82±1.192	19.66±0.717 37.82±1.82
CAPROS® 100 mg/kg	10.05*±0.161 22.94±0.396	18.20*±0.84 39.60±1.279	17.98±0.750 39.82±0.781
CAPROS® 500 mg/kg	9.04±0.191 22.42±0.322	17.62±0.645 38.46±1.241	18.73±0.663 37.2±1.50

(Values are Mean+SE, n=10). Superscript* indicates statistical significance in comparison to control group. * denote P<0.05)

Table-1C Daily feed (gm) and water (ml) intake of Male rabbits under oral CAPROS® administration for 90 days

Group/Treatment	16-30 Days	46-60 Days	76-90 Days
Control	142.6±11.49 244.0±9.466	106.1±3.306 245.3±8.486	97.57±3.750 252.5±5.16
CAPROS® 100 mg/kg	120.3±9.419 267.6±11.63	110.12±2.949 268.4±3.990*	100.4±2.315 251.8±5.983
CAPROS® 500 mg/kg	133.04±4.04 261.5±8.745	130.85±4.038 244.7±4.81	107.0±2.75 266.31±4.363

(Values are Mean+SE, n=5). Superscript* indicates statistical significance in comparison to control group. * denote P<0.05)

Table-1D Daily feed (gm) and water (ml) intake of Female rabbits under oral CAPROS® administration for 90 days

Group/Treatment	16-30 Days	46-60 Days	76-90 Days
Control	137.2±11.92 270.2±8.033	104.3±4.16 265.7±7.866	99.90±2.32 254.8±5.69
CAPROS® 100 mg/kg	124.5±11.75 252.3±9.581	108.4±4.316 282.3±8.55	100.5±3.66 240.5±9.902
CAPROS® 500 mg/kg	130.60±4.839 252.4±10.71	128.55±1.606** 248.7±5.329	99.40±3.26 258.0±9.518

(Values are Mean+SE, n=5). Superscript* indicates statistical significance in comparison to control group. ** denote P<0.01)

Table-2A Body weight gain of Male rats under oral CAPROS® administration for 90 days

Group/Treatment	**Initial body wt. (gm)**	**Day 30 (gm)**	**Day 60 (gm)**	**Day 90 (gm)**	**Final gain (gm)**
Control	92.70±3.201	114.6±1.57	174.2±4.46	174.0±6.944	+81.3
CAPROS® 100 mg/kg	86.00±2.74	122.9±2.51	171.1±3.71	191.9±6.86	+105.00
CAPROS® 500 mg/kg	91.50±2.40	190.8±14.6	144.5±2.33	173.9±1.48	+82.4

(Values are Mean+SE, n=10)

Table-2B Body weight gain of Female rats under oral CAPROS® administration for 90 days

Group/Treatment	Initial body wt. (gm)	Day 30 (gm)	Day 60 (gm)	Day 90 (gm)	Final gain (gm)
Control	95.20±2.78	111.5±1.18	135.0±4.61	141.7±4.68	+46.5
CAPROS® 100 mg/kg	86.30±0.70	109.1±1.900	144.7±5.47	144.5±3.71	+58.2
CAPROS® 500 mg/kg	69.57±0.80	102.8±2.19	120.7±3.13	129.5±4.13	+59.50

(Values are Mean+SE, n=10)

Table-2C Body weight gain of Male rabbits under oral CAPROS® administration for 90 days

Group/ Treatment	Initial body wt. (kg)	Day 30 (kg)	Day 60 (kg)	Day 90 (kg)	Final Gain (kg)
Control	1.83±0.04	1.81±0.10	2.07±0.03	2.08±0.05	+0.25
CAPROS® 100 mg/kg	2.01±0.13	2.06±0.12	2.17±0.14	2.35±0.18	+0.34
CAPROS® 500 mg/kg	1.75±0.08	1.80±0.07	2.30±0.18	2.08±0.15	+0.33

(Values are Mean+SE, n=5)

Table-2D Body weight gain of Female rabbits under oral CAPROS® administration for 90 days

Group/ Treatment	Initial body wt. (kg)	Day 30 (kg)	Day 60 (kg)	Day 90 (kg)	Final Gain (kg)
Control	2.14±0.04	2.23±0.04	2.28±0.03	2.31±0.12	+0.17
CAPROS® 100 mg/kg	2.06±0.04	2.11±0.08	2.19±0.06	2.08±0.13	+0.02
CAPROS® 500 mg/kg	2.08±0.14	2.07±0.14	1.95±0.15	2.17±0.15	+0.09

(Values are Mean+SE, n=5)

Table 3A : Blood profile of Male rats under oral CAPROS® administration for 90 days.

Group /Treatment	Hb (gm/dl)	Total RBC (x 10^6/µl)	Total WBC (x 10^3/µl)	Platelets (x 10^5/µl)	Differential Leukocyte counts			
					Neutro-phils (%)	Lympho-cytes (%)	Eosino-phils (%)	Mono-cytes (%)
Control	13.40±0.45	3.75±0.12	8.3±0.53	3.54±0.27	12.7±1.88	86.80±2.12	0.40±0.22	0.20±0.13
CAPROS® 100 mg/kg	13.22±0.45	4.3±0.28	9.00±0.57	3.45±0.23	19.5±3.21	79.6±2.13	0.40±0.16	0.50±0.22
CAPROS® 500 mg/kg	14.95±0.38*	4.28±0.11**	7.61±0.71	2.62±0.43	18.3±1.96	79.3±2.89	1.7±0.69	0.80±0.29

Values are Mean±SE, n=10, Superscript * indicates statistical significance in comparison to control group.
**P<0.01
*P<0.05

Table 3B : Blood profile of Female rats under oral CAPROS® administration for 90 days.

Group /Treatment	Hb (gm/dl)	Total RBC (x 10^6/µl)	Total WBC (x 10^3/µl)	Platelets (x 10^5/µl)	Differential Leukocyte counts			
					Neutro-phils (%)	Lympho-cytes (%)	Eosino-phils (%)	Mono-cytes (%)
Control	11.73±0.31	3.57±0.15	6.85±0.41	3.3±0.33	14.2±2.39	85.5±2.44	0.3±0.15	0
CAPROS® 100 mg/kg	13.09±0.62	4.59±0.06***	6.07±0.30	4.01±0.18	13.1±2.65	86.3±2.66	0.6±0.22	0
CAPROS® 500 mg/kg	13.55±0.22***	4.0±0.08*	7.29±0.29	3.24±0.09	13.2±1.74	85.9±1.98	0.9±0.55	0

Values are Mean±SE, n=10, Superscript * indicates statistical significance in comparison to control group.
***P<0.001
*P<0.05

Table-3C Blood profile of Male rabbits after oral CAPROS® administration for 90 days

Group/ Treatment	Hb (gm/dl)	Total RBC (x 10^6/µl)	Total WBC (x 10^3/µl)	Platelets (x 10^5/µl)	Differential leukocyte counts			
					Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
45 DAY								
Control	12.0±0.14	3.82±0.18	5.57±0.50	2.46±0.10	11.2±2.74	87.2±3.51	1.6±0.98	0
CAPROS® 100 mg/kg	14.46±0.57**	4.37±0.21	5.78±0.46	2.94±0.14*	15.6±3.85	83.4±3.85	1.0±0.32	0
CAPROS® 500 mg/kg	15.42±0.35***	4.52±0.27	5.58±0.28	2.64±0.16	14.0±3.73	85.2±3.84	0.6±0.40	0.20±0.20
90 DAY								
Control	12.83±0.53	3.74±0.08	7.14±0.09	2.76±0.05	17±2.97	78.8±2.32	2±0	2.2±0.58
CAPROS® 100 mg/kg	14.32±0.81	4.14±0.22	6.44±0.43	2.4±0.17	26.2±2.92	70.2±2.96	1.8±0.49	1.8±0.37
CAPROS® 500 mg/kg	13.31±0.41	5.3±0.18***	6.63±0.13	2.24±0.27	20.8±1.74	75.8±2.15	2.2±0.8	1.2±0.37

(Values are Mean+SE, n=5). Superscript* indicates statistical significance in comparison to control group. *, **, * denote P<0.05, P<0.01 and P<0.001, respectively.)**

Table-3D Blood profile of Female rabbits after oral CAPROS® administration for 90 days

Group/ Treatment	Hb (gm/dl)	Total RBC (x 10^6/µl)	Total WBC (x 10^3/µl)	Platelets (x 10^5/µl)	Differential leukocyte counts			
					Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
45 DAY								
Control	12.67±0.42	3.92±0.22	5.37±0.36	2.42±0.1	7.4±2.15	91.6±4.97	1.0±0.63	0.0
CAPROS® 100 mg/kg	13.14±0.21	3.61±0.17	5.28±0.27	2.88±0.31	23.2±6.63	74.6±5.63	1.6±0.68	0.6±0.24
CAPROS® 500 mg/kg	14.04±0.37*	4.08±0.11	5.62±0.67	2.22±0.27	15.6±3.34	83.4±3.71	0.60±0.40	0.40±0.24
90 DAY								
Control	10.99±0.81	3.48±0.11	5.78±0.29	2.66±0.02	24.0±2.30	73.2±2.08	2.0±0.49	0.8±0.58
CAPROS® 100 mg/kg	13.25±0.91	3.94±0.26	6.42±0.23	3.06±0.32	21.0±1.22	76.4±0.51	1.8±0.37	1.8±0.49
CAPROS® 500 mg/kg	12.57±0.41	4.95±0.05***	7.70±0.89	2.82±0.11	26.2±0.34	70.4±0.68	2.0±0.45	1.4±0.40

(Values are Mean+SE, n=5). Superscript* indicates statistical significance in comparison to control group. *, **, * denote P<0.05, P<0.01 and P<0.001, respectively.)**

Table 4A : Biochemical parameters of Male rats under oral CAPROS® administration for 90 days.

Group/ Treatment	Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Calcium (mg/dl)	Total Lipid (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	SGOT (units/ml)	SGPT (units/ml)	ALP (KAU)
Control	101.05±2.48	6.93±0 11	3.52±0.11	22.80±1.21	0.59±0.29	11.15±0.42	91.99±5 70	96.79±4.14	53.02±6.73	62.1±5.89	23.4±2.56	7.03±1.93
CAPROS® 100 mg/kg	105.66±0.58	5.53±0.75	3.02±0.26	19.63±1.03	1.0±0.23	9.08±1.06	109.33±6.93	87.54±1.58	34.42±5.97	42.5±7.72	24.9±0.72	11.54±3 71
CAPROS® 500 mg/kg	97.86±3.14	6.16±0.39	3.14±0.18	19.04±0.62*	0.29±0.03	9.74±0.63	92.7±8.12	94.82±3.19	48.67±5.31	47.8±4.72	22.9±1.59	11.33±2.2

Values are Mean±SE, n=10, Superscript * indicates statistical significance in comparison to control group.
*P<0.05

Table 4B : Biochemical Parameters of Female rats under oral CAPROS® administration for 90 days.

Group/ Treatment	Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Calcium (mg/dl)	Total Lipid (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	SGOT (units/ml)	SGPT (units/ml)	ALP (KAU)
Control	89.78±2.53	6.03±0.15	3.22±0.23	24.77±0.88	1.03±0.02	10.60±0.38	119.21±7.59	92.36±3.53	43.72±2 51	63 3±4 74	12.7±1.78	11.17±1.52
CAPROS® 100 mg/kg	83.94±4.19	6.51±0.35	3.84±0.23	24.79±0.78	0.78±0.17	9.98±0.29	97.12±7.57	89.68±3.37	51.14±2.61	58.0±6.22	14.9±2.44	14.66±1.55
CAPROS® 500 mg/kg	89 02±1.03	5.85±0 09	3.33±0.06	21.96±1.28	0.53±0.04***	9.46±0.43	114.70±4.56	86.90±1.66	35.69±2.91	67.3±4.34	16.4±3.04	12.68±4.08

Values are Mean±SE, n=10, Superscript * indicates statistical significance in comparison to control group.
***P<0.001

Table-4C Biochemical parameters of Male rabbits after oral CAPROS® administration for 90 days

Group/ Treatment	Glucose (mg/dl)	Total lipid (mg/dl)	Total Cholesterol (mg/dl)	HDL Chole-sterol (mg/dl)	GOT (µ/ml)	GPT (µ/ml)	Alkaline Phosph-atase (KAU)	BUN (mg/dl)	Creati-nine (mg/dl)	Albumin (g/dl)	Total Protein (g/dl)	Calcium (mg/dl)
45 DAY												
Control	129.0 ± 5.83	253.6 ± 8 36	61.35 ± 16 18	24.95 ± 4 57	48.8 ± 4.23	22.0 ± 2.26	4.56 ± 0.42	22.8 ± 1.21	1.79 ± 0.16	3.93 ± 0.19	5.68 ± 0.26	11.15 ± 0.54
CAPROS® 100 mg/kg	129.3 ± 9.26	255.74 ± 25.59	86.4 ± 5.89	26 7 ± 4.58	40.4 ± 7 73	32.2 ± 4.52	6.18 ± 0.68	29 27 ± 2.97	1.11 ± 0 36	3.09 ± 0.42	5.57 ± 0.08	10.92 ± 0 38
CAPROS® 500 mg/kg	135.09 ± 3.52	287.67 ± 50 0	74.78 ± 12.23	42.58 ± 6.13	29.4** ± 2.06	20.0 ± 0.63	2 26 ± 0.92	19.65 ± 0.96	1.31 ± 0.17	3.73 ± 0.11	6.74 ± 0.53	12 02 ± 0.33
90 DAY												
Control	150.7 ± 6.68	168.94 ± 43.26	81.76 ± 7.83	29.94 ± 1.52	65.2 ± 15.6	58.6 ± 0.87	4.94 ± 0.41	27.01 ± 3.85	1.10 ± 0.09	4.46 ± 0.05	6.53 ± 0.24	9.11 ± 0.35
CAPROS® 100 mg/kg	139.48 ± 2.76	162.74 ± 45.72	78 33 ± 7.28	39.48 ± 5.40	45.0 ± 8.06	68.6 ± 10.36	6.22 ± 0.62	27.19 ± 0.80	1.19 ± 0.11	4.25 ± 0.27	5.90 ± 0.28	10.13 ± 0.81
CAPROS® 500 mg/kg	136.46 ± 18.48	82.61 ± 13.93	81.61 ± 3.89	27.63 ± 2.2	73.0 ± 11.81	58.0 ± 2.66	4.46 ± 0.16	21.84 ± 1.11	1.13 ± 0 05	3.95 ± 0.23	5.99 ± 0.20	12.8*** ± 0.27

(Values are Mean±SE, n=5). Superscript* indicates statistical significance in comparison to control group. ** and *** denote P<0.01, P<0.001, respectively.)

Table-4D Biochemical parameters of Female rabbits after oral CAPROS® administration for 90 days

Group/ Treatment	Glucose (mg/dl)	Total lipid (mg/dl)	Total Cholesterol (mg/dl)	HDL Chole-sterol (mg/dl)	GOT (μ/ml)	GPT (μ/ml)	Alkaline Phosph-atase (KAU)	BUN (mg/dl)	Creati-nine (mg/dl)	Albumin (g/dl)	Total Protein (g/dl)	Calcium (mg/dl)
45 DAY												
Control	131.79 ± 6.23	260.82 ± 21.28	97.02 ± 21.12	34.79 ± 2.95	45.6 ± 7.71	20.0 ± 2.30	5.38 ± 0.59	26.20 ± 0.42	1.70 ± 0.28	3 67 ± 0 34	6.04 ± 0.34	10.29 ± 0.13
CAPROS® 100 mg/kg	113.50 ± 5.10	284.30 ± 26.55	89.0 ± 7.68	37.56 ± 5.72	22.6 ± 7.63	31.4 ± 4.74	4.17 ± 0.27	24.41 ± 2.29	1 15 ± 0 13	2.97 ± 0.10	5.47 ± 0.10	9.90 ± 0.22
CAPROS® 500 mg/kg	139 65 ± 8.09	226.07 ± 27.35	50.04 ± 6.92	33.86 ± 0.53	18.0* ± 3.08	20.2 ± 1.88	3.44 ± 0.75	19.35*** ± 0.46	1 55 ± 0.04	3.77 ± 0.10	5.96 ± 0.08	12.14** ± 0 39
90 DAY												
Control	134.96 ± 1.28	128 48 ± 9.12	71.76 ± 5.14	31.34 ± 0.51	44 8 ± 6.81	53.8 ± 3.6	4.86 ± 0.89	27.61 ± 1.09	1.3 ± 0 04	4.08 ± 0.07	6.67 ± 0.72	8.01 ± 0.25
CAPROS® 100 mg/kg	146 62 ± 7.76	118.58 ± 10.40	86.43 ± 5 39	39.10 ± 7.12	41.6 ± 4.96	56.4 ± 4.46	4 70 ± 0.48	24.19 ± 1.69	1.27 ± 0.21	3 87 ± 0.13	6.46 ± 0.61	11.57** ± 0.18
CAPROS® 500 mg/kg	136.58 ± 11.32	128.11 ± 32.36	95.15 ± 10.52	32.75 ± 3.97	39.6 ± 12.76	66 0 ± 9 51	2 50 ± 0.63	23 06 ± 2 46	1.37 ± 0.04	4.29 ± 0.15	5.41 ± 0.93	11.94** ± 0.13

(Values are Mean+SE, n=5). Superscript* indicates statistical significance in comparison to control group. *, ** and *** denote P<0.05, P<0.01 and P<0.001 respectively.)

Table-5A **Organ weight (gm/100g body wt.) of Male rats under oral Capros administration for 90 days**

Group/ Treatment	Liver (gm)	Spleen (gm)	Kidney (gm)	Heart (gm)	Lungs (gm)	Adrenals (gm)
Control	4.97±0.22	0.72±0.05	1.22±0.031	0.57±0.024	1.20±0.04	0.017±0.001
Capros 100 mg/kg	5.23±0.28	0.76±0.07	1.33±0.03	0.60±0.02	1.11±0.04	0.016±0.0007
Capros 500 mg/kg	4.82±0.09	0.73±0.03	1.17±0.02	0.57±0.02	1.24±0.08	0.015±0.0008

(Values are Mean ± SE, n=10)

Table-5B **Organ weight (gm/100g body wt.) of Female rats under oral Capros administration for 90 days**

Group/ Treatment	Liver (gm)	Spleen (gm)	Kidney (gm)	Heart (gm)	Lungs (gm)	Adrenals (gm)
Control	4.74±0.219	0.79±0.06	1.07±0.028	0.48±0.010	1.138±0.017	0.022±0.001
Capros 100 mg/kg	4.66±0.29	0.72±0.031	1.12±0.037	0.49±0.01	1.25±0.05	0.019±0.001
Capros 500 mg/kg	4.73±0.10	0.74±0.03	1.01±0.02	0.47±0.01	1.24±0.08	0.019±0.007

(Values are Mean ± SE, n=10)

Table-5C **Organ weights (gm/kg body wt.) of Male rabbits under oral Capros administration for 90 days**

Group/ Treatment	Liver (gm)	Spleen (gm)	Kidney (gm)	Heart (gm)	Lungs (gm)	Adrenals (gm)
Control	28.66±2.53	0.089±0.003	4.84±0.10	4.27±0.52	1.61±0.06	0.0894±0.008
Capros 100 mg/kg	21.64±1.77	0.13±0.02	4.43±0.58	4.67±0.82	1.51±0.04	0.089±0.001
Capros 500 mg/kg	26.19±3.01	0.158±0.03	5.45±0.62	4.88±0.70	1.74±0.10	0.06±0.01

(Values are Mean ± SE, n=5)

Table-5D **Organ weights (gm/kg body wt.) of Female rabbits under oral Capros administration for 90 days**

Group/ Treatment	Liver (gm)	Spleen (gm)	Kidney (gm)	Heart (gm)	Lungs (gm)	Adrenals (gm)
Control	26.04±1.80	0.06±0.002	4.34±0.05	3.62±0.11	1.64±0.04	0.0526±0.002
Capros 100 mg/kg	21.90±1.65	0.18±0.06	4.67±0.43	4.36±0.58	1.54±0.08	0.075±0.006
Capros 500 mg/kg	24.56±2.65	0.10±0.02	4.77±0.56	4.69±0.71	1.73±0.02	0.10±0.03

(Values are Mean ± SE, n=5)

APPENDIX H

Testimonial on Safety of *Emblica officinalis*
Dr. Tuhin Kanti Biswas

Telephone Nos. (91-33) 2554 5418 (OfC.)
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2554 5019 (Res. Unit)

Research Unit
B. R. Roy State Ayurvedic Medical College & Hospital
GOVERNMENT OF WEST BENGAL
[illegible]
[illegible], PATA DANGA, [illegible] KOLKATA [illegible]

Ref. Nat/JBR/N-DM/ 46 /06 **Date : November 7, 2006**

To Whom it May Concern

Emblica officinalis (Amlaki) as a single herb or in combination with some other herbs is under use in continuity for several diseases in Ayurvedic system of medicine in India. The fruit of Amlaki is not only used to treat adult men and women but also children, lactating mothers, in some cases during pregnancy and old men. There is no contraindication of Amlaki mentioned in any authentic Ayurvedic texts. It is also used in healthy subject to prevent the diseases. In our hospital, both outdoor and indoor patients are often treated with Amlaki and/or Amlaki formulations for several years. Some delicious food prepared from Amlaki is very popular in India. No adverse effect of Amlaki and/or its other formulation was reported in the prescribed dose (3 – 12.5 g/day orally) as also mentioned in Ayurvedic texts (Bhavprakash Nigantu). Hence the herb *Emblica officinalis* can be regarded as safe herb.

(Dr. Tuhin Kanti Biswas)
Principal-Investigator
Clinical Project on Diabetes
Natreon Inc. India

APPENDIX I

Expert Panel Credentials

Robert S. McQuate, Ph.D.

Richard C. Kraska, Ph.D., DABT

Wayne R. Bidlack, Ph.D.

Pages 000136-000152 removed under Freedom of Information exemption 6.

SUBMISSION END

Reference List for Industry Submission, GRN 000295

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000059 - 000060	Kaiyadeva	Pathyapathya Vibhodhakah	1979	NA	Number 64, pg 47
000061 - 000062	Ojha, J.; Mishra, U.	Dhanvantri Nighantu	1985	NA	p.38
000063 - 000064	Tripathi, I.	Raja Nighantu of Pandit Narahari	1982	NA	pg 372
000065 - 000066	Pandey, G.S.; Chunekar, K.C.	Bhavaprakasa Nighantu	1986	Indian Materia Medica	

NA- Not applicable

From: Bob McQuate
To: Fasano, Jeremiah;
cc: sanni@globepharma.com;
Date: Monday, November 23, 2009 1:57:04 PM
Attachments: FDA Withdraw Ltr 112309.doc

Dear Dr. Fasano,

On behalf of Natreon, Inc, attached is our letter that requests that FDA cease its evaluation of GRN 295 which addresses the safety of the aqueous extract of Emblica officinalis.

We will undertake appropriate modifications to our common use in foods approach for the subject material and will resubmit for your further review and evaluation.

Thank you.

Bob
Robert S. McQuate, Ph.D.
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November 23, 2009

Dr. Jeremiah Fasano
Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review (HFS-255)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification 295 --- *Emblica officinalis*

Dear Dr. Fasano:

On behalf of Natreon, Inc., we request that you cease the evaluation of the above-referenced GRAS notice addressing the safety of the aqueous extract of *Emblica officinalis*.

We may elect to resubmit a modified notification at a future date.

Thank you.

Sincerely,

(b) (6)

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