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# Ester-C® Calcium Ascorbate
# Summary of Technical and Bioavailability Data
## (Excerpts from Dossier Submitted to EU)

## TECHNICAL DATA

Ester-C® Calcium Ascorbate (referred to as calcium ascorbate/threonate herein) is a powdered product containing the calcium salt of L-ascorbic acid (vitamin C) and L-threonic acid (an aldonic acid derived from vitamin C oxidation), along with minimal amounts of residual calcium carbonate and water. Calcium ascorbate/threonate is formed when ascorbic acid and calcium carbonate are neutralized when mixed together in water and then dried. The calcium salt is principally formed together with small amounts of the calcium salt of threonic acid, a naturally occurring metabolite derived from the spontaneous oxidation and metabolism of vitamin C *in vivo* and *in vitro*. The proportion of each component in the mixture is listed in Table 1.

**Table 1**
**Components of Calcium Ascorbate/Threonate**

| Component | Average % in Calcium Ascorbate/Threonate |
|---|---|
| Calcium L-ascorbate dihydrate | 95.52 ± 0.75 |
| Calcium L-threonate | 1.22 ± 0.08 |
| Calcium Carbonate | 1.60 ± 0.78 |
| Free Moisture | 1.13 ± 0.28 |
| Total | 99.47 ± 1.89 |

## SPECIFICATIONS

**Calcium Ascorbate/Theonate Specification**

| Description | Zila Specification | Method |
|---|---|---|
| **Assay** | | |
| L-ascorbic acid | 77.37 – 80.47% | Iodometric titration per Zila SOP 510.001[1] |
| Calcium | 8.54 – 9.94% | ICPMS per Zila SOP 526.001[1] |
| L-threonic acid | NLT 0.9% | HPLC per Zila SOP 512.001[1] |
| Free Moisture/Loss on Drying | 0.2 – 1.8% | Halogen Moisture Balance per Zila SOP 514.001[1] |
| **Identification** | | |
| IR Spectra | Positive match | FTIR per Zila SOP 517.001[1] |
| **Purity** | | |
| PH | 5.9 – 7.1 | 1% aqueous solution per Zila SOP 518.001[1] |
| Lead | NMT 3 ppm | ICPMS per Zila SOP 527.001[1] |
| Total Plate Count | NMT 1000 cfu/g | USP <2021> |
| Total Yeast and Mold | NMT 100 cfu/g | USP <2021> |
| *P. aeruginosa* | Negative/10g | USP <2021> |
| *E. coli* | Negative/10g | USP <2021> |
| *S. aureus* | Negative/10g | USP <2021> |
| *Salmonella spp.* | Negative/10g | USP <2021> |

## BIOAVAILABILITY

The profound biological effects of high doses of vitamin C have led many researchers and health professionals to appreciate that large doses of vitamin C behave differently in the body than smaller, nutritional doses. Convincing arguments have recently been presented for raising the dietary intake of vitamin C above present recommended levels to amounts that promote optimal function of the dozens of biochemical reactions in which ascorbate is known to participate as an essential co-factor.

The body changes vitamin C into numerous metabolites that may have physiological actions that are different from those of vitamin C itself. And these metabolites, whether manufactured in the body or ingested along with supplemental vitamin C, may influence how the vitamin itself is transported and utilized. The level of these metabolites in the body may not rise significantly, however, until very large doses of vitamin C are ingested.

Laboratory studies have suggested the mechanism by which the vitamin C metabolite, threonate, is directly involved which might cause increased blood levels of vitamin C. Dr. Anthony Verlangieri at the University of Mississippi used isolated cells in culture to model how vitamin C is utilized by various body tissues. His research group has used cells from laboratory animals as well as humans to study influences on vitamin C utilization. They observed that exposing cells to calcium threonate increased the uptake of ascorbic acid by these cells (Fay & Verlangieri 1991; Fay, Bush & Verlangieri 1994). Verlangieri's group had previously shown that glucose and ascorbate probably share a common transport system for shuttling these molecules through the membrane into cells (Fay, Bush & Verlangieri 1990). This transport mechanism is thought to involve membrane-bound receptors for ascorbate. These lines of evidence suggest that threonate may facilitate cellular uptake of ascorbate by influencing cell membrane receptors.

A number of studies have been conducted to explore the capabilities of calcium ascorbate/threonate and to document its biological actions. These studies are discussed in detail below.

**Human data**

A focused study of the cellular uptake from calcium ascorbate/threonate was performed in San Diego, California by Dr. Howard Hunt, Professor Emeritus of the University of California San Diego, and Dr. Thomas Rice of the Life Management Group with a group of men enrolled in a corporate fitness program. Their objective was to see if calcium ascorbate/threonate would increase tissue levels of vitamin C in infection-fighting and immunological cells, as measured by uptake of ascorbate into the white blood cells. The subjects began with a 2-week washout period to stabilize their vitamin C intake at low levels.

Three groups of 18 men were selected by a complete randomization scheme to select groups that were equivalent with respect to several demographic variables (age, sex, etc.). Each subject received a one-gram oral dose of vitamin C in one of three product forms: ascorbic acid, calcium ascorbate/threonate (1% threonate), or calcium ascorbate/threonate with 3% threonate (an experimental product not available commercially). Dosages were adjusted to ensure that each subject received exactly one gram of ascorbate equivalents delivered by the various product forms. White blood cells were isolated from blood samples removed at intervals of 0, 1, 2, 4, and 24 hours from the time of administration. Following density-gradient isolation of the white cell fraction, total ascorbate was analyzed by high-performance liquid chromatography (HPLC). The double-blind protocol assured that subjects, investigators, and analysts were unaware of the nature of the supplement until all the data were gathered, decoded, and analyzed. The protocol received approval of a duly-constituted institutional review board.

Hunt and Rice discovered that, with time, the ascorbate in all supplement groups steadily accumulated in white cells, but that both the standardized calcium ascorbate/threonate groups reached levels considerably above the final baseline level attained with ordinary ascorbic acid. At 24 hours, the higher ascorbate levels in the white blood cells reached statistical significance with calcium ascorbate/threonate; white cell ascorbate in the 3% threonate group, while higher than that in the ascorbic acid group, did not reach the level of statistical significance.

Hunt and Rice concluded that supplementation with calcium ascorbate/threonate provides a demonstrably effective way to build vitamin C reserves in the important immuno-competent cells of the blood, even when modest doses (one-gram) of ascorbate are used (Hunt & Rice 1995).

It has been suggested that the plasma level of vitamin C reflects the vitamin in transit, on the way to be delivered to critical target cells. Thus, studies of cellular accumulation and biochemical functions are important in interpreting advantages offered by different vitamin C preparations. In a study completed at the University of California, Los Angeles, white blood cell levels of vitamin C were determined after oral administration of ascorbic acid, placebo, and two preparations of calcium ascorbate/threonate.

Three groups of five subjects were randomised in a double-blind cross-over clinical study. After an initial washout period of low vitamin C diet, patients were given one of three vitamin C preparations or placebo, in random sequence, separated by washout periods. With each administration, blood samples were collected at 0, 1, 2, 3, 4, 8 and 24 hours after oral administration of the vitamin. The vitamin C levels were determined by HPLC on the plasma and white cell preparations.

The findings indicated that the three different vitamin C preparations did not lead to significant differences in plasma and white cell uptake of vitamin C. However, the two calcium ascorbate/threonate preparations led to significantly longer retention of vitamin C in the white cells, compared to pure ascorbic acid. The longer retention of vitamin C in white cells was apparent 24 hours after oral administration. The lowest levels of white cell vitamin C were observed among smokers, who showed more rapid declines in vitamin C levels compared to non-smokers, after administration of all vitamin C supplements. Smokers retained significantly higher levels of white cell vitamin C after administration of the calcium ascorbate/threonate preparations (Bernal, Velasquez & Supangan).

**Animal data**

In one animal trial, Dr. Verlangieri gavaged two groups of rats with equal amounts of either pure ascorbic acid or calcium ascorbate/threonate. He tested their blood and urine levels of vitamin C for four hours after administration. The animals given calcium ascorbate/threonate had higher blood levels of vitamin C, reflecting its greater absorption. Vitamin C was not detected in the urine of the test group until long after it was discovered in the urine of the group given ascorbic acid. This delay in excretion suggested better bodily retention of ascorbate before it was "spilled over" into the urine. Verlangieri's study provided preliminary evidence that calcium ascorbate/threonate was absorbed more readily and excreted less rapidly than ordinary ascorbic acid in rats (Bush & Verlangieri 1987).

Except for the guinea pig, some bats, and monkeys, all lower mammals manufacture their own vitamin C. However, one strain of rat - the Japanese ODS rat - is defective in its ability to manufacture its own ascorbic acid, and it is widely used by many vitamin C researchers to study this nutrient in a laboratory setting. These rats develop scurvy without a utilizable source of vitamin C in their diets. Dr. Verlangieri and his colleagues took advantage of this fact to evaluate the bioavailability of calcium ascorbate/threonate. One group of mildly scorbutic ODS

rats was administered calcium ascorbate/threonate. A minimum anti-scorbutic dose of calcium ascorbate/threonate was determined after 24 days. When an equivalent amount of pure ascorbic acid was administered, they found it not to be anti-scorbutic, as assessed by scoring the severity of signs of scurvy. The results from this sensitive nutritional test system indicated that calcium ascorbate/threonate possessed considerable potency in the scorbutic rat model (Verlangieri, Fay & Bannon 1991).

**Veterinary data**

While it is appreciated that most animals synthesize their own vitamin C to satisfy nutritional needs within their tissues, vitamin C has non-nutritional properties in higher dosages. For example, the anti-inflammatory and antioxidant properties of large doses of vitamin C are widely recognized. Many veterinarians and pet owners use calcium ascorbate/threonate because it is a gentle way of administering vitamin C to dogs and horses without gastrointestinal upset. Because they have found that calcium ascorbate/threonate is gentler in the stomachs of animals, it can be given at the higher doses necessary to achieve desired non-nutritional actions.

Some very interesting results have been obtained by veterinarians who have searched for innovative and safe ways to treat intractable conditions in dogs and horses. The value of calcium ascorbate/threonate as an addition to the conventional clinical treatments has been demonstrated in several preliminary studies.

Because dogs are among the majority of mammals which manufacture their own ascorbic acid, veterinarians would not ordinarily think of giving them supplemental vitamin C. Dogs have been shown to manufacture five-to-six times as much vitamin C as the Recommended Dietary Allowance for humans, but they still have one of the lowest synthetic capacities in the animal kingdom. Some practitioners speculate that dogs, particularly older ones, do not always manufacture optimal levels of vitamin C and that lower tissue levels of vitamin C may exacerbate chronic joint inflammation and muscle stiffness.

Dr. Geir Erik Berge, a veterinarian in Oslo, Norway, gave 100 disabled dogs 30 mg/kg of calcium ascorbate/threonate three times daily for six months. To qualify for the study, a dog had to have one of the following chronic conditions involving the joints and connective tissue: severe joint injury, arthrosis, spondylosis, hip dysplasia, older disc-prolapse, muscle atrophy as a result of functional loss, or senile wear-and-tear in support and motion systems.

Dr. Berge measured the effect of supplementation in improving the condition of the dogs after one week, six weeks, and six months through clinical evaluations using a scoring system and reports from the dogs' owners. Because of the chronic nature of these joint disorders and their resistance to conventional therapy, Dr. Berge did not include a control group of animals, since their outcomes could have been predicted. Berge's study demonstrated significant results. After one week of treatment with calcium ascorbate/threonate, 75% of the ailing dogs showed dramatic improvement in their conditions. This percentage improvement remained relatively stable for the rest of the study; by the six-month mark, 78% of the previously suffering dogs had experienced a significant reduction in symptoms. Berge concluded that dogs manufacture suboptimal concentrations of vitamin C in some tissues under the stress of certain ailments. He

recommended high-dosage supplementation with calcium ascorbate/threonate to help correct those deficits (Berge 1990).

Guided by Berge's success with degenerative syndromes in dogs, Dr. L. Phillips Brown, DVM conducted a similar study with dogs housed at the Best Friends Animal Sanctuary in Kanab, Utah. Dr. Brown administered calcium ascorbate/threonate, pure ascorbic acid, or a placebo twice daily to each group of dogs for three weeks. Response to treatment was graded with the Average Mobility Improvement Score (AMIS), using a four-point scale with "0" representing no response and "3" representing a very good response. Treatment was then discontinued for three weeks and the groups were crossed over, so that each group received a different treatment. Treatment and scoring were performed two more times in this fashion. When all improvement scores were added up, Dr. Brown found that dogs receiving the calcium ascorbate/threonate showed an AMIS score of 1.5, while dogs receiving only ascorbic acid showed an AMIS score of 0.5. The average score of the placebo was 0.1, indicating that no significant improvement could be expected if no intervention was performed. Dr. Brown concluded that calcium ascorbate/threonate was more effective than ordinary ascorbic acid for improvement of mobility difficulties (Brown 1994).

Horses also manufacture endogenous ascorbic acid, yet there is growing evidence that the amount produced may not be sufficient to counter the effects of age, husbandry, and athletic demands. Dr. N. Lee Newman, DVM realized that vitamin C supplementation might help with the treatment of degenerative joint disease (DJD) in horses she frequently saw in her large-animal veterinary practice in Middletown, Virginia. In 1992, she began clinical trials of oral calcium ascorbate/threonate for horses diagnosed with a variety of DJD conditions exhibiting moderate to severe lameness.

Dr. Newman recorded radiographic evidence and degree of response to treatment using the American Association of Equine Practitioners Lameness Classification Scores. At the conclusion of the 18-month trial period, over 90% of the horses had shown good to excellent response to the calcium ascorbate/threonate supplement. More significantly, 80% of the "improved" horses remained sound and usable even after the supplement was discontinued (Newman 1995).

Dr. Newman also specialized in the study of chronic obstructive pulmonary disorder (COPD) in horses. COPD is a common progressive condition of unknown cause characterized by dyspnea, cough, and exercise intolerance. Many of its symptoms appear related to human asthma and allergy. COPD has no known cure or therapy, and intractability of the disease may warrant euthanasia.

Dr. Newman performed a clinical field trial to evaluate the effect of supplemental calcium ascorbate/threonate to alleviate the signs of COPD in horses. Six horses were selected for the study. All had exhibited symptoms of COPD for more than one year and were resistant to various management regimens and drug therapies administered by a veterinarian. Calcium ascorbate/threonate was administered at a dose of approximately 20-30 g once or twice a day. Clinical change was scored by owners in consultation with Dr. Newman, using a 5-point scale of improvement ranging from no change to excellent improvement.

Clinical improvement was seen within 45 days in all supplemented horses. Over the course of the one-year study, two horses were rated as good ("4") and four horses were rated as excellent ("5") (Newman 1997).

### *In Vitro* (Human) Data

A study to determine the effects of calcium ascorbate/threonate on the cells most directly affected by periodontal disease was completed at the Department of Dental Public Health and Hygiene and the Department of Stomatology at the University of California San Francisco School of Dentistry. Drs. Dorothy Rowe and David Richards sought to determine the effect of vitamin C on the restorative process by which human periodontal ligament cells produce alveolar bone and cementum to enmesh new periodontal ligament fibers and thereby permit a repopulation of the root surface. Formation of mineralized nodules by cultured cells is a well-established *in vitro* representation of the capacity of cells to form bone *in vivo*. Their bioassay included ascorbate as a critical component, not only because human cells require an exogenous source of vitamin C, but also because ascorbate is a differentiation factor for many animal and human cells.

Dr. Rowe and her colleagues at the University of California San Francisco School of Dentistry cultured periodontal ligament cells in a medium containing calcium ascorbate as either the USP grade or as calcium ascorbate/threonate. Quantitative assessment of mineralized tissue production was determined over a four to five week period by optical scanning measurement of the surface area of the cell cultures occupied by mineralized nodules. By the third week of their culture experiments, they observed noticeable differences in the way that USP calcium ascorbate and calcium ascorbate/threonate affected the number and size of nodules. After four to five weeks of culture, statistical analysis showed that, in two separate experiments performed at a concentration of 50 µg/mL, the area occupied by mineralized nodules in the cultured periodontal ligament cells exposed to calcium ascorbate/threonate was statistically significantly greater than that achieved by the same concentration of USP calcium ascorbate. Dr. Rowe concluded that the calcium threonate in calcium ascorbate/threonate influenced the cellular utilization of ascorbate in human periodontal ligament cells to permit a more efficient production of mineralized tissues (Rowe et al. 1999).

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