

**Memorandum**

0403 5 SEP 30 P2:17

Date: SEP 13 2005

From: Consumer Safety Officer, Division of Dietary Supplement Programs , Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

Subject of the Notification: "Diosmin (95/5) Complex \_\_\_\_\_

Firm: \_\_\_\_\_ Stragen Pharma SA \_\_\_\_\_

Date Received by FDA: \_\_\_\_\_ June 23, 2005 \_\_\_\_\_

90-Day Date: \_\_\_\_\_ September 21, 2005 \_\_\_\_\_

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Victoria Lutwak

1995S-0316

RPT293



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, Maryland 20741

Paul D. Rubin, Esq.  
Patton Boggs LLP  
2550 M St., NW  
Washington, DC 20037

SEP 6 2005

Dear Mr. Rubin:

This is to inform you that the notification, dated June 22, 2005 that you submitted on behalf of your client, Stragen Pharma SA, pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)) was filed by the Food and Drug Administration (FDA) on June 23, 2005. Additional information dated July 14, 2005 was received July 18, 2005. Your notification concerns the substance that you identify as "Diosmin (95/5) Complex" that you intend to market as a new dietary ingredient in a dietary supplement product.

According to your notification, "Diosmin (95/5) Complex" will be marketed in a tablet containing 600 mg of this new dietary ingredient as well as other non-dietary ingredients such as binders. The conditions of use that will be suggested or recommended on the label include: "600 mg/day (1 tablet per day)," "Maximum recommended duration of use: 3 months" and "Not recommended for use by children of pregnant or nursing women."

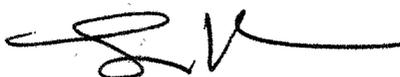
Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement containing a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under 21 U.S.C. 350b (a) (2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is considered to be adulterated under 21 U.S.C. 342(f) (1) (B) (section 402(f)(1)(B) of the Act) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

Your notification presents an issue for FDA to consider, namely, whether a product containing your new dietary ingredient meets the definition of a dietary supplement in 21 U.S.C. 321(ff) (section 201(ff) of the Act). This letter is to alert you within the 75-day notification period that FDA has concerns about whether your product can lawfully be marketed as a dietary supplement. FDA intends to complete its evaluation to determine whether your product is a dietary supplement within the meaning of 21 U.S.C. 321(ff), and send you a response to your notification explaining FDA's decision. Please note that a lack of a response to a notification within the 75-day timeframe does not constitute a finding by the agency that the ingredient or a product that contains the ingredient is safe or is not adulterated under 21 U.S.C. 342. See 21 C.F.R. 190.6(f).

Your notification will be kept confidential for 90 days after the filing date of June 23, 2005. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

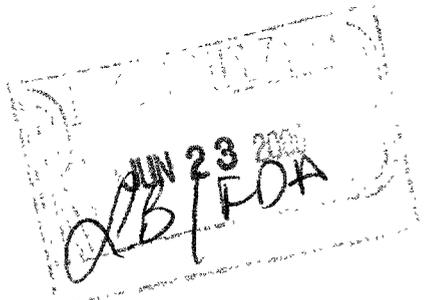
If you have any questions concerning this matter, please contact Linda S. Pellicore, Ph.D. at (301) 436-2375.

Sincerely yours,



Susan J. Walker, M.D.  
Director  
Division of Dietary Supplement Programs  
Office of Nutritional Products, Labeling  
and Dietary Supplements  
Center for Food Safety and Applied Nutrition

June 22, 2005



Paul D. Rubin  
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Division of Standards and Labeling Regulations  
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD, 20740-3835

Re: New Dietary Ingredient Submission for Stragen's Diosmin (95/5) Complex

Dear Sir or Madam:

On behalf of our client, Stragen Pharma, SA (Stragen), Patton Boggs LLP is hereby enclosing a New Dietary Ingredient (NDI) submission for Stragen's Diosmin (95/5) Complex.

As detailed in the attached submission, Stragen's Diosmin (95/5) Complex contains 95% diosmin and 5% hesperidin, and is virtually identical to another formulation the Food and Drug Administration (FDA) has already favorably reviewed under the NDI process. Specifically, in 2000, Nutratch, Inc. (Nutratch) submitted an NDI for its Diosmin Complex, a 90% diosmin/10% hesperidin formulation. FDA ultimately reviewed this submission without comment and, to our knowledge, Nutratch's 90/10 Diosmin Complex has been subsequently marketed in the United States.

Stragen's NDI submission contains virtually all of the same information – including the same comprehensive studies - the Agency has already reviewed in the context of Nutratch's 2000 NDI submission. These include clinical trials, animal studies and *in vitro* studies, which have demonstrated that diosmin complex formulations have an established safety profile at dosages as high as 6g and for durations of use as long as 1 year. Both Nutratch's and Stragen's formulations are recommended for use at less than 1g and for a duration of not longer than 3 months.

In addition, this NDI submission also contains: (1) additional studies conducted after the Nutratch submission was filed that further support the safety profile of Stragen's Diosmin (95/5) Complex; (2) information regarding European drug approvals for Stragen's Diosmin (95/5) Complex; and (3) European sales data for Stragen's Diosmin (95/5) Complex.

Division of Standards and Labeling Regulations

June 22, 2005

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All of the information contained in this NDI submission clearly supports the favorable safety profile of this ingredient. Based upon the extensive similarities to the Nutratech 90/10 Diosmin Complex NDI submission the Agency has already favorably reviewed, as well as the additional information Stragen has provided in support of ingredient safety, FDA should find that the safety profile of Stragen's Diosmin (95/5) Complex is at least as favorable as, if not more favorable than, that of Nutratech's 90/10 Diosmin Complex. The Agency should, therefore, find that Stragen's Diosmin (95/5) Complex is reasonably expected to be safe under the conditions of use recommended or suggested in its labeling.

This NDI submission contains certain trade secret and commercial and/or financial information that Stragen designates as confidential under 21 C.F.R. §§ 20.61(d) and 190.6(e). Confidential materials have been marked "confidential."

Please call me if you have any questions or concerns regarding Stragen's attached submission.

Sincerely,



Paul D. Rubin, Esq.  
Counsel to Stragen Pharma S.A.

**Diosmin  
(95/5)  
Complex  
600 mg Tablet**

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## **DIOSMIN (95/5) COMPLEX: 95% Diosmin/ 5% Hesperidin**

The following new dietary ingredient submission is submitted to the FDA by Stragen Pharma, SA. Pursuant to 21 C.F.R. § 190.6, Stragen provides the following information:

*Address of manufacturing firm:* 3, rue Hugo-de-Senger  
P. O. Box 617  
CH-1211 Geneva 4  
Switzerland

*Name of New Dietary Ingredient:* Diosmin (95/5) Complex

*Level of New Dietary Ingredient:* 600 mg

*Recommended Conditions of Use:* 600 mg/day (1 tablet per day)  
Maximum recommended duration of use: 3 months  
Not recommended for use by children or pregnant or nursing women.

*History of Use Establishing Safety:* See detailed discussions, below.

### Basis for Concluding Diosmin (95/5) Complex is Reasonably Expected to be Safe

This document presents a New Dietary Ingredient (NDI) submission for Stragen's Diosmin (95/5) Complex.

In April 2000, Nutratch, Inc. (Nutratch) submitted an NDI to the Food and Drug Administration (FDA or Agency) for its Diosmin Complex, a formulation consisting of 90% diosmin and 10% hesperidin. Following receipt of a response letter from the FDA, Nutratch resubmitted its NDI with additional data to support a finding that Diosmin Complex was reasonably expected to be safe. This NDI was resubmitted in August 2000 without comment from the Agency and, to our knowledge, Nutratch's 90/10 Diosmin Complex has been subsequently marketed in the United States.

Stragen's Diosmin (95/5) Complex is reasonably expected to be safe because it is virtually identical to Nutratch's 90/10 Diosmin Complex, which the FDA has already reviewed. Minor differences between the two formulations indicate that Stragen's Diosmin (95/5) Complex should have at least as favorable a safety profile as the Nutratch 90/10 Diosmin Complex the Agency already reviewed. In addition, Stragen's Diosmin (95/5) Complex has been widely approved by European drug officials - under strict drug approval requirements - and has been safely marketed in Europe since the late 1990s.

As explained herein, Stragen's formulation has a higher level of purity than Nutratch's formulation. Stragen's formulation consists of 95% diosmin and 5% hesperidin - whereas Nutratch's formulation consists of only 90% diosmin and 10% hesperidin. Stragen has been able to reduce the amount of hesperidin to obtain a more pure diosmin formulation. Furthermore, Stragen's Diosmin (95/5) Complex is recommended for the same three-month duration of use as the Nutratch formulation the FDA already reviewed. Moreover, like

Nutraceutical's formulation, Stragen's Diosmin (95/5) Complex is not intended for use by children or pregnant or nursing women and will be so labeled.

Similarly, the proposed daily dosage for Stragen's Diosmin (95/5) Complex is entirely consistent with the dosage of the Nutraceutical 90/10 diosmin formulation. Specifically, Stragen's proposed daily dosage for its Diosmin (95/5) Complex is 600 mg, while the recommended daily dosage for Nutraceutical's 90/10 formulation is 500 mg. Both dosage levels are well within the levels tested repeatedly in the studies that FDA has already reviewed as part of Nutraceutical's submission. In fact, as noted herein, most of the over twenty clinical trials addressing the safety of diosmin-related complexes were conducted on formulations containing a minimum daily dose of 1,000 mg Diosmin Complex. 500 mg and 600 mg dosages are both well below the tested levels that have already been proven to be safe. In addition, the safety of Stragen's Diosmin (95/5) Complex is also demonstrated by the millions of tablets that have been sold and ingested throughout Europe since 1998. Confidential sales data are attached. (See Attachment A).

- This NDI submission contains the same comprehensive studies that the Agency has already reviewed in the context of Nutraceutical's 2002 NDI submission. These include clinical trials, animal studies and *in vitro* studies, which have demonstrated that diosmin complex formulations have an excellent safety profile at dosages as high as 6g and for durations of use as long as 1 year.<sup>1</sup>

In addition to the studies contained in the Nutraceutical 90/10 Diosmin Complex NDI submission, this NDI submission for the 95/5 Diosmin Complex also contains additional studies conducted after the Nutraceutical submission was filed that further support the safety profile of Stragen's 95/5 Diosmin Complex. Furthermore, this NDI submission contains information regarding the drug approvals in Europe for the Stragen 95/5 Diosmin Complex. All of this information clearly supports the favorable safety profile of this ingredient.

Based upon the extensive similarities to the Nutraceutical 90/10 NDI submission the Agency has already reviewed, as well as the additional information Stragen has provided in support of ingredient safety, FDA should find that the safety profile of Stragen's Diosmin (95/5) Complex is equivalent to, if not superior to, that of the 90/10 Diosmin Complex already reviewed by the agency. The Agency should, therefore, find that Diosmin (95/5) Complex is reasonably expected to be safe under the conditions of use recommended or suggested in its labeling.

This NDI submission contains certain trade secret and commercial and/or financial information that Stragen designates as confidential under 21 C.F.R. §§ 20.61(d) and 190.6(e). Confidential materials have been identified as such with a "Confidential" stamp.

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<sup>1</sup> The vast majority of these studies were conducted on 90/10 formulations.

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## 1 BACKGROUND

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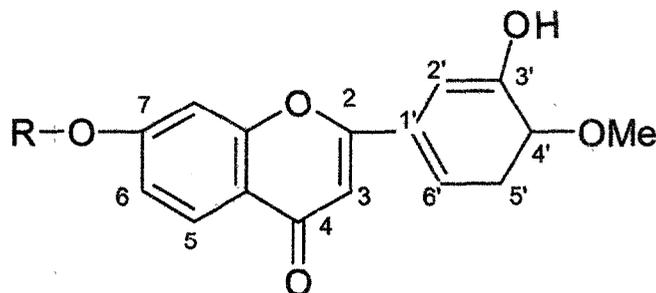
### 1.1 Background: Flavonoids – Diosmin and Hesperidin

For more than a century, flavonoids have been recognized as a plant pigment. Flavonoids are the most common group of plant polyphenols. They provide most of the flavor and color to both fruits and vegetables. The majority of flavonoids existing in plants are attached to sugars (glycosides) and therefore they have a tendency to be water-soluble. Flavonoids can be found in practically all parts of many plant species, including fruit, vegetables, nuts, seeds, leaves, flowers and bark.

Hesperidin is the major flavonoid found in sweet orange and lemon, principally in the rinds. An abundant and inexpensive by-product of Citrus cultivation, hesperidin is listed by the National Nutritional Foods Association as an ingredient in use before October 15, 1994

Diosmin is a flavonoid that can be isolated from various plant sources or derived from the flavonoid Hesperidin. Diosmin is the major active constituent of Buchu leaf (*Barosma betulina*, Rutaceae) and is also found in other *Rutaceae* species.

Chemically, Diosmin is a flavone derivative, which is defined as the 7-rhamnoglucoside of 5,7,3'-trihydroxy-4'-methoxyflavone.



Diosmin (R = 7-rhamnoglucoside)

CAS 520-27-4

Diosmin can be manufactured by extracting hesperidin from citrus rinds and converting the hesperidin to diosmin. The molecular structure of diosmin differs from that of hesperidin, specifically because of the presence of a double bond between two carbon atoms in the diosmin's central carbon ring.

### 1.2 History of Global Diosmin Marketing

Diosmin has a long history of use in the United States and abroad as both a drug and a dietary supplement. Please note that the intended uses described in this section are provided as background only and do not reflect the intended use of a dietary supplement sold in the U.S. that would contain Diosmin (95/5) Complex. As Nutratech pointed out in its submission for its 90/10 Diosmin Complex, diosmin is available under many brand names around the world.

In fact, to date, diosmin has been approved for use in more than 20 countries. (See Attachment B).

Diosmin was introduced in Europe early in the 19<sup>th</sup> century as both a diuretic and urinary antiseptic. For over 30 years, diosmin has also been used as a phlebotonic and vascular-protecting agent. Diosmin was first isolated in 1925 from *Scrophularia nodosa*, and first introduced as a therapeutic agent in 1969.

A diosmin-hesperidin formulation was first launched in European countries as a vegetal extract drug product (DAFLON<sup>®</sup>) in 1971 to treat chronic venous insufficiency (CVI) functional symptoms. In 1986, a 500 mg Purified and Micronized Flavonoid Fraction (PMFF) containing 90% diosmin and 10% hesperidin and flavonoid-related substances, DAFLON 500<sup>®</sup>, was launched in France to treat CVI. As Nutratch indicated in its NDI submission, by 1992 a number of these diosmin-hesperidin combinations were being marketed in 57 countries, including eight countries in Western Europe. (See Attachment C).

Since 2002, the 4<sup>th</sup> Edition of the European Pharmacopoeia (January 2002) has included a monograph for diosmin, obtained by hemi-synthesis. The hemi-synthetic process under the monograph is conceptually similar to the iodine-assisted oxidation process utilized by Stragen to produce its Diosmin (95/5) Complex, as will be discussed below. This monograph defines diosmin specifications (90 to 102% on a dry weight basis) and the chemical structure and specifications of the flavonoids related substances. It also specifies a 1% limit for unknown impurities. (See Attachment D).

In the United States, diosmin is used in dietary supplement formulas. Several dietary supplement manufacturers presently market products containing diosmin in a complex with hesperidin. As noted above, one of these, Nutratch's "Diosmin Complex," was subject to an NDI submission filed and reviewed by the FDA in 2002. Nutratch's product is a dietary supplement formulation consisting of 90% Diosmin and 10% Hesperidin at a maximum dose of 500 mg per day and three-month duration of use for adults. Nutratch demonstrated the safety of Diosmin Complex by providing the FDA with animal studies and approximately 25 clinical studies that tested a minimum daily dose of 1000 mg of Diosmin Complex 90/10. After an initial revision, this NDI was submitted to the Agency without comment and, to our knowledge, Nutratch's Diosmin Complex has subsequently been marketed.

Today, diosmin-hesperidin formulations are used worldwide, not only to treat CVI, but also for a wide range of other venocapillary disorders, including varicose veins, venous stasis ulcers, subconjunctival and retinal hemorrhage, and gingival bleeding. This extensive worldwide commercial use of diosmin - since 1971 - as well as numerous clinical studies demonstrating a lack of toxicity, confirms that diosmin is safe for use in humans.

### **1.3 Worldwide Approvals for Stragen's Diosmin (95/5) Complex**

Stragen's Diosmin (95/5) Complex has been approved and marketed in France since 1998 under the trademarks Diosmine Merck 600 mg<sup>®</sup> and Veineva 600 mg<sup>®</sup>, and in Poland since 2002 under the trademark Otrex 600 mg<sup>®</sup>.<sup>2</sup> (See Attachments E<sub>1</sub>-E<sub>3</sub>). All three are approved Stragen Diosmin 95/5 Complex products that are identical to the Stragen Diosmin (95/5)

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<sup>2</sup> A modified tablet (only the shape of the tablet is different) went on the market in 2004. Attached sales data reflect sales from 2004 forward.

Complex that is the subject of this NDI. Millions of tablets have been sold in both countries, and the safety profile has been exemplary.

Since the launch of the French products in 1998, Stragen is not aware of any adverse event reports. Since the launch of the Polish product in 2002, Stragen is aware of only three adverse event reports – all three of these events were minor, all three issues were resolved quickly, and in one of the three cases it was deemed unlikely that the adverse events were causally related to the Diosmin (95/5) Complex. (See Attachments F<sub>1</sub>-F<sub>3</sub>). The fact that only three minor adverse event reports have been brought to Stragen's attention while millions of tablets have been sold since 1998 indicates that Stragen's Diosmin (95/5) Complex can be reasonably expected to be safe.

#### **1.4 Characteristics of Stragen's Diosmin (95/5) Complex**

Stragen's Diosmin (95/5) Complex dietary supplement:

- *Contains a minimum of 95% diosmin and a maximum of 5% of hesperidin and flavonoid-related substances;*
- *Is substantially similar to both Nutratech's Diosmin Complex product and to the diosmin and hesperidin formulations studied in the clinical trials presented with this submission;*
- *Is more pure (i.e. has more pure diosmin) than other diosmin/hesperidin formulations that have been tested extensively in numerous clinical trials and shown to have no toxic effect at significantly higher dosage levels;*
- *Complies with all specifications of the European Pharmacopoeia.*

##### **1.4.1 General Description**

Stragen's Diosmin (95/5) Complex is available in Europe and the recommended duration of use is 1 dose per day for a maximum recommended duration of three months. Like Nutratech's product, Stragen's Diosmin (95/5) Complex is not recommended for use in children or pregnant or nursing women and will be so labeled.

Stragen's Diosmin (95/5) Complex 600 mg tablets are light yellow in color and oblong in shape, with break-marks on both sides. The theoretical mass of the tablet is 720 mg. The tablets are available in blister-trays, each containing 15 units. The blister-trays are packed in a folding carton containing 30 tablets per carton.

The composition of one tablet of Diosmin (95/5) Complex 600 mg is provided in Table 1 below.

Table 1 Composition of one tablet of Stragen's Diosmin (95/5) Complex 600 mg

Name of ingredients	Function	Reference to standards
Diosmin (95/5) Complex	Active substance	Eur. Pharm. <sup>1</sup>
Povidone	Binder	Eur. Pharm. <sup>1</sup>
Cellulose, microcrystalline	Diluent	Eur. Pharm. <sup>1</sup>
Starch, maize	Disintegrant	Eur. Pharm. <sup>1</sup>
Magnesium stearate <sup>2</sup>	Lubricant	Eur. Pharm. <sup>1</sup>
Water*	wetting agent	Eur. Pharm. <sup>1</sup>

<sup>1</sup> The specifications and analytical methods will be updated to reflect the current edition of a pharmacopoeia monograph at the time of manufacture or analysis.

<sup>2</sup> Derived from vegetarian source

\* Excipient removed during the manufacturing process

#### 1.4.2 Container Closure System

Diosmin (95/5) Complex 600 mg tablets are packed in pharmaceutical push-through blisters consisting of a hard temper and heat-seal lacquered aluminum foil sealed against a Polyvinyl Chloride (PVC) film.

Aluminum foil (from outside to inside):

- Primer based on acrylate
- Aluminum 1050 in accordance with DIN EN 546; hard temper, one side dull, one side bright, thickness: 20 µm
- Primer based on acrylate PVC terpolymer
- Heat-seal-lacquer based on acrylate PVC terpolymer

PVC film:

Thickness: 250 µm  
Surface: Glossy on both sides  
Color: Transparent

Pack sizes consist of two blister trays, containing 15 tablets, being packed into a folding carton with the patient information leaflet.

#### 1.4.3 Comparison to Nutratech's Diosmin Complex

As noted previously, there are two major distinctions between Stragen's Diosmin (95/5) Complex and Nutratech's Diosmin Complex. First, Stragen's product is a 95% diosmin and 5% hesperidin formulation, while Nutratech's Diosmin Complex is 90% diosmin and 10% hesperidin. Second, Stragen's product is recommended at a 600 mg dose, while Nutratech's is recommended at 500 mg. Neither distinction negatively impacts the safety profile of Stragen's product.

### **Formulation and Manufacturing Process**

First, the distinction in formulation reflects the fact that Stragen's product has a higher degree of purity. Generally, as discussed above, diosmin is derived from hesperidin using a vegetal extraction process. This process involves many challenges, including the shortage of available raw vegetal and the variation in diosmin content in source material depending on harvesting location, season, and growing conditions.

Traditional vegetal extraction attempts to extract diosmin directly from the source plant. The extraction process, which utilizes a solvent, also results in the extraction of numerous other vegetal compounds, including tannin, gums, and other Flavonoids, making it very difficult to achieve 95% purity when extraction is done on an industrial scale. In contrast, Stragen's hemi-synthetic process allows for a better control of impurities.

The hemi-synthetic extraction process used by Stragen helps to overcome the challenges of vegetal extraction. As with vegetal extraction, the hemi-synthetic process begins with a natural source. In this case, hesperidin is extracted from citrus fruits. The purity of the hesperidin is critical for achieving a high level of purity in the extracted diosmin. Therefore, Stragen utilizes a hemi-synthetic process of assisted iodine oxidation of hesperidin. This process allows Stragen to achieve a purity level for its starting material (hesperidin) of greater than 95%. Two oxidation products – Linarin and Isorhoifolin – are typical flavonoid impurities found in Diosmin resulting of Hesperidin impurities oxidation. Monitoring the levels of these products allows Stragen to monitor the level of purity of the hesperidin.

The starting material chosen is hesperidin, and Stragen chooses a hesperidin starting material with a high degree of purity. Its oxidation by iodine leads to diosmin. Finally, the crude diosmin obtained from the hemi-synthetic process is "washed" in an alkaline medium, which allows Stragen to separate the diosmin from additional impurities and isolate it by filtration. Moreover, a final purification process allows Stragen to control the purity of the final product.

The result is that Stragen's 95% diosmin product has a higher level of purity – that is, it exhibits a lower level of impurity – than 90% diosmin products currently on the market. Given its higher level of purity, Stragen's Diosmin (95/5) Complex can be reasonably expected to be at least as safe as 90% diosmin/10% hesperidin products such as the Nutratech product reviewed by the Agency.

The Appendix to this submission contains additional information on Stragen's diosmin raw material, stability studies, and formulation specifications.

### **Dosage**

Second, Stragen's proposed 600 mg dose is well within the dosage level that has been proven to be safe and non-toxic. In its submission to the Agency for its 500 mg product, Nutratech provided numerous clinical studies that assessed the safety of diosmin. These studies, which are submitted and discussed in this submission (see Sections 3 and 4), involved daily doses of 1000 mg to as much as 3000 mg administered for up to one year. These studies demonstrated that diosmin/hesperidin formulations that are less pure than Stragen's formulation have no toxic effect with many times the daily dose. Therefore, Stragen's proposed product – a maximum oral daily dose of 600 mg administered for no more than

three months – is well within the levels that have been clinically studied and can be reasonably expected to be safe for use in humans.

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## **2 OVERVIEW OF SAFETY/TOXICITY AND PHARMACOLOGY**

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### **2.1 Overview**

Nutraceutical's submission also provided information on approximately 35 studies, conducted over 30 years with more than 10,000 participants, all of which demonstrated the safety and efficacy of its 90% diosmin and 10% hesperidin formulation. Based on these studies, Nutraceutical concluded that its Diosmin Complex was reasonably expected to be safe under recommended conditions of use. The FDA received Nutraceutical's August 2000 submission without comment, and, to our knowledge, Nutraceutical's Diosmin Complex has subsequently been marketed.

In this submission, Stragen provides and/or discusses all of the animal and clinical studies that Nutraceutical referenced in its NDI submission (see Sections 3 and 4, below) and which are also applicable to the Stragen (95/5) Diosmin Complex.

### **2.2 New Studies**

In addition, Stragen provides eight additional studies, which, to our knowledge, FDA has not yet reviewed. These studies, virtually all of which were published after Nutraceutical's submission, further demonstrate the excellent safety profile of diosmin-hesperidin formulations and provide additional evidence that such combinations are reasonably expected to be safe and effective. These studies can be found at:

- Reference 25 (Jantet, 2002)
- Reference 26 (Belcaro, et al., 2002)
- Reference 27 (Danielsson, et al., 2002)
- Reference 28 (Maruszynski, et al., 2004)<sup>3</sup>
- Reference 29 (Roztocil, et al., 2003)
- Reference 30 (Simka, et al., 2003)
- Reference 31 (Ramelet, 2001)
- Reference 32 (Lyseng-Williamson, et al., 2003).

Finally, Stragen has also provided information on 20 additional pharmacology and pharmacokinetic studies. Three of these studies have been published since 2001, while the remaining studies are older studies. These studies were not included in Nutraceutical's submission, and, to our knowledge, have not been reviewed by the FDA. These studies provide further evidence of the pharmacological and pharmacokinetic profile of diosmin/hesperidin formulations.

Importantly, all of the new studies identified above are discussed and incorporated into the narrative sections associated with clinical trials, pharmacology, etc. as appropriate below.

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<sup>3</sup> This study evaluated a 95/5 diosmin/hesperidin formulation.

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### 3 TOXICITY STUDIES IN ANIMALS

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Toxicity studies evaluating the effect of diosmin/hesperidin formulations in animals have documented an excellent safety profile.

Heusser and Oswald (1977) conducted several studies examining the subchronic, chronic and teratogenic toxicities of diosmin. In a first study, oral treatment with diosmin, at 200 mg/kg daily, for 50 days, was assessed in 20 white rats. A second study also examined chronic toxicity in 22 white mice. Oral treatment with diosmin was administered at a dose of 620 mg/kg daily for 196 days. The observations from both experiments indicated that there were no toxic effects. These findings were confirmed by the blood count, the macroscopic and histological assessment of the organs, the weight development and biochemical examination (glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and urea). In another experiment, diosmin oral treatment (doses between 50 and 250 mg/kg) was given to male and female mini-pigs for 180 days. There were no systematic deviations in clinical, biochemical or hematological values, suggesting that there was no toxicological effect of diosmin.<sup>Ref. 1</sup>

In a teratogenicity study conducted by Heusser and Oswald, mother white mice were given diosmin at a daily dose of 50 mg/kg from the 4<sup>th</sup> to the 12<sup>th</sup> postcoital day. The fetuses were delivered by laparotomy on the 19<sup>th</sup> day after conception. After laparotomy, the mean number of animals per litter was 12.3 in the control group and 11.5 in the diosmin treated group. One hundred and twenty-six fetuses in each group were assessed and their skeletons were also examined. No pathological findings were observed.<sup>Ref. 1</sup> In another group of mice, diosmin was administered in the same dose and fetuses were delivered by spontaneous birth. The fetuses were examined macroscopically and the development of the animals was carefully monitored. Postnatal mortality (20 days) was 19% in the control group and 26% in the diosmin group. Weight gain, length development, gross behavior, hair growth and opening of the eyes were similar in both groups. No significant differences between groups in organ weights, macroscopic and histological findings were observed.<sup>Ref. 1</sup> In a similar study, mother white rats were administered a daily dose of 100 mg/kg of sodium salt from the 4<sup>th</sup> until the 14<sup>th</sup> postcoital day. The fetuses were delivered by laparotomy on the 21<sup>st</sup> day after fertilization. The mean number of animals per litter was 13.9 in both the control and diosmin groups. The average weight was 3.56 and 3.17 g respectively. After examination of their skeletons, no pathological findings were observed in the fetuses.<sup>Ref. 1</sup> In another group of rats, fetuses were delivered by spontaneous birth. The average number of animals per litter was 12.9 in the control group and 11.6 in the diosmin group. Mortality within the 20 postnatal days was respectively 11% and 20%. There was no significant difference between the two groups in terms of weight gain, length development, gross behavior, hair growth, opening of eyes, organ weights, macroscopic and histological examinations.<sup>Ref. 1</sup> Heusser and Oswald concluded from their toxicological experiments that diosmin is well tolerated, even at very high doses, by the different animal species studied.

Animal toxicity studies of diosmin have also been carefully reviewed and summarized by Meyer (1994)<sup>2</sup> and Buckshee et al. (1997).<sup>Ref. 3</sup> The major findings are detailed below. Diosmin Complex (90 % diosmin and 10 % hesperidin) was administered to mice, rats and primates as single oral dosing, as well as repeated oral dosing (13 weeks and 26 weeks). These represent respectively 180 times and 35 times the recommended daily dose in humans.

No toxic or lethal effect could be observed. The 50 % Lethal Dose (LD<sub>50</sub>) was impossible to determine both in the mouse and in the rat, because of the excessively low toxicity; it was assessed as greater than 3000 mg/kg for the active principle. <sup>Refs. 2, 3</sup>

The good gastrointestinal acceptability was confirmed in the Wistar rat, at oral doses representing 12, 24 and 48 times the recommended daily dose. No impairment of the reproductive function was found in the rat after administration of an oral dose, representing 37 times the recommended daily dose. Fertility, embryotoxicity, perinatal, and postnatal development of the generation born from treated parents were not affected. The absence of genetic toxicity was shown by the following tests: bacteria gene mutation, analysis of metaphases in human lymphocytes in culture, in vitro eukaryote system gene mutation, in vivo clastogenic lesions, and DNA repair.

Transplacental passage in the rat was assessed for a single dose of 10 mg/kg of diosmin and was minimal (0.003 % per fetus, of the dose administered to the mother). Passage into breast milk was assessed as 1 % of the dose administered to the mother. Accumulation in the uterus was 0.02 %. Autoradiography of pregnant females showed that the compound was distributed essentially in the intestine and secondarily in the kidneys. However, there was no uptake of the compound by the genital organs of the mother. <sup>Ref. 3</sup>

Meyer concluded that these studies, evaluating the possible toxicity on the digestive tract, the lactation and the reproductive function, demonstrated the excellent safety of Diosmin Complex in animals.

Hitzenberger (1997) also described several toxicology studies of Daflon 500 mg (90 % diosmin and 10 % hesperidin). Acute symptoms were studied in both mice and rats (oral administration up to a maximum of 3000 mg/kg). However, LD<sub>50</sub> could not be determined. No deaths were observed during the 15-day observation period and no substance-related changes were detected during the autopsy. <sup>Ref. 4</sup> Macaca monkeys were administered an oral dose of 4500 mg/kg. No relevant toxicity symptoms were observed. In addition, subchronic toxicity was tested on rats for a period of 13 weeks. Maximum dosage of 600 mg/kg per day was given without any toxicological symptoms. <sup>Ref. 4</sup> Chronic toxicity was studied on rats for a period of 26 weeks, at a dosage of 600 mg/kg per day. No substance-related changes were observed. <sup>Ref. 4</sup> A similar administration schedule was also used in cynomolgus monkeys, with the same overall results. <sup>Ref. 4</sup> Mutagenicity was examined with various tests and no effect could be shown. Reproduction toxicological test on rats and rabbits, as well as peri- and post-natal toxicity studies and further teratogenic studies on rabbits were negative. Furthermore, fertility was not impaired. <sup>Ref. 4</sup>

In conclusion, chronic toxicity, teratogenicity, mutagenicity, fertility and embryotoxicity studies have clearly demonstrated that diosmin has an excellent tolerability profile in animals at dose ranges far superior to the recommended dosage regimen in humans.

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## 4 CLINICAL TRIALS – SAFETY IN HUMANS

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Numerous clinical trials have demonstrated the safety of diosmin in humans. All of these studies were performed on a 90% diosmin, 10% hesperidin complex – a less pure formulation than Stragen's Diosmin (95/5) Complex. These studies provide strong evidence that, even at a lower level of purity, diosmin is safe for use in humans. Therefore, there is strong support for the conclusion that Stragen's more pure formulation can be reasonably expected to be safe for human use.

Details of the clinical trials discussed below can be found in Attachment G. Study reprints are provided at Attachment H.

Laurent and colleagues (1988) conducted two double-blind placebo-controlled randomized trials using Diosmin Complex (90% diosmin and 10% hesperidin) versus placebo. Two hundred patients were entered into these studies (174 females and 26 males), experiencing either organic (n=83) or functional (n=117) chronic venous insufficiency (CVI). Subjects were treated with 1000 mg/day for two consecutive months. A good safety profile of the product was observed both clinically and biochemically. Specifically, variations in blood parameters were within accepted physiological limits. No allergic reaction or drug interactions were observed. Side effects seen in the two groups were of the same type and occurred with comparable frequency. These were, in Diosmin Complex and placebo groups, respectively, as follows: nausea (4 and 4 cases), headache (1 and 4 cases), gastric pain (2 and 2 cases), insomnia (1 and 1 cases), hypotension (1 and 1 cases), metrorrhagia (0 and 1 case). Among these patients, only three dropped out of the trial as a result of a side effect: one in the Diosmin Complex group (epigastric pain) and two in placebo group (one due to nausea and the other due to hypotension).<sup>Ref. 5</sup>

Cospite, et al. (1989) reported a randomized double-blind controlled trial comparing micronized Diosmin Complex (1000 mg/day) with a daily dose of 900 mg of non-micronized formulation. Ninety patients with CVI of the lower limbs (stabilized for one year) were included in this study. The treatment period was for two months. Two patients withdrew from the trial: one in the micronized Diosmin Complex group for a non-medical reason and one in the non-micronized group for epigastric pain (which resolved after treatment termination). Satisfactory tolerance was observed, with stable hemodynamic parameters (blood pressure, pulse rate and respiratory rate) and no significant variation in laboratory parameters. Clinical acceptability was judged satisfactory by 93 % of the patients and 79 % of the clinicians. Twelve cases of epigastric pain were reported: five in the non-micronized diosmin group and seven in the micronized Diosmin Complex group.<sup>Ref. 6</sup>

Tsouderos (1989) presented the results of a study that included 20 patients who had been suffering from CVI for at least one year. This study evaluated the activity of 1000 mg Diosmin Complex as a single dose, compared to a placebo. The results showed that there was no significant change in cardiac index, capillary filtration index, blood pressure, cardiac or respiratory rate. Tsouderos also reported the results of a double blind randomized controlled trial of the effect of Diosmin Complex (1000 mg/day) compared to placebo, over a two-month treatment period. Eighteen patients with functional venous insufficiency were examined in each group. Assessments were undertaken for capillary filtration, arterial output,

respiratory and cardiac rates and systolic and diastolic blood pressure. There was no statistically significant difference between Diosmin Complex and placebo groups. <sup>Ref. 7</sup>

Guillot et al. (1989) reported the results of a multicenter study investigating the safety of Diosmin Complex (1000 mg daily) over one year of continuous administration. Two hundred and fifteen patients (187 females and 28 males), who were suffering from functional symptoms of venous insufficiency, were enrolled into this trial. One hundred and seventy patients completed this study. Forty-five patients were excluded from the final analysis, because of: side effects (n=4, gastralgia), non observance of the protocol (n=10), drop out (n=19) and causes external (n=12) to the trial (moving out, surgery, pregnancy, etc.). These adverse events were judged as probably not related to treatment. Among the 170 completers, clinical side effects were observed in only 20 patients: gastralgia (n=7), dizziness (n=4), gynecological signs (n=7) and cutaneous eruption (n=2). Hematological parameters (red blood cells, leucocytes and hemoglobin) remained unchanged. Hepatic enzymes (SGPT, SGPT and  $\gamma$ -GT), alkaline phosphatase, Quick test and fibrin showed no modifications. Blood urea varied between 0.32 and 0.34 g/L. Creatinine decreased from  $87.7 \pm 1.6$  to  $84.0 \pm 1.8$   $\mu\text{mol/L}$ . Lipid fluctuations, urea, glucose, magnesium, phosphate and calcium remained within normal ranges during the 12-month administration. <sup>Ref. 8</sup>

Thanapongsathorn, et al. (1992) presented a double-blind, placebo-controlled study enrolling 100 patients with hemorrhoids. The treatment lasted 14 days and consisted of a conventional bulk laxative plus either 6000 mg/day of Diosmin Complex for the first four days and then 2000 mg/day for the next ten days or placebo. Two patients in the placebo group withdrew from the study due to clinical deterioration. No side effects of Diosmin Complex were detected during this study. <sup>Ref. 9</sup>

Galley and Thiollet (1993) undertook a randomized double-blind, placebo-controlled study to evaluate the safety of micronized Diosmin Complex (90% diosmin and 10% hesperidin). One hundred patients, all with symptomatic capillary fragility, were randomized and received either Diosmin Complex 1000 mg daily or placebo for six weeks. Four patients withdrew from the study. Two withdrawals from the placebo group (vertigo and drowsiness) may have been placebo-related, while one from the Diosmin Complex group (nausea) may have been drug-related. The rate of side effects spontaneously reported by the patients was the same in both groups. These events were mild and their incidence was low. The reported side effects were the following in the Diosmin and placebo groups respectively: nausea (4 versus 1), gastralgia (1 and 1), dyspepsia (2 and 3), drowsiness (0 and 2), vertigo (0 versus 1) and cramps in the lower limbs (1 versus 0). Mean systolic and diastolic blood pressure remained unchanged under either treatment. <sup>Ref. 10</sup>

Cesarone, et al. (1993) reported the results of a pilot study in which 43 patients with venous hypertension were administered Diosmin Complex at a daily dose of 1500 mg or 1000 mg. In addition, ten healthy subjects were also administered Diosmin Complex at a daily dose of 1500 mg. The duration of administration was four weeks. After treatment with Diosmin Complex, a dose-related decrease in capillary filtration was observed. The treatments were well tolerated and the patients did not report any unwanted effects. <sup>Ref. 11</sup>

In 1994, Geroulakos and Nicolaidis published a review, subsequently reedited in a similar format (Nicolaidis and Geroulakos, 1995), which presented an overview and summary of the studies described above (Laurent et al., 1988, Tsouderos, 1989, Cospite, et al., 1989). This review summarized data collected on 183 patients with CVI treated with Diosmin Complex

or placebo. They concluded that clinical side effects were rare and led to treatment discontinuation for only three patients.<sup>Refs. 12, 13</sup>

Cospite (1994) also examined the safety of Diosmin Complex, in a double-blind, placebo-controlled trial, for the treatment of acute hemorrhoids. One hundred patients suffering from an acute hemorrhoid attack were included in the study and were treated with Diosmin Complex or placebo. Diosmin was administered for seven days, with a dose of 3000 mg daily for the first four days and 2000 mg per day during the following three days. No patient withdrew because of an adverse event. Acceptability was good in both groups with none of the patients reporting any major side effects. However, seven patients experienced at least one side effect: four patients in the Diosmin group (three occurrences of gastralgia, two occurrences of diarrhea, one abdominal pain case, and one headache) and three patients in the placebo group (one occurrence of gastralgia, one dyspepsia, and one nausea). Blood pressure remained normal over the study and showed no change attributable to treatment. Acceptability of the treatment was judged satisfactory by 46 patients in each group. The investigators also reported a similar rate of good acceptability of the treatment.<sup>Ref. 14</sup>

Godeberge (1994) reported the results of a double-blind, placebo-controlled trial of Diosmin Complex including 120 patients, all suffering from internal hemorrhoids. The patients received Diosmin Complex 1000 mg daily or placebo for two months. Five patients (two in the Diosmin group and three in the placebo group) withdrew from the study because of side effects, all of which were resolved after discontinuation of treatment. Diosmin administration was well tolerated. The reported side effects, generally transient and of mild intensity, were anxiety, shivering, oppressive feeling across the chest and epigastric pain. The frequency of side effects was similar in both groups. Blood pressure did not change significantly during the two-month treatment.<sup>Ref. 15</sup>

Amato (1994) reported a randomized, double-blind, multicenter trial assessing the pharmacodynamic and clinical activities of micronized Diosmin Complex, in comparison with a non-micronized Diosmin formulation. Ninety patients with CVI of the lower limbs, stabilized for one year, were entered into this study. The patients received either 1000 mg of micronized Diosmin or an equivalent dose of non-micronized Diosmin, in two doses, each day, during two months. Among the 90 patients included in the study, two dropped out (one in the micronized Diosmin group for a non-medical reason and one in the non-micronized Diosmin group for epigastric pain). Clinical tolerance was satisfactory. Five cases of epigastric pain were reported in the non-micronized group and seven in the micronized Diosmin group. All these events resolved spontaneously without any change of the dosage. The clinical acceptability of micronized Diosmin Complex was regarded as satisfactory by 93 % of the patients and 79 % of the investigators. The clinical and laboratory acceptability was similar in both groups.<sup>Ref. 16</sup>

Meyer (1994) reported a review of 12 mid-term and long-term clinical trials. The pooled data for these studies included 2850 patients treated with Diosmin Complex (1000 mg daily) from six weeks up to one year, 225 patients treated with a placebo and another 85 patients treated with non-micronized Diosmin formulation. Clinical acceptability of Diosmin Complex was found to be good. Only 10% of the patients treated with Diosmin Complex developed side effects, in comparison to 13.9% of the patients taking placebo and 13% of patients taking non-micronized Diosmin. Side effects were similar in nature and among the different treatment groups. Specifically, 6.9% of the patients treated with Diosmin Complex reported gastrointestinal side effects including abdominal pain, gastric discomfort, epigastric pain,

nausea, dyspepsia, vomiting, diarrhea. Another 1.7% of patients treated with Diosmin Complex reported autonomic disorders including insomnia, drowsiness, vertigo, headache, tiredness, anxiety, cramps, palpitations and hypotension. Other reported side effects included: pruritus (one case in placebo group), menometrorrhagia (2 cases in the placebo group and 1 case in the Diosmin Complex group), epistaxis (one case in the Diosmin Complex group) and skin problems (2 cases in the Diosmin Complex group). These last two cases were apparently not related to treatment and resolved in three weeks while the treatment was continued. The proportion of patients dropping out of trials because of side effect was 1.1% in the Diosmin Complex group, in comparison to 3.2% in the placebo group and 4.8% in the non-micronized Diosmin group. Clinical acceptability was assessed at the end of the trials and was considered good by the investigators for 90% of the patients in both Diosmin groups, and by 86% and 87% of the patients in the placebo and Diosmin groups, respectively.<sup>Ref. 2</sup>

As part of his review, Meyer also summarized several trials that highlighted the safety and tolerability of Diosmin Complex. Specifically, the review included:

- One trial that monitored systolic and diastolic blood pressure in 215 patients over one year. The results demonstrated that no change was observed during treatment with a daily dose of 1000 mg Diosmin Complex.
- An incidence of side effects in the elderly population (70 years and over) that was not significantly different from that of the total population. Results showed that the incidence of adverse events in the elderly population was equal to 16.3% and 15.9% in the Diosmin Complex and in the placebo groups, respectively.
- An incidence of side effects in the Diosmin Complex group and in the placebo group that did not differ significantly in patients with hypertension, atherosclerosis, diabetes, neurologic/ psychiatric disease or alcoholism.
- No evidence of drug incompatibility, drug interaction or photosensitizing action of Diosmin Complex when combined with other drugs used to treat concomitant disorders.
- No change in the safety profile observed when comparing a treatment duration of up to two months with a prolonged treatment of six months to one year.
- No side effects seen in studies assessing the administration at higher dose levels. For example, 18 patients were treated with 3000 mg/day of Diosmin Complex for 28 days. In other studies, 10 patients were treated with a daily dose of 2000 mg Diosmin Complex for one month and 18 patients administered a single dose of 2000 mg Diosmin Complex.

Finally, laboratory parameters were used to help assessing the safety of Diosmin Complex. This was reported in a one-year multicenter trial described in Meyer's review. These parameters were: complete blood count, hemoglobin, packed cell volume, prothrombin, creatinine, urea, albumin, fasting blood glucose, total cholesterol, HDL and LDL-cholesterol, HDL/LDL cholesterol ration, triglycerides, uric acid, calcium, phosphorus, magnesium, transaminases (ASAT, ALAT), gammaglutamyltransferase, alkaline phosphatase and fibrinogen. None of the parameters were modified during treatment. However, there was a slight decrease in plasma creatinine, seen in 65.5 % of patients. In addition there was a non-

significant fall in fibrinogen levels in 65.2 % of patients. Nevertheless both parameters remained within physiological range. <sup>Ref. 2</sup>

Belcaro, et al. (1995) reported the results of a three-month double-blind randomized study, which allocated patients into three groups with different daily doses of Diosmin Complex: 500 mg (n=34), 1000 mg (n=33) or 2000 mg (n=37). All 104 patients included in the trial were affected by mild CVI. Fourteen patients dropped out of the study: nine for reasons not related to treatment, two lost to follow-up and three because of an adverse event. Side effects leading to withdrawal occurred in one patient in group 1 (inguinal pain) and in two patients in group 3 (gastralgia and cystitis). The treatment was discontinued and the adverse events disappeared. For all patients, hematological and biochemical parameters remained stable over the study period. <sup>Ref. 17</sup>

Ho and colleagues (1995) conducted a randomized controlled trial of Diosmin Complex in patients undergoing hemorrhoidectomy. Two hundred and twenty-eight patients were included in the study. One hundred and fourteen patients served as controls and another 114 patients received Diosmin Complex for one week after surgery (3000 mg daily for three days and then 1500 mg daily for the next four days). No side effects from the postoperative Diosmin Complex administration were reported. <sup>Ref. 18</sup>

In 1995, Godeberge reported a review of 5 studies assessing Diosmin Complex in patients with hemorrhoids. This review included 299 patients. In all trials, Diosmin Complex was very well tolerated. The side effects were generally transient and of mild intensity. These included anxiety, shivering, an oppressive feeling across the chest and epigastric pain. The frequency of side effects was similar in both the treated and control groups, and no side effect required any specific treatment. No evidence of drug interaction was observed in any of the studies. <sup>Ref. 19</sup>

A multicenter double-blind randomized placebo-controlled trial of Diosmin Complex was reported by Guilhou et al. (1997). Patients with venous leg ulcer were randomized between Diosmin Complex (1000 mg/day) or placebo, for a two-month treatment, while also undergoing conventional therapy (elastic compression). Among the 105 randomized patients with available data, it was reported that six patients withdrew from the study for reason other than ulcer healing: two patients in the Diosmin Complex group (one due to phlebitis and one because of non-compliance) and four patients in the placebo group (three due to mild cutaneous adverse event and one for personal reasons). The treatment was well tolerated. In the Diosmin group, two venous thromboses were reported, but these events were judged by the investigators as unlikely to be related to treatment. The other side effects reported in the Diosmin group were: skin changes around ulcer (n=1), asthenia (n=1), headaches (n=1) and exacerbation of chronic colopathy (n=1). In the placebo group, the adverse events included eczema (n=2), urticaria (n=1), pruritus of the scalp (n=1) and local pain (n=1). No side effect was clearly related to treatment. <sup>Ref. 20</sup>

Le Dévéhat and colleagues (1997) evaluated the use of Diosmin Complex at a daily dose of 1000 mg in a single-center, double-blind, placebo-controlled trial. The study assessed the effect of Diosmin Complex on microcirculatory and hemorheologic parameters, white blood cell count and neutrophil activation. Seventy-seven patients suffering from CVI were included in the trial and treated with Diosmin Complex (1000 mg daily) (n=39) or placebo (n=38) for two months. Eleven healthy volunteers who were not taking any medication were also included as controls. Results showed a significant reduction in the stasis-induced red

blood cell (RBC) aggregation index in the Diosmin Complex group ( $p=0.03$ ). A significant difference between groups ( $p<0.001$ ) was shown using a linear combination of RBC aggregation, RBC count, microcirculatory blood flux (BF), amplitude and frequency of vasomotion. There was no change in the number of total leucocytes, neutrophils and monocytes after two months of treatment with Diosmin Complex or placebo. <sup>Ref. 21</sup>

Serfaty and Magneron (1997) reported the findings of an open-label multicenter study evaluating micronized purified flavonoid fraction (MPFF) on women with premenstrual syndrome (PMS). The women were given a dose of 1000 mg per day for approximately three months. In total, 1724 women initially started the study treatment. Over the study period, 60 women (3.5 %) had some digestive disturbances, 14 (0.8%) experienced menomethrorragia, 11 (0.6%) had a flare-up of venocapillary symptomatology or peripheral vasodilatation, 6 (0.3%) had headache or migraines, and 5 had various other disturbances. Among the 1724 patients included, 251 failed to complete the study. In most cases (241 patients), the reason for discontinuation was not related to safety (e.g. consent withdrawal, non-compliance, contraindicated therapy, house move). Ten women dropped out of the study due to an adverse event: menometrorragia (3), gastric disturbance (2), migraine (1), ovarian cyst (1), intercurrent disease requiring antibiotics (1), and hospitalization (2) (appendectomy and hepatitis). PMS disappeared completely in 37.4 % of the 1473 women who completed the trial. Acceptability and tolerance of Diosmin Complex was judged good or excellent by 98% of the women and 97% of the investigators. <sup>Ref. 22</sup>

Buckshee, et al. (1997) reported an open study assessing the use of micronized Diosmin Complex in the treatment of internal hemorrhoids of pregnancy. Fifty pregnant women with acute hemorrhoids were administered Diosmin Complex for a median of eight weeks before delivery and four weeks after delivery. The treatment was divided in three phases. In the first phase, a loading dose was given for seven days (3000 mg daily for 4 days and 2000 mg daily for 3 days). In the second (antenatal) and third (postnatal) phases, a maintenance dose of 1000 mg daily was given up to delivery and for 30 days thereafter. Among the 50 women recruited, 47 completed the loading treatment phase; 44 the antenatal maintenance phase and 41 the post-natal maintenance phase. Over the entire study period, five patients were lost to follow-up and four patients withdrew from the study: two for reasons unrelated to treatment, one due to nausea in the loading phase and one because of diarrhea during the loading phase. Side effects not leading to withdrawal included nausea and diarrhea and occurred in five patients (four during the loading phase and one in the maintenance phase). Heart rate, blood pressure and biochemical variables showed no significant change with treatment during pregnancy and were normal at the end of the study. No ultrasonic fetal abnormalities were detected during the study. One intrauterine death occurred due to a cord around the neck of the fetus. At delivery, gross placental insufficiency was detected in 6 (13.6%) women. The median maturity of the infant at birth was 39 weeks and the median weight was 2.9 kg. One infant had a single umbilical artery. At the end of the postpartum treatment, 38 infants were breast fed or supplemented artificially and the median weight gain was 1 kg. In conclusion, treatment was well accepted, and did not affect pregnancy, fetal development, birth weight, infant growth and feeding. <sup>Ref. 3</sup>

In 1997, Hitzengerger presented an overview and summary of several clinical trials assessing the safety of Diosmin Complex. This review included principally studies already described in this document (Laurent et al., 1988; Cospite et al., 1989; Guillot et al., 1989; Ho et al., 1995; Guilhou et al., 1997) but did not bring any new relevant element. <sup>Ref. 4</sup>

Manuel y Keenoy et al. (1999) investigated the use of Diosmin Complex in a group of 28 Type I diabetic patients. In this double-blind, placebo-controlled study, treatment duration was three months, with a daily dose of 2000 mg. Patients in the placebo group also received Diosmin Complex after the three-month placebo administration. The investigators measured parameters of glycation and oxidative stress, both before and after the intervention. Results showed a decrease in HbA<sub>1c</sub> (from 8.85 ± 1.57 to 8.47 ± 1.40 %, p=0.017), an increase in glutathione peroxidase activity (from 119 ± 68 to 145 ± 42 U/l hemolysate, p=0.015) and an increase in the lag time of the copper-induced *in vitro* oxidability on non-HDL lipoproteins (from 96 ± 24 to 111 ± 28 min, p=0.005). The Diosmin Complex treatment was well tolerated by the 28 patients, and no adverse event was observed or mentioned by any of the patients. <sup>Ref. 23</sup>

Jantet (2000, 2002) reported the results of the RELIEF study (Reflux assessment and Quality of life improvement with micronized Flavonoids) in CVI. This controlled multi-center study was performed in patients with or without venous reflux, across 23 countries. Patients were separated into two comparative groups, depending on whether or not they presented with venous reflux. The patients were all treated with micronized purified flavonoid fraction (MPFF), which consists of 450 mg of micronized diosmin and 50 mg of flavonoids expressed in hesperidin per tablet, for six months (1000 mg daily). A total of 5052 patients were screened and 4527 received the MPFF treatment. 91% of the patients and 93% of the investigators judged the overall acceptability as good or excellent. <sup>Refs. 24, 25</sup>

Belcaro, et al. (2002) reported the findings from a prospective randomized study, which compared Diosmin Complex with HR (Venoruton<sup>1000</sup>, Paroven, 0-[beta-hydroxyethyl]-rutosides). Ninety patients with severe venous hypertension were included and randomized to receive either oral HR (1 g sachet, twice daily) or Diosmin Complex (1500 mg daily) for eight weeks. No side effects due to the treatment were observed. Compliance and tolerability were very good. None of the patients discontinued the treatment or withdrew from the study. <sup>Ref. 26</sup>

Danielsson, et al. (2002) reported a double-blind, randomized placebo-controlled trial with patients experiencing symptomatic chronic venous disease (CVD). Patients were randomly allocated to a 60-day administration of MPFF (Daflon® 500) or placebo (500 mg twice daily). Out of the 101 trial subjects, only four patients withdrew: two patients because of nausea (one in the MPFF group and one in the placebo group), one patient because of pregnancy (in the placebo group) and one patient for a reason unrelated to the therapy (in the MPFF group). Mild side effects were reported by 12% of the patients in the MPFF group and by 4% in the placebo group. Patients overall opinion of the treatment was excellent or good in 40% of the MPFF group and 26 % of the placebo group. <sup>Ref. 27</sup>

Maruszynski and colleagues (2004) conducted a double-blind randomized study in women with symptoms of lower limb CVI. One hundred and twenty-six patients were included to receive hemi-synthetic Diosmin (600 mg, once a day) (group A) or MPFF (500 mg twice daily) (group B) over a period of four weeks. The safety of both drugs was confirmed by good treatment tolerance and by a limited number of adverse drug reactions. Six patients withdraw prematurely from the study, three as a result of mild adverse events. Over the entire study, six adverse events were reported in four patients; none of these were classified as serious. Among these, two events in group A (calf, hands and feet edema; body rash) and three events in group B (calf edema; body rash; dryness of the mouth) were reported as potentially associated to the treatment. <sup>Ref. 28</sup>

Roztocil, et al. (2003) reported the results of a multicenter randomized study in patients with venous leg ulcers (diameter between 2 and 10 cm). The patients in the control group (n=68) remained on conventional compression therapy, while the patients in the investigational group (n=82) also received 1000 mg of Diosmin Complex daily. Treatment duration was six months with the option to stop if the ulcer was fully healed. Seven patients (five in the control group and two in the Diosmin group) withdrew from the study due to complications unrelated to therapy. No change in body weight, heart rate or blood pressure was observed during the study. In addition, no side effects related to treatment were reported. The acceptability was reported as excellent by 84.9% of the patients in the Diosmin Complex group. <sup>Ref. 29</sup>

In addition, several reviews have also recently summarized the extensive use of Diosmin Complex in clinical trials in patients with venous leg ulcers (Simka and Majewski, 2003; <sup>Ref. 30</sup> Ramelet, 2001 <sup>Ref. 31</sup>), CVI or hemorrhoids (Lyseng-Williamson and Perry, 2003 <sup>Ref. 32</sup>). The findings indicated no problems with the safety of Diosmin.

In conclusion, extensive research demonstrates Diosmin's excellent safety profile. The abundance of clinical trials reviewed above provides substantial confirmation that this product is safe for use in humans. Because Stragen's product is simply a more pure version of the formulation that was tested in these studies, and because Stragen's 600 mg proposed dose is significantly lower than the dosages utilized in these studies, there is a strong basis upon which to conclude that Stragen's Diosmin (95/5) Complex is safe for human use.

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## 5 DOSE CONSIDERATIONS

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- **Stragen's Diosmin (95/5) Complex contains 95% diosmin and 5% hesperidin.**
- **Diosmin Complexes containing either 90% or 95% diosmin and 10% or 5% hesperidin have been the subject of numerous clinical trials, animal studies, and in vitro studies.**
- **In clinical trials, Diosmin Complexes have been given in doses up to 6 g per day orally for up to 12 months.**
- **The recommended 600 mg daily dose for Stragen's Diosmin (95/5) Complex is approximately 60% of the usual recommended therapeutic dose for the standard 90% diosmin and 10% hesperidin formulation and is well within the safety limits that have already been extensively tested.**

### **Diosmin (95/5) Complex: Dosing Determination**

Doses for Diosmin (95/5) Complex used as a dietary supplement have been calculated after an assessment of animal and human clinical trial data and drug dosing regimens in Europe.

The recommended drug dose in Europe for standard Diosmin Complex formulations is 1000 mg to 3000 mg per day. In clinical trials, Daflon 500 mg<sup>®</sup>,<sup>4</sup> for example, has been given for up to 1 year and in doses up to 6 g per day. The usual diosmin drug dose for adults with CVI is 900 mg daily. The usual diosmin drug dose for acute hemorrhoid attacks is 2700 mg daily for the first 4 days, then 1800 mg/day for 3 days and 900 mg daily thereafter. For chronic hemorrhoids, the dose is 900 mg diosmin per day. Diosmin has been used in numerous clinical trials lasting from 2 months to 1 year, and loading doses of 3 g per day for 4 days have been given without incident. (See Attachment G).

As noted, doses for diosmin, used as a dietary supplement, were calculated after an assessment of animal and human clinical trial data. As a dietary supplement, Diosmin (95/5) Complex will be marketed as a 600 mg tablet and labeled with a recommended dose of 600 mg per day (1 tablet per day) orally (approximately 60% of the dose typically used in clinical studies) for adults only for no longer than 3 months.

- The recommended daily dose for Diosmin (95/5) Complex as a dietary supplement is 600 mg per day (1 tablet per day).
- A 600-mg dose of Diosmin (95/5) Complex is 23% to 60% of the recommended drug dose.
- A 600-mg dose of Diosmin (95/5) Complex is 10% of the maximum drug dose given in a clinical trial.
- The recommended daily dose of Diosmin (95/5) Complex as a supplement is approximately 25% to 60% of the recommended drug dose.

Finally, in reviewing dosage determinations, it should be noted that Diosmin (95/5) Complex is not intended for use by children or by pregnant or nursing women and will be so labeled. By way of background, however, it may be noted that the safe use of diosmin for the treatment of pregnant or nursing women with hemorrhoid disease or venous insufficiency has not been established in large-scale clinical trials. However, no deleterious effects have been reported in pregnant women or their offspring after administration during pregnancy.<sup>Ref. 2</sup>

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## 6 CLINICAL PHARMACOLOGY, PHARMACOKINETICS AND METABOLISM

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### 6.1 Clinical Pharmacology

Diosmin is a phlebotonic agent, which increases venous tone, improves lymphatic drainage and protects microcirculation from inflammatory processes and apoptosis. While many other interesting properties have been found, this overview focuses on the three properties noted above, which are directly related to diosmin use in the treatment of the functional symptoms of CVI and acute hemorrhoids crisis.

Interest in the possible health benefits of flavonoids has increased owing to their potent antioxidant, antiestrogenic and free radical scavenging properties, as well as their observed

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<sup>4</sup> As noted above, Daflon 500 mg is a 500 mg Purified and Micronized Flavonoid Fraction (PMFF) containing 90% diosmin and 10% hesperidin and flavonoid-related substances.

biological effect in vitro on the modulation of enzymatic activity and also the inhibition of cellular proliferation. These properties are commonly described to help explain their potential use in reducing the occurrence of different pathologies, including cardiovascular disease and cancer (Garg et al., 2001; <sup>Ref. 33</sup> Ross and Kasum, 2002 <sup>Ref. 34</sup>).

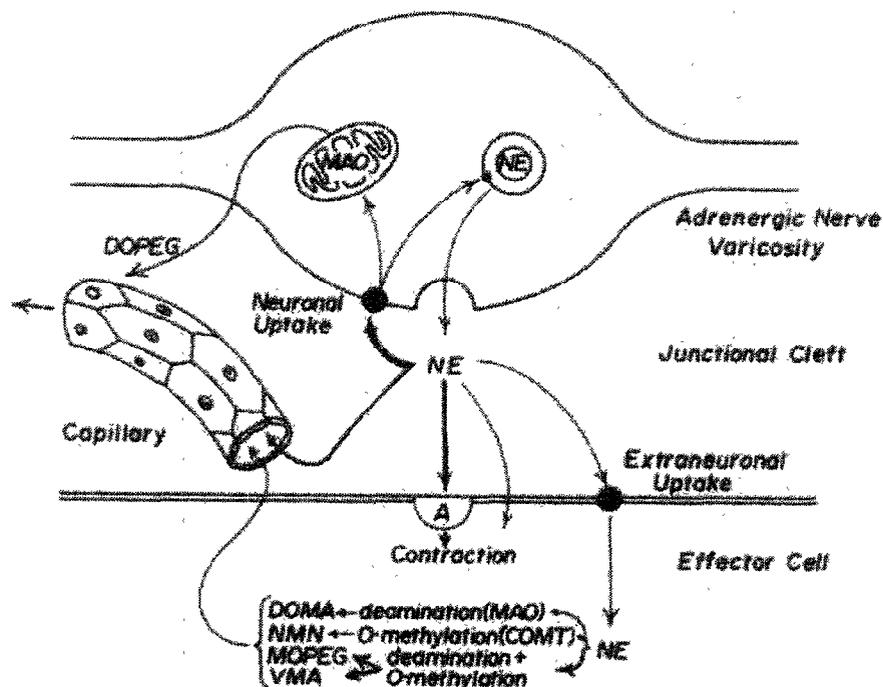
### 6.1.1 Mechanism of Action

#### 6.1.1(a) Venous Tone

Venous tone is controlled by several nerve endings. The noradrenergic and cholinergic nerve endings, respectively, play major roles in contractile and relaxing response. They act on the corresponding smooth muscle cell wall receptor, through the release of their specific neuromediator (norepinephrine and acetylcholine) by the nerve influx in the junctional cleft. The vein contractile response to norepinephrine is mediated by alpha subtype 1 and 2 post junctional receptors (Vanhoutte, 1984). <sup>Ref. 35</sup>

Flavahan (1988) showed that norepinephrine receptor responsiveness depends on the peripheral body temperature (alpha 2 type), with warming inducing a venous dilation and cooling the contrary, results consistent with the "heavy legs" syndrome. <sup>Ref. 36</sup>

Norepinephrine is removed by uptake in the nerve ending where it is enzymatically degraded by the intraneuronal monoamine oxidase (MAO), but most is recycled to the storage vesicles; diffusion to the capillaries; and uptake by the effector cells and enzymatic degradation by the enzymes monoamine oxidase and catechol-O-methyltransferase (COMT). The metabolites of norepinephrine are inactive and diffuse to the extracellular fluid and the capillaries.



Source: P. Vanhoutte, *Inter. Angio.* 1984, 3, n°1, 40-46

Keys:

- NE = Norepinephrine
- A = adrenergic receptors
- MAO = monoamine oxidase
- DOPEG = 3,4-dihydroxyphenyl-glycol
- COMT = catechol-O-methyltransferase
- DOMA = 3,4-dihydroxymandelic acid
- NMN = normetanephrine
- MOPEG = 3-methoxy-4-dihydroxyphenylglycol
- VMA = 3-methoxy-4-hydroxymandelic acid

Heusser and Oswald (1977) reported findings of studies conducted on saphenous vein strips of dog. Diosmin blocked the inactivation of exogenous norepinephrine and caused a slow and contractile response of an oil-immersed strip, which was not attributable to the release of norepinephrine. Monoamine oxidase inhibition appeared not to be included in the action of diosmin. An inhibition of catechol-O-methyltransferase (COMT) could not be excluded and was even probable. <sup>Ref. 1</sup>

In an *in vitro* study, Juteau and colleagues (1995) used isolated varicose veins to test the effect of diosmin and norepinephrine under acidosis conditions. The results showed that diosmin induced a shift to the left of the concentration-response curves of norepinephrine. This potentiation was significant in both normal and varicose veins and was increased in proportion with the pathological status of the venous rings. <sup>Ref. 37</sup>

It can be concluded that diosmin reinforces venous tone by prolonging the activity of parietal norepinephrine, even under acidosis conditions. Local acidosis depresses reactivity of vascular smooth muscle, especially the response of human isolated saphenous veins to exogenous norepinephrine.

The following studies show the effect of diosmin or its metabolite diosmetin, on:

- COMT inhibition,
- Inhibition of amine re-uptake,
- Calcium contraction effectiveness,

All of these properties are consistent with an increase of venous tone.

- *In vivo* COMT inhibition (Boudet and Peyrin, 1986) <sup>Ref. 38</sup>

The ability of intraperitoneal diosmin (100, 200, 400 mg/kg for 5 days) to inhibit venous catechol-O-methyltransferase (COMT) activity was compared to tropolone in various veins from 50 male Sprague-Dawley rats (150-170 g), divided into 5 groups (between 200 and 400 mg/kg). Diosmin had a COMT inhibitory effect, though lower than tropolone. Furthermore, diosmin (400 mg/kg) increased the urinary excretion of normetanephrine (NMN) by 56 % and that of 3-methoxy 4-hydroxyphenylglycol (MHPG) in a dose-dependent way. This suggests that diosmin may exert an activating effect on sympathetic activity. Both mechanisms (local inhibition of COMT and enhanced sympathetic activity) may contribute to increase norepinephrine levels in the synaptic clefts of the vascular wall and explain the vasoconstrictor effect of diosmin.

- *In vitro/ex vivo* COMT inhibition (Araujo et al., 1991)<sup>Ref. 39</sup>

Varicose saphenous veins have also been used to study the *in vivo* metabolism of norepinephrine. Eleven female patients suffering from varicose disease were allocated to control (n=5) and treated group of 600 mg diosmin twice a day, orally, for 10 days (n=6) before surgery. Fragments of the excised saphenous veins were incubated with <sup>3</sup>H-norepinephrine for 60 minutes, with an interval between surgery and incubation inferior to 30 minutes. Column chromatography and liquid scintillation counting were used to measure <sup>3</sup>H-norepinephrine and its metabolites. In the treated group, accumulation of <sup>3</sup>H-norepinephrine was significantly reduced and the formation of metabolites decreased by approximately 50%. The present results show that oral administration of diosmin has evident effects on the *in vitro* metabolism of norepinephrine by the varicose tissue.

- *In vitro* amine reuptake inhibition (Codignola et al., 1992;<sup>Ref. 40</sup> Sher et al., 1992<sup>Ref. 41</sup>)

Human neuroblastoma cells of sympathetic origin were used as a model to study the effects of diosmin and its metabolite diosmetin on amine reuptake systems. Neuroblastoma cells take up <sup>3</sup>H-dopamine in a specific and time-dependent manner. Whereas diosmin had no effect, its aglycone, diosmetin inhibited <sup>3</sup>H-dopamine uptake in a dose-dependent manner (IC<sub>50</sub> = 4 μM). Furthermore, diosmetin also inhibited <sup>3</sup>H-serotonin uptake. These results demonstrate that diosmetin acts as an antagonist of plasma membrane amine transporters at the molecular level and suggest that inhibition of amine reuptake at the level of peripheral sympathetic nerve terminals could be responsible for the increased vascular tone observed *in vivo* after oral administration.

- Sensitivity to calcium was demonstrated by Savineau and Marthans (1994)<sup>Ref. 42</sup>

Savineau and Marthans (1994) investigated the effect of diosmin on the Ca<sup>2+</sup> sensitivity of the smooth muscle contractile apparatus in strips from the isolated rat femoral vein. Diosmin shifted the concentration-response curve to Ca<sup>2+</sup> to the left. At a dose of 1 μM, diosmin increased the contractile response evoked by 0.15 μM Ca<sup>2+</sup> from 26.3 % to 78.9 % of the maximal Ca<sup>2+</sup>-induced response. This research demonstrates that the venotonic action of diosmin involves an increase in the Ca<sup>2+</sup> sensitivity of the contractile apparatus. This direct modulation could therefore explain, at least in part, the venotonic action of diosmin.

### 6.1.1(b) Lymphatic System

Diosmin stimulates lymphagogue activity. It improves the drainage of interstitial tissues by increasing lymphatic flow and lymphatic oncotic pressure.

The lymphatic activity of diosmin was tested in dogs (Labrid, 1995). Diosmin induced a lymphatic flow increase that was correlated with the administered doses. The maximal increase of lymphatic volume reached 191 % after i.v. administration of diosmin at 12.5 mg/kg. A correlation between lymphatic flow increase and pulsatility was demonstrated. The oncotic pressure was increased. These results suggest that diosmin induces a lymphagogue effect in the dog related to an increase of the lymphatic oncotic pressure.<sup>Ref. 43</sup>

After infusion of  $^{14}\text{C}$ -diosmin in the dog, an active blood-lymph transfer of diosmin was observed during a 15–100 min period after infusion. The improvement of lymphatic drainage displayed by diosmin seems to be an important component of its beneficial effect on perivascular edema. <sup>Ref. 43</sup>

### 6.1.1(c) Inflammatory Processes

Diosmin opposes microcirculatory deterioration by inhibiting the expression of certain mediators involved in leukocyte or endothelial adhesion. Consequently, diosmin reduces both the activation and the adhesion of leukocytes as well as their migration through the microcapillary endothelium. This results in a decrease in pericapillary inflammation via inhibition of the release of the principal mediators of inflammation, principally free radical and prostaglandins. Therefore capillary permeability is normalized and capillary resistance is strengthened.

- **Free radical scavenger property**

Diosmin is also a free radical scavenger of reactive oxygen metabolites involved in tissue destruction occurring in inflammatory reaction (Cypriani et al., 1993). <sup>Ref. 44</sup> Therefore, it protects vein cell wall membrane from acute stress, but also from aging, by inhibiting the activity of the lipoxygenase (Dumon et al., 1994; Melin et al., 1996). <sup>Refs. 45, 46</sup> This activity ahead of the inflammatory reaction, due to the free radical scavenging property of diosmin, is linked to diosmin's capacity to inhibit the well-known inflammation mediators prostaglandins (E2 and F2 $\alpha$ ) and thromboxane (B2) (Jean and Bodinier, 1994). <sup>Ref. 47</sup>

- **Effect on edema and inflammation**

Edema and inflammation are the consequence of venous insufficiency and stasis.

- Effect of diosmin on edema reduction (Casley-Smith and Casley-Smith, 1985): <sup>Ref. 48</sup>

The administration via a stomach tube of 0, 50, and 200 mg/kg diosmin suspended in 0.8 % Tylose to 36 male Wistar rats ( $250 \pm 25$  g) divided into 3 groups, was studied on high-protein edemas obtained with lung contusion. Diosmin considerably reduced the interstitial edema and tissue disorganization with a greater effect at the higher dose. Conversely, in the rat leg edema model, studied in 45 female Wistar rats ( $200 \pm 25$  g) with the same doses, the lower concentration (50 mg/kg) was more active. In the acute lymphedema of the leg studied in 45 male Wistar rats ( $200 \pm 25$  g), both concentrations were active. At high doses, diosmin induced the release of mediators in the rat foot, whereas in other tissues, it reduced many forms of high-protein edemas.

- Anti-inflammatory activity of diosmin (Freneix-Clerc et al., 1994, Dumon et al., 1994): <sup>Refs. 49, 45</sup>

Carrageenan and  $\text{CCl}_4$  induced acute phase inflammatory reactions in the rat, characterized by a marked increase in serum  $\alpha_1$ ,  $\alpha_2$  and  $\beta$  globulins. Diosmin injected intraperitoneally (150 mg/kg/week) for 8 weeks prior to carrageenan or  $\text{CCl}_4$  injection, displayed a marked inhibitory activity on the production of inflammatory glycoproteins mediated by cytokines.

## 6.2 Pharmacokinetics and Metabolism

The pharmacokinetic parameters of diosmin have been studied in several animal species (i.e. rats, dogs, rabbits, and monkeys). Although the findings of one study conducted in rats are reported below, this section will focus on the available pharmacokinetic data of diosmin in humans.

In a study conducted by Oustrin and colleagues (1977),<sup>Ref. 50</sup> <sup>3</sup>H-labelled diosmin was administered both i.v. and orally to Wistar rats respectively at a dose of 15 mg/kg and 30 mg/kg. Absorption by gastrointestinal tract was rapid, with a peak of plasma concentration between one and two hours after administration. The metabolism was active and did not allow a temporary accumulation in the organs. Almost all organs examined only had 0.1% to 0.2% of the original activity of the organ after 48 hours, except the liver with 1%. Elimination took place both in the urine and the feces. After i.v. administration, the substance was eliminated predominantly in the urine, while after oral administration it was eliminated almost equally in the urine and in the feces, during the first 24-hour period. In the following 24-hour period, the feces carry the greater proportion of diosmin or its metabolites.

Binding to the vascular wall has been observed, but a long time after oral administration. The delayed binding could imply that it involves the metabolites and not the initial parent molecule. This supports the conclusion of other authors that the phlebotonic action of diosmin seems to be due to the metabolites themselves.

Following oral administration to humans, diosmin is not absorbed, but metabolized in the gastro-intestinal track (GI track) as other flavonoids.

Cova and colleagues (1992)<sup>Ref. 51</sup> conducted a study in 5 healthy volunteers (2 males and 3 females) who received 10 mg/kg p.o. of non-micronized diosmin. No parent compound was detected in the plasma (sensitivity limit of 20 ng/mL). Only diosmetin, the diosmin aglycone, was found in the plasma, with a maximum concentration reached after one hour. Plasma levels started to decrease slowly after 2 hours, constantly after 24 hours and were still detectable after 48 hours. The corresponding pharmacokinetic parameters are presented in Table 2.

Table 2 Pharmacokinetics parameters (mean  $\pm$  SD) after a single oral administration of non-micronized diosmin (10 mg/kg) to 5 healthy volunteers

Parameters	Mean $\pm$ SD
C <sub>max</sub> (ng/mL)	417 $\pm$ 94.1
T <sub>1/2<math>\beta</math></sub> (h)	31.5 $\pm$ 8.6
MRT (h)	36.6 $\pm$ 9.9
AUC <sub>(0-48h)</sub> ng/mL.h	5617.1 $\pm$ 1518.4
TCL (L/h)*	1.32 $\pm$ 0.42
Vd (L)*	62.1 $\pm$ 7.9

\* Total clearance and volume of distribution were computed assuming complete bioavailability.

Diosmetin results from the hydrolysis of the  $\beta$ -glycoside bond by  $\beta$ -glycosidases. These enzymes have been extensively examined. They are located in the small intestine mucosa (Nemeth et al., 2003).<sup>Ref. 52</sup> This hydrolysis represents a rate limiting step for the absorption of the aglycone. Its absorption level is very low according to the Summary of Product Characteristics (SPC) of Daflon<sup>®</sup> 500 mg, in which the drug is described as being excreted urinary at a level of 14 % after administration of <sup>14</sup>C-radiolabelled micronized diosmin.

The pharmacokinetic parameters measured by Cova and colleagues (1992)<sup>Ref. 51</sup> are consistent with:

- an enterohepatic circulation (in view of the long plasma elimination half life ranging from 26 to 43 hours), involving sulfate and glucuronide derivatives as shown by Perego et al. (1993) on perfused rat liver;<sup>Ref. 53</sup>
- an extensive uptake of the compound by tissues (large volume of distribution compared to the administered dose of 10 mg/kg). This supports the conclusion of other authors showing that the phlebotonic action of this compound seems to be due to binding to the vascular wall.
- a very active metabolism (total body clearance of 1.32 L/h) to be related to the absence of diosmin as well as diosmetin in urine.

In contrast with the total absence of urinary elimination for both diosmin and its aglycone diosmetin, its metabolites are eliminated in urine (mainly as glucuronic acid conjugates). The predominant metabolite detected in urine samples was m-hydroxy-phenylpropionic acid, which is mainly eliminated in its conjugated form. In addition, smaller amounts of other phenolic acids, corresponding to 3-hydroxy-4-methoxybenzoic acid and 3-methoxy-4-hydroxyphenylacetic acid and 3,4-dihydroxybenzoic acid were detected. The presence of degradation products such as alkyl-phenolic acids confirms a metabolic pattern similar to other flavonoids.

Similar findings, related to the elimination process were found in a study conducted by Servier and reported by Hitzenberger (1997).<sup>Ref. 4</sup> Twelve healthy volunteers were administered a single dose of 250 mg diosmin (25  $\mu$ Ci). Due to limitations, for ethical reasons, on permissible amounts of radioactivity, it was not possible to detect radioactive substances in the plasma, but only in the urine and feces. In the urine, the measured radioactive levels were  $13.8 \pm 2.9\%$  of the administered dose, and in the feces it was  $80.5 \pm 3.5\%$ . Neither diosmin, nor diosmetin were found in the urine. Only metabolites were found. The metabolites consisted of hippuracid, hydroxyhippuracid and cinnamoyglycinacid. Additional metabolites were phenylpropanacid, m-hydroxy-p-methoxy-phenyl- $\beta$ -hydroxypropan-, and m-phenyl-hydroxypropanacid. Unmetabolized compounds were found only in the feces. Hitzenberger concluded from these results that the first step of the metabolism takes place in the intestinal flora and consists of demethoxylation, demethylation or hydroxylation. Oxidation and conjugation processes take place in the liver.<sup>Ref. 4</sup>

Garner et al. (2002)<sup>Ref. 54</sup> conducted a study to assess the overall absorption of diosmin after oral administration. In a double-blind cross-over study design, 12 healthy male volunteers received a single oral dose of 500 mg of non-micronized <sup>14</sup>C-diosmin or 500 mg of PMFF (Daflon 500<sup>®</sup>) enriched with <sup>14</sup>C-diosmin (25 nCi). Absorption from the gastrointestinal tract was estimated at  $32.7 \pm 18.8\%$  and  $57.9 \pm 20.2\%$ , respectively for the two compounds during a period of 168 hours post-dose. A huge inter-individual variability was observed. Because

unabsorbed diosmin is not excreted in the urine, absorption was evaluated based on the cumulative urinary elimination of radioactivity. An important fraction of the administered dose was excreted as unchanged (unabsorbed) diosmin and diosmetin via the feces ( $80.3 \pm 25.1\%$  and  $50.9 \pm 24.2\%$  respectively for the two compounds) (Table 3).

Table 3 Excretion of non-micronized and micronized  $^{14}\text{C}$ -diosmin into the urine and feces. Percentage of the total radioactivity of a single oral 500 mg dose excreted in the urine and in the feces in 12 healthy male volunteers.

Time period (h)	Percentage of total radioactivity	
	Urine* (mean $\pm$ SD)	Feces (mean $\pm$ SD)
0 – 24	15.9 $\pm$ 6.0	13.7 $\pm$ 20.3
0 – 48	26.3 $\pm$ 12.0	54.5 $\pm$ 43.0
0 – 72	28.5 $\pm$ 12.9	66.0 $\pm$ 34.2
0 – 168	32.7 $\pm$ 18.8	80.3 $\pm$ 25.1

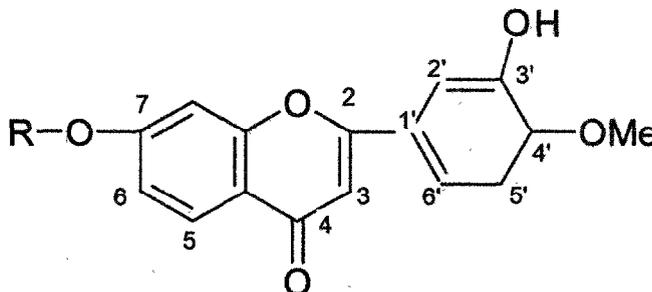
\* Indicates the % of the dose that was absorbed from the gastrointestinal tract. Excreted solely as metabolites.

\*\* Indicates the % of the dose that was excreted as unchanged (unabsorbed) diosmin and diosmetin.

Most pharmacokinetic properties of the diosmin described by Garner et al. (2002),<sup>Ref. 54</sup> Cova et al. (1992)<sup>Ref. 51</sup> and available data on Daflon 500<sup>®</sup> from the manufacturer have also been summarized in a review by Lyseng-Williamson and Perry (2003).<sup>Ref. 32</sup>

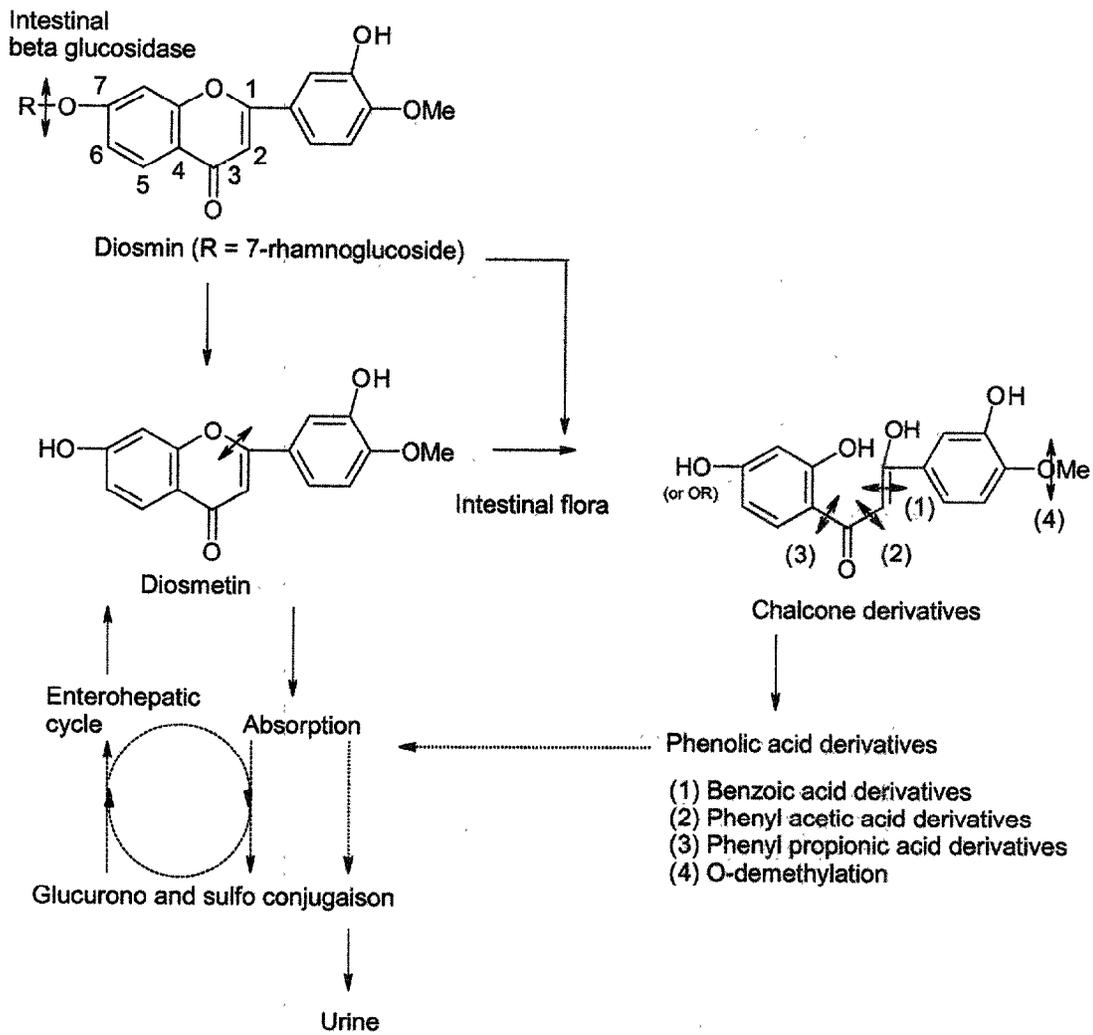
From a structural point of view, phenolic acid derivatives found in urine are resulting of the cleavage of the  $\gamma$  pyrone ring with a further oxidation involving:

- carbon "2" leading to 3-4 dihydroxy benzoic acid and 3 hydroxy 4 methoxy benzoic acid,
- carbon "3" leading to 3 methoxy 4 hydroxy phenyl acetic acid,
- carbon "4" leading to m-hydroxyphenyl propionic acid.



Diosmin (R = 7-rhamnoglucoside)

## Diosmin metabolism



These oxidations could be associated with a phenolic o-demethylation.

This metabolic pathway can take place in the colon, where phenolic derivatives are absorbed. It has been shown that for flavonoids, they result of the action of bacterial colonic flora. These phenolic acid metabolites are common to food flavonoids. That can explain, taking into account the total metabolism of diosmin and diosmetin, the absence of toxicity of the product at its recommended dosage regimen.

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## 7 SUMMARY

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Stragen's Diosmin (95/5) Complex is a 95% diosmin and 5% hesperidin formulation that should be assessed in comparison with Nutratch's Diosmin Complex (90% diosmin and 10% hesperidin). Nutratch's NDI for its Diosmin Complex was reviewed by the FDA as a dietary supplement with a maximum dose of 500 mg per day and a three-month duration of use for adults only.

Stragen's proposed dietary supplement, Diosmin (95/5) Complex:

- contains a minimum of 95% diosmin and a maximum of 5% hesperidin and flavonoid-related substances;
- complies with all specifications of the European Pharmacopoeia and in particular, with less than 1% of unknown substances.

Branded diosmin/hesperidin formulations have been used safely worldwide for decades. These formulations – particularly in 90% diosmin/10% hesperidin combinations, have been the subject of numerous clinical studies, animal studies and *in vitro* studies. All of these studies have demonstrated good levels of tolerance and high safety profiles, even at significant dosage levels. In particular, clinical trials have utilized dosages as high as 6000 mg daily for up to one year. These positive results have been seen in both healthy participants and those with conditions such as CVI.

Stragen's Diosmin (95/5) Complex has a higher level of purity than the formulations in these clinical studies. Its proposed dosage level – 600 mg for a duration not longer than three months- is comparable to the 500 mg dosage Nutratch submitted and is well within the dosage range tested in a myriad of clinical studies. Moreover, Stragen's Diosmin (95/5) Complex has been widely approved in Europe and millions of tablets have been sold throughout Europe since 1998, demonstrating a history of safe use. Based on the history of diosmin use and the strong safety record that exists for diosmin/hesperidin formulations, Stragen believes that its Diosmin (95/5) Complex is reasonably expected to be safe under the conditions of use recommended in the labeling that Stragen will use for this dietary ingredient.

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## 9 APPENDIX – ADDITIONAL INFORMATION ON DIOSMIN

### 9.1 Diosmin Raw Material

#### 9.1.1 Raw Material Specifications

Stragen's Diosmin raw material is obtained from Hesperidin using hemi-synthesis and complies with the 4<sup>th</sup> edition of the European Pharmacopoeia (January 2002) specifications (described in Table 4, below). Two suppliers, (B.) and (F.), have been selected for their compliance with these specifications.

The residual solvents are specific to each manufacturer and the corresponding specification complies with the International Conference on Harmonization (ICH) guidelines.

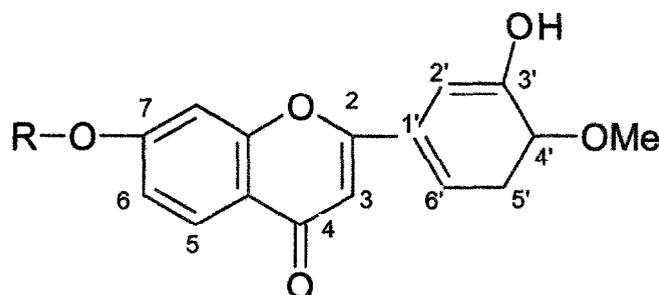
Table 4 European Pharmacopoeia (January 2002) (4<sup>th</sup> edition) specifications of Diosmin obtained by hemi-synthesis from Hesperidin

TESTS	European Pharmacopoeia IV Ed. Specifications
Appearance	grayish-yellow or light yellow hygroscopic powder
Water content	≤ 6%
Identification of Diosmin (I.R. / HPLC)	positive
Heavy metals	not more than 20ppm
Sulfated ash	not more than 0.2%
Iodine Residue	not more than 0.1%
Methanol residue (F) or Pyridine residue (B)	≤ 0.1% or ≤ 0.02%
Assay of Diosmin (on anhydrous basis)	90.0% to 102.0% (anhydrous substance).
Related Substances: - Acetoisovanillone - Hesperidin - Isorhoifolin - Linarin - Diosmetin - Each other impurity - Sum of other impurities (including Acetoisovanillone)	≤ 1.0% ≤ 5.0% ≤ 3.0% ≤ 3.0% ≤ 3.0% ≤ 1.0% ≤ 1.0%
Total impurities	≤ 10.0%

### 9.1.2 Raw Material Chemical Structure

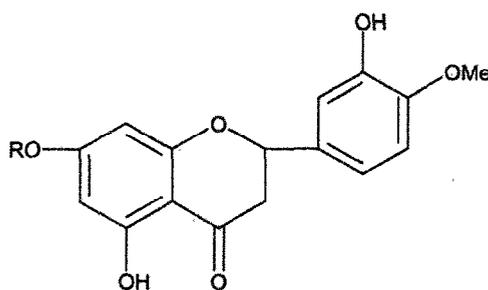
The chemical structure of the diosmin and the flavonoid related substances are as follows:

- **Diosmin (CAS 520-27-4)** has the chemical name:  
7-[[6-O-(6-Deoxy- $\alpha$ -1-mannopyranosyl)- $\beta$ -D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one.



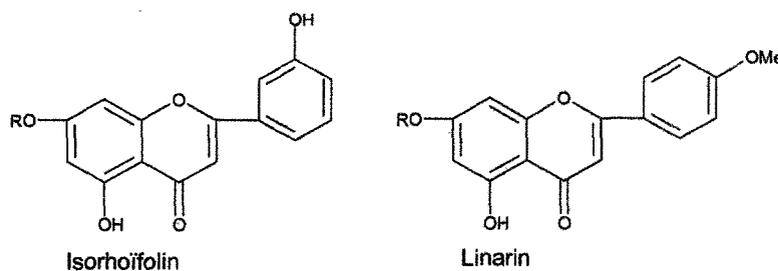
Diosmin (R = 7-rhamnoglucoside)

- **Hesperidin (CAS 520-26-3)**, the starting material, is 2-3 dihydro diosmin, a well known flavanon found in citrus rinds.



Hesperidin

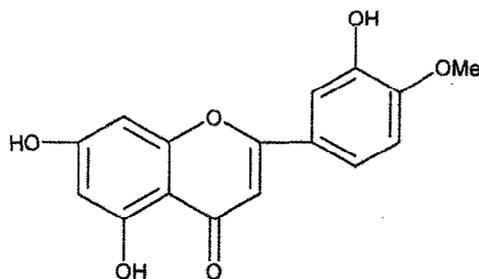
- **Isorhoifolin and linarin (CAS 480-36-4)** are diosmin derivatives. The former lacks the methoxy group in 4', and the later lacks the hydroxyl group in 3'. They result from the oxidation of narirutin and didymin, respectively, both of which are already present in the hesperidin starting material.



Isorhoifolin

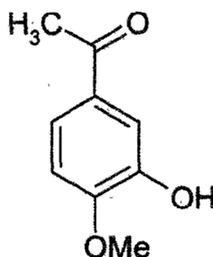
Linarin

- **Diosmetin (CAS 520-34-3)** is the diosmin aglycone and diosmin's main metabolite.



Diosmetin

- **Acetoisovanillone** is a phenolic compound resulting from the hydrolysis of the chalcone derivative of diosmin. The same type of degradation occurs in human metabolism of diosmin.



Acetoisovanillone

### **9.1.3 Raw Material Container Closure System**

The primary packaging material used by both suppliers consists of low-density polyethylene bags. Secondary packaging consists of fiber drums.

### **9.1.4 Raw Material Analytical Procedures**

All analytical procedures are described in the European Pharmacopoeia monograph, except the pyridine residual content, which is fully described and validated in the Drug Master File (DMF) of manufacturer (B.).

### **9.1.5 Raw Material Reference Standards or Materials**

For both suppliers, reference standards are adjusted against European Pharmacopoeia Diosmin Certified Reference Standards (CRS).

### 9.1.6 Raw Material Batch Analysis

Three (F) batches and two (B) batches of diosmin have been analyzed and all results are within the required specifications. The corresponding certificates of analysis are enclosed. (See Attachments I<sub>1</sub>-I<sub>3</sub>).

### 1.5.7. Raw Material Stability Studies

Stability studies performed on the active substance from both suppliers confirmed the absence of significant variation after six months under accelerated conditions, as well as after 24 months under long-term conditions. Therefore, a 36-month re-control period should be sufficient to ensure the good quality of the substance.

#### *Supplier (B.):*

Stability studies have been performed on three batches (DF-2385, DF-2391, DF-2438) from Supplier (B.) according to the following ICH conditions:

- Long-term conditions: 25°C / 60 % Relative Humidity (RH);
- Accelerated: 40°C / 75 % RH.

The parameters evaluated are described in Table 5, below.

Table 5 Parameters evaluated in stability studies on 3 batches from supplier (B.)

SPECIFICATIONS	LIMITS
Appearance	grayish-yellow or light yellow hygroscopic powder
Identification IR/HPLC	positive
Melting point	272°C – 283°C
Water content (KF)	≤ 6.0%
Diosmin content	90.0% - 102.0%
Impurities :	
- Acetoisovanillone	≤ 1.0%
- Hesperidin	≤ 5.0%
- Isorhoifolin	≤ 3.0%
- Linarin	≤ 3.0%
- Diosmetin	≤ 3.0%
- Sum of other impurities (including acetoisovanillone)	≤ 1.0%
Total impurities	≤ 10.0%

The results at 24 months, 25°C / 60% RH, as well as the results obtained after six months of storage at 40°C / 75 % RH, all confirm the absence of any product degradation, other than a trend for some water content increase. This increase, which, importantly, always remained within specifications, was stabilized after 12 months of storage. (See Attachments J<sub>1</sub>-J<sub>2</sub>).

In addition, a previous study (non-ICH) has been undertaken on eight Supplier (B.) batches, stored at approximately 25°C. After 60 months of storage, the results showed no significant variation on the tested parameters.

**Supplier (F.)**

Stability studies have been undertaken on two batches (1709 and 1725) from Supplier (F.) in accordance with the following ICH conditions:

- Long-term conditions 25°C / 60 % RH (see Attachments K<sub>1</sub>, K<sub>2</sub>);
- Intermediate conditions 30°C / 60 % RH (see Attachment K<sub>3</sub>);
- Accelerated 40°C / 75 % RH (see Attachment K<sub>4</sub>).

The parameters evaluated are described in Table 6.

Table 6 Parameters evaluated in stability studies on 2 batches from supplier (F.)

SPECIFICATIONS	LIMITS
Appearance	grayish-yellow or light yellow hygroscopic powder
Water content (KF)	≤ 6.0%
Diosmin content	90.0% - 102.0%
Impurities:	
- Acetoisovanillone	≤ 1.0%
- Hesperidin	≤ 5.0%
- Isorhoifolin	≤ 3.0%
- Linarin	≤ 3.0%
- Diosmetin	≤ 3.0%
- Other impurities + acetoisovanillone	≤ 1.0% (none above 1%)
- Total impurities	≤ 10.0%

The results obtained at 24 months confirm that all results are in accordance with the requested specifications. (See Attachment K<sub>1</sub>).

In addition, a previous study was conducted on one batch from Supplier (F.) stored only under ICH long-term conditions. The results, after 60 months of storage, showed no significant variation on the tested parameters. (See Attachment K<sub>2</sub>).

## 9.2 Stragen's Diosmin Complex Stability Study

The following batches of Stragen's Diosmin (95/5) Complex have been manufactured and corresponding samples used for the stability study:

Batch number:	Batch size (units):	Manufacturing date:	Stability storage date:
0129417	100,000	06/2001	08/2001
0129459	300,000	06/2001	08/2001

### • Study conditions:

The stability was tested in accordance with ICH conditions:

- Long-term conditions ( $25 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$  RH) were tested at 3, 6, 9, 12, 18, 24, 36, 48 and 60 months. Results are available up to 24 months for both batches at Attachment L<sub>1</sub>.
- Accelerated conditions ( $40 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  RH) were tested at 3 and 6 months. Study has been completed and results are available at Attachment L<sub>2</sub>.
- Intermediate conditions ( $30 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$  RH) were tested at 3 and 6 months. Analyses at 9 and 12 months were not required given that the results observed under accelerated conditions were satisfactory. Results are presented at Attachment L<sub>3</sub>.

The parameters tested during stability studies are described in Table 7.

Table 7 Parameters tested in stability studies conducted on manufactured batches of Stragen's Diosmin (95/5) 600 mg

TEST	SPECIFICATIONS	
	At Release	On Stability
Appearance	Tablets have a yellow gray to clear yellow color and an oblong shape	Tablets have a yellow gray to clear yellow color and an oblong shape
Identification of Diosmin HPLC UV spectrum at pH = 13	positive positive	positive positive
Water content (Eur Pharm. 2.5.12)	$\leq 8\%$	$\leq 8\%$
Disintegration time (Eur. Pharm. 2.9.1)	$\leq 15$ min	$\leq 15$ min
Tablets average weight	684.0 – 756.00 mg/tablet (95 – 105% of 720mg)	684.0 – 756.00 mg/tablet (95 – 105% of 720mg)
Mass uniformity (Eur. Pharm. 2.9.5)	Complies to Eur Pharm. 2002	Complies to Eur Pharm. 2002

TEST	SPECIFICATIONS	
	At Release	On Stability
Hardness (Eur. Pharm. 2.9.8)	≥ 125 N	≥ 125 N
Active ingredient Diosmin (HPLC)	540.0 – 612.0 mg/tablet (90 - 102% of 600 mg)	540.0 – 612.0 mg/tablet (90 - 102% of 600 mg)
Total microbial count	< 10 <sup>4</sup> CFU/g	< 10 <sup>4</sup> CFU/g
Yeast and moulds	< 10 <sup>2</sup> CFU/g	< 10 <sup>2</sup> CFU/g
Enterobacteriaceae	< 10 <sup>2</sup> CFU/g	< 10 <sup>2</sup> CFU/g
E. Coli	Absent in 1 g	Absent in 1 g
Salmonella sp.	Absent in 10 g	Absent in 10 g
Staphylococcus aureus	Absent in 1 g	Absent in 1 g

The general findings from the stability studies related to the finished product are as follows.  
(See Attachment L for full details):

- There is an increase in water content and in tablet average weight. However this increase is stabilized after nine months of storage and, importantly, always remains within specifications.
- There is no significant modification in the appearance nor in the disintegration time, regardless of storage conditions.
- At six months, there was no significant variation in the content of diosmin and its related substances in the batches stored at 40°C / 75% RH and 30°C / 60% RH. This finding also applies to the batches stored at 25°C / 60% RH at 24 months. All values remain within acceptable limits. Specifically, the supposed degradation product of Diosmin, its aglycone Diosmetin, remains unchanged in all conditions.
- Specifications for 24-month stability testing requires hesperidin to be present at levels less than or equal to 5%.
- Across storage conditions, the product complies with the European Pharmacopoeia microbiological specification requested for a non-sterile product.

### 9.3 Specifications of Stragen's Diosmin (95/5) Complex

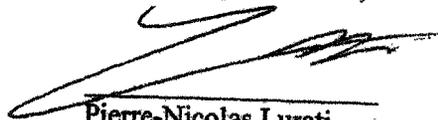
The specifications at release time are described in Table 8.

Table 8. Specifications at release time of Stragen's Diosmin (95/5) batches

TEST	LIMITS
Appearance	grayish-yellow or light yellow hygroscopic tablets with an oblong shape
Identification of Diosmin HPLC	positive
UV spectrum at pH = 13	positive
Water content (Eur. Pharm. 2.5.12)	≤ 8 %
Disintegration time (Eur Pharm. 2.9.1)	≤ 15 min
Tablets average weight	684.0 – 756.00 mg/tablet (95 – 105% of 720mg)
Mass uniformity (Eur. Pharm. 2.9.5)	Complies to Eur. Pharm. 2002 (2.9.5)
Hardness (Eur. Pharm. 2.9.8)	≥ 125 N
Active ingredient Diosmin (HPLC)	570.0 – 612.0 mg/tablet (95 – 102% of 600 mg)
Total microbial count	< 10 <sup>4</sup> CFU/g
Yeast and moulds	< 10 <sup>2</sup> CFU/g
Enterobacteriaceae	< 10 <sup>2</sup> CFU/g
E. Coli	Absent in 1 g
Salmonella sp.	Absent in 10 g
Staphylococcus aureus	Absent in 1 g

This NDI has been submitted by Stragen Pharma, SA. Please let us know if you have any questions.

Respectfully submitted,



Pierre-Nicolas Lurati  
Regulatory & Technical Affairs Manager  
Stragen Pharma SA