GR

ORIGINAL SUBMISSION

Stepan

Stepan Company

Northfield, Illinois 60093
Telephone 847 446 7500

May 24, 2006

REC'D MAY 2 6 2006

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

Dear Sir or Madam:

SUBJECT: GRAS Notification Enclosed

Enclosed is a notice of a GRAS exemption claim on behalf of Stepan Company for the use of Erythritol Distearate as a surface active agent or micro-encapsulant in food applications. Included in this submission is the original copy as well as two duplicate copies. Please feel free to contact me should any questions arise.

Respectfully,

Tracy Strilich
Senior Regulatory Chemist
Stepan Company

Printed on Recycled Paper

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GRAS Exemption Notification

Erythritol Distearate for Use in Food As a Surface Active Agent or Micro-Encapsulant

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RECD MAY 26 2006

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PROPOSED

GRAS Notice

GRN No.	Notifier	Substance	INTENDED USE	GRP #	*BASIS*	*Date* of Filing	*Closure*
	Stepan Company	Erythritol Distearate	*To be used in food as a surface active agent or micro-encapsulant at a level not to exceed the amount required for the technical effect*		Scientific procedures	May 24, 2006	

Stepan

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Telephone 847 446 7500

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Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Sir or Madam:

SUBJECT: GRAS Notification –
Exemption Claim for Erythritol Distearate

In accordance with FDA proposed rule of April 17, 1997 (62 FR 18938), relating to the filing of a generally recognized as safe (GRAS) notification, we hereby claim that the use of Erythritol Distearate as a surface active agent or micro-encapsulant in food used at a level not to exceed the amount required for the technical effect is generally recognized as safe and exempt from premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. In conformity with the requirements outlined in the proposed rule, the following information is included (*in triplicate*) with this exemption claim.

GRAS EXEMPTION CLAIM

We hereby claim that the use of Erythritol Distearate as a surface active agent or micro-encapsulant in food used at a level not to exceed the amount required for the technical effect is exempt from the premarket notification requirements of the Federal Food, Drug, and Cosmetic Act because of determination that such use of Erythritol Distearate is generally recognized as safe.

(1) Identification of the notifier:

Tracy Strilich
Senior Regulatory Chemist - Product Safety and Compliance
Stepan Company
22 West Frontage Road
Northfield, IL 60093
Telephone: 847-501-2416
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Regulatory Representative:

William A. Olson, Ph.D.
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192-5755
Telephone: 703-590-7337
Faxline: 703-580-8637
E-Mail: cfrsrv@aol.com

(2) Common or usual name of the substance:

Erythritol Distearate

(3) Applicable conditions of use:

(a) Foods in which the substance is to be used:

Erythritol Distearate is intended to be used in food applications as a surface active agent or micro-encapsulant at a level required to achieve the desired technical effect.

(b) Purposes for which the substance is used:

Erythritol Distearate is a surface active agent or micro-encapsulant.

(c) Description of the population expected to consume the substance:

Erythritol Distearate is expected to be used by the general public.

(4) Basis for GRAS determination, i.e., through experience based on common use in food (pre-1958) or through scientific procedures:

The basis of the GRAS determination is through scientific procedures.

(5) Availability of Data and Information (references)

The referenced material that is the basis for this GRAS determination is available for the Food and Drug Administration's review and copying at reasonable times at the Product Safety and Compliance Department of Stepan Company, 22 Frontage Road, Northfield, IL 60093, or will be sent to FDA upon request.

5-24-2006

Stepan Company

Stepan EDS - GRAS

Identity of the Substance

A. Chemical Name:

Octadecanoic acid, ester with (2R, 3S) – 1,2,3,4 – butanetetrol, rel- (CAS Registry Number 126967-61-1)

B. Common Name:

Erythritol Distearate

C. Trade Name:

STEPAN® EDS

D. Definition:

Erythritol Distearate occurs as pale yellow flakes. It is prepared via two routes; esterification of erythritol and stearic acid or transesterification of erythritol and methyl stearate. Erythritol Distearate is a random ester of erythritol and two molar equivalents of fatty acids.

E. Description:

Pale yellow flakes

Chemical Description, Manufacture, Specifications, and Stability

Erythritol Ester Chemistry

There are several methods for manufacturing erythritol esters. The major processes include esterification and transesterification. The basic chemical reactions for these two methods are shown in Example 1 below:

$$\text{Erythritol} + \text{Fatty acids} \rightleftharpoons \text{Erythritol Esters} + \text{Water} \uparrow$$

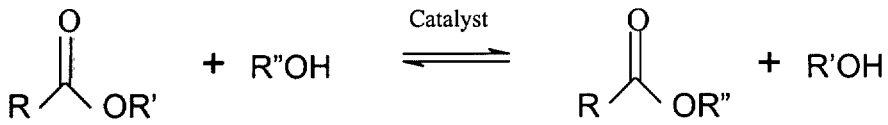
$$\text{Erythritol} + \text{Fatty acid methyl esters} \overset{\text{Catalyst}}{\rightleftharpoons} \text{Erythritol Esters} + \text{Methanol} \uparrow$$

Example 1

Esterification is the reaction of a carboxylic acid with an alcohol. The reaction results in the formation of an ester and water. Acids, bases and heat can catalyze esterification reactions and due to the reaction equilibrium, water is distilled to isolate the desired end product, Scheme 1.

Scheme 1

A transesterification reaction is the transfer of an alkoxy group located on a carboxylic ester with one located on an alcohol. The reaction results in the formation of a new ester and a new alcohol moiety. Acids or bases can catalyze transesterification reactions and, due to the reaction equilibrium, the newly formed low boiling alcohol can be distilled to isolate the desired end product, Scheme 2.

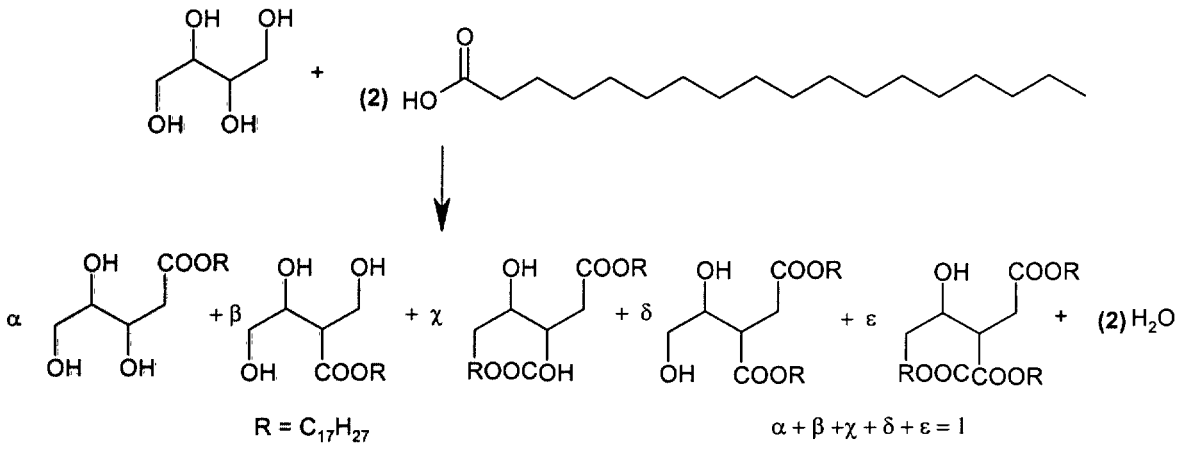


Scheme 2

Esterification of Erythritol and Stearic Acid

In this reaction, erythritol and stearic acid are being esterified to produce erythritol esters. The reaction is catalyzed using heat with a 2:1 mole ratio of stearic acid to erythritol. This reaction yields an indefinite ratio of erythritol monostearate, erythritol distearate,

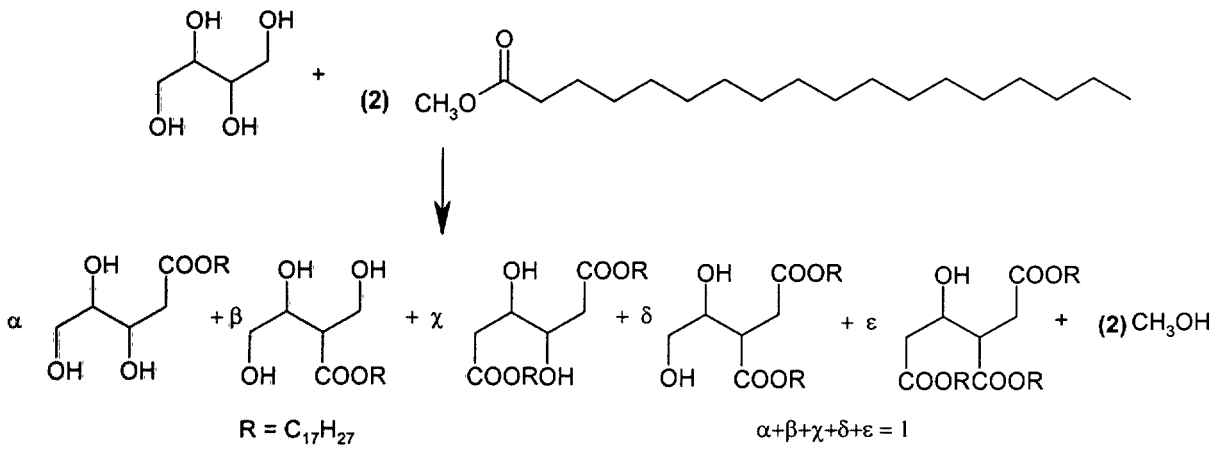
erythritol tristearate and water, Scheme 3. The water formed during the reaction is distilled out of the vessel to isolate the final product. The predominant product will be erythritol distearate due to reactivity and selectivity of the reactants. This diagram and others that follow depict a general representation of the esters produced in the end composition. Esterification in this scheme and those below happen by a random nature and the final randomized ester is a mixture of monoesters, diesters, and triesters.



Scheme 3

Transesterification of Erythritol and Methyl Stearate

Another method for producing the desired erythritol esters is by transesterifying erythritol and a stearate ester, such as methyl stearate. The reaction is catalyzed using a base catalyst such as potassium carbonate, and a 2:1 mole ratio of methyl stearate to erythritol. This reaction yields an indefinite ratio of erythritol monostearate, erythritol distearate, erythritol tristearate and methanol, Scheme 4. The methanol formed during the reaction is distilled out of the vessel to isolate the final product. The predominant product will be erythritol distearate due to reactivity and selectivity of the reactants. The residual catalysts, in the form of potassium soaps, remain in the end product mixture and are not detrimental to the finished product.

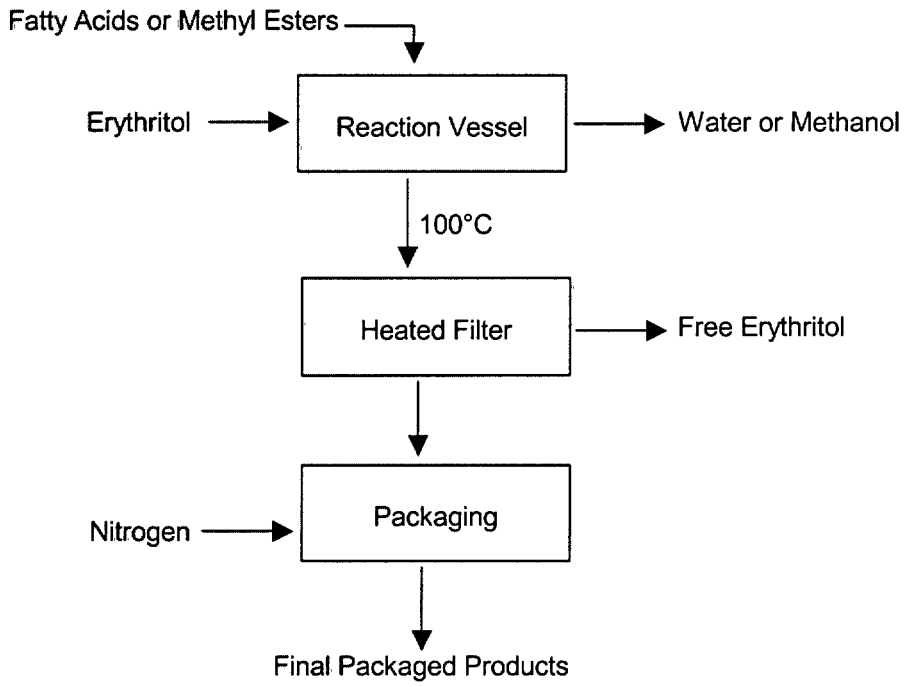


Scheme 4

Process Description

The process for manufacturing erythritol esters when done by esterification takes place in three basic steps: reaction, filtering, and packaging.

Figure 1



A block diagram of the process is contained in Figure 1. The reaction vessel is charged with preheated fatty acid or methyl ester, to which granular erythritol is added with

agitation. It is important to note that the melt point of erythritol is 122°C and will be in a suspension/slurry with the fatty acid or methyl ester until the reactor is heated to above the melt point. The reactor will be slowly heated to a final temperature of 230°C. At about 190°C, the reaction begins to generate water (or alcohol) that is removed via a total condenser. A partial condenser is also used to return any acids vapor or methyl ester to the reaction that may be carried by the reaction vapors into the reactor overheads. The batch will be held at 230°C until it is determined through acid value and hydroxyl value testing that the fatty acid or methyl ester has been essentially fully consumed. Once the reaction is complete the batch will be cooled to 100°C. A small amount of erythritol, approximately 1%, will remain unreacted and can be removed by filtration at this point; however, a small degree of erythritol remaining in the product is not detrimental. Once filtered, the erythritol ester is packaged in a flaked, prilled or drummed media for distribution and use.

The preferred route is to prepare erythritol distearate through the fatty acid condensation process as this avoids the complication of catalyst addition and subsequent removal.

Analytical Specifications

Lipid esters can be fully characterized by performing the four following analytical tests:

1. Acid Value
2. Saponification Value
3. Hydroxyl Value
4. Fatty acid profile by GLC

Acid Value

Acid value is indicative of the level of free, unreacted fatty acid remaining in the finished erythritol distearate product. Acid value is defined as the number of milligrams of potassium hydroxide per gram of a substance required to neutralize the acids contained in that substance. The method is adapted from AOCS (American Oil Chemist Society) Method Cd 3d - 63; a copy of the Stepan method is appended. The theoretical acid value for erythritol distearate is diminishingly small. The specification acid value for erythritol distearate suitable for use in foods is a maximum of 5.0 mg/Kg KOH.

Saponification Value

Saponification value is indicative of the degree to which the polyol, in this case erythritol, is esterified and indicative of the equivalent weight of any lipid ester. Polyol esters can be esterified to different degrees, e.g. mono, di, tri or higher esters, depending on the number of fatty acid groups covalently bonded to the polyol. In addition, fatty acids, as obtained from natural sources, come as mixtures within a range or fraction of a range; i.e. stearic acid is obtained as a mixture of saturated C_{16} and C_{18} fatty acids (palmitic and stearic). If one of two conditions is not met, if either the degree of esterification is too high or low or if the stearic acid does not comply with the correct fatty acid profile, then the

saponification value is not likely to comply with the specification set forth in this document for an erythritol distearate suitable for use in foods.

Saponification value is defined as the milligrams of potassium hydroxide per gram of ester required to neutralize any free fatty acid and to saponify (to make soap from) the esterified fatty acids. The method is adapted from AOCS Method Cd 3 – 25; a copy of the Stepan method is appended. The theoretical saponification value for erythritol distearate is 175 mg KOH / g. The specification saponification value range for erythritol distearate suitable for use in foods is 158-190 mg KOH / g.

Hydroxyl Value

Hydroxyl value is the hydroxyl content of an alcohol or polyol. In the case of erythritol, there are four hydroxyls contained within the molecule. On average, erythritol distearate, a mixture of mono, di and tristearate esters of erythritol, has two remaining free hydroxyls and two esterified hydroxyls. Hydroxyl value is also indicative of equivalent weight, and if the conditions of degree of esterification or the fatty acid profile are not correct, then hydroxyl value is not likely to comply with the specification set forth for erythritol distearate suitable for use in foods.

Hydroxyl value is defined as the milligrams of potassium hydroxide per gram of hydroxyl containing substance equivalent to the hydroxyl content of that substance. Hydroxyl value is performed by measuring the degree of unreacted acetate resulting from complete acetylation of a sample of the hydroxyl containing substance. The method is adapted from AOCS Method Cd 13 – 60; a copy of the Stepan method is appended. The theoretical hydroxyl value for erythritol distearate is 154 mg KOH / g. The specification hydroxyl value range for erythritol distearate suitable for use in foods is 120-200 mg KOH / g.

Fatty Acid Profile

The fatty acid profile is indicative of the distribution of fatty acids in any lipid ester. It is performed with a suitable gas chromatograph (GLC), using appropriate packed or capillary columns and appropriate detectors suitable for measuring the presence of fatty acids. In addition to identifying the presence of specific fatty acids, the relative amount of each fatty acid is determined by proportioning the peak area for each component to the total area of all components in the chromatogram; the area percent. The method is adapted from AOCS Method Cd 1 – 62; a copy of the Stepan method is appended. The specification fatty acid profile for the fatty acids in erythritol distearate suitable for use in foods is provided below in Table 1.

Acid	Carbon Chain	Specification
Myristic	C_{14}	2% Maximum
Myristoleic	C_{14}	1% Maximum
Palmitic	C_{16}	45% Maximum
Palmitoleic	C_{16}	1% Maximum

Stearic	C_{18}	51% Minimum
Oleic	C_{18}	5% Maximum
Linoleic	C_{18}	3% Maximum
Linolenic	C_{18}	2% Maximum
Icosanoic	C_{20}	1% Maximum

Table 1

Residual Alcohol (Methanol)

Preparing erythritol distearate by the transesterification process results in a release of alcohol rather than water, as is the case for the direct esterification process. To ensure the integrity of the final composition, it is important to verify that little or no residual alcohol remains in the product prior to quality release. This test is performed by headspace GLC of the composition. It is performed with a suitable gas chromatograph, using an appropriate capillary column and detectors suitable for measuring the presence of lower alcohols. A copy of the Stepan Method is appended. The theoretical amount of residual alcohol in erythritol distearate is diminishingly small. The specification for residual alcohol (ethanol or methanol) in erythritol distearate suitable for use in foods is a maximum of 0.1% when transesterification is the method of manufacturing.

Residual Methyl or Ethyl Ester

Preparing erythritol distearate by the transesterification process requires monitoring the level of unreacted alcohol ester (methyl or ethyl) to ensure the integrity of the final composition. It is important to verify that the starting ester is essentially fully consumed prior to quality release. This test is performed by GLC of the composition in a manner similar to that for obtaining the fatty acid profile; without breaking down the composition into its individual fatty acids. It is performed with a suitable gas chromatograph, using an appropriate capillary column and detectors suitable for measuring the distribution of fatty acid esters. A copy of the Stepan Method is appended. The theoretical amount of residual methyl or ethyl stearate in erythritol distearate is diminishingly small. The specification for residual methyl or ethyl stearate in erythritol distearate suitable for use in foods is a maximum of 0.5% when transesterification is the method of manufacturing.

A summary of the analytical tests and their specification ranges are listed below in Table 2.

Test	Stepan Method Number	Specification
Acid Value	514-O	5 mg / kg KOH Maximum
Saponification Value	516-O	158 – 190 mg KOH / g
Hydroxyl Value	053-O	120 – 200 mg KOH / g
Fatty Acid Profile	499-I	
C_{14} Myristic Acid	499-I	2% Maximum
C_{14} Myristoleic Acid	499-I	1% Maximum
C_{16} Palmitic Acid	499-I	45% Maximum
C_{16} Palmitoleic Acid	499-I	1% Maximum
C_{18} Stearic Acid	499-I	51% Minimum
C_{18} Oleic Acid	499-I	5% Maximum
C_{18} Linoleic Acid	499-I	3% Maximum
C_{18} Linolenic Acid	499-I	2% Maximum
C_{20} Icosanoic Acid	499-I	1% Maximum
Residual Alcohol (Methanol)	149-M	0.1% Maximum
Residual Methyl/Ethyl Ester	103-G	0.5% Maximum
Heavy Metals as Lead[6]	FCC *	2 mg / Kg Maximum

Table 2

Stability

The stability of erythritol distearate was measured in two separate stability tests. The first stability test was conducted at ambient temperature. Erythritol distearate was manufactured and analyzed, held at room temperature, RT, for 11 months and reanalyzed. Results are listed below in Table 3.

Time	0 months	11 months
StorageTemperature	@ RT	@ RT
Acid Value	0.7	1.4
Hydroxyl Value	165.4	165.3

Table 3

The second stability test, an accelerated stability study, was conducted at an elevated temperature. Erythritol distearate was manufactured and analyzed, held at a constant temperature of 90°C for 9 months and reanalyzed. Results are listed below in Table 4.

Time	0 months	9 months
StorageTemperature	@ 22°C	@ 90°C
Acid Value	2.1	7.5
Hydroxyl Value	154.1	147.1

Table 4

No significant change can be observed from the above data; therefore, erythritol distearate is a stable product. The stability of erythritol distearate is equivalent to standard monoglycerides, diglycerides, and triglycerides.

Use Information

Food ingredients such as flavors or leavening agents can be rendered ineffective in applications such as baking, when exposed to high temperatures for prolonged periods of time. In order to allow baked food to capture the flavor or leavening for maximum effect, a lipid-encapsulated component is not released until the shell of the capsule melts. With the introduction of esters of erythritol to the food industry, the limitation of solid fats melting in the range of 60 to 70°C is eliminated because erythritol esters melt at higher temperature ranges. This enables the food formulator to use a safe lipid similar to esters of glycerol in food compositions that require processing at higher temperatures; it allows for the retention of flavor or leavening, possibly antioxidants, or any technical effect in foods that is best exhibited when the food product is exposed to temperatures in excess of 70°C.

As outlined above in the chemistry section, the presently described technology relates to edible fatty acid ester compositions based upon erythritol. Erythritol esters are structurally similar to glycerol esters, namely monoesters and diesters of erythritol are nearly identical to monoesters and diesters of glycerol with the exception of a second carbon and hydroxyl moiety. We believe that by replacing glycerol as the backbone of the fat component, we obtain a more polar lipid and, that by the presence of increased polarity, we observe higher melting points for this fat component. We have also observed that diglycerides follow this same physical behavior, namely, that diglycerides exhibit a higher melting point than their parent fat and also greater than the corresponding monoglyceride. This appears to hold true for erythritides as well, and as such we have identified the ideal composition for a high melting lipid to be composed of the diesters of erythritol. As is the nature of partial fatty esters, these findings are based upon averages of the composition because in the presence of diglycerides always exist monoglycerides and triglycerides; i.e. in the presence of predominately dierythritides, monoerythritides and trierythritides exist. Erythritol distearate appears to give the best compromise of utility and melting point in that it achieves a melting point of about 80°C.

Partial esters of glycerol are ubiquitous in food products, acting primarily as:

- Surface active agents; emulsifiers, dispersants, agglomeration controlling agents, wetting agents, aeration agents (foamers) and anti-foams
- Lubricants, to reduce stickiness or tackiness
- Component of shortening, anti-staling
- Crystal modification
- Components of microencapsulated ingredients, specifically as the shell component

In every aspect, they are used to achieve one of the technical effects as listed here and possibly others, at a level in the food required to achieve that technical effect. Typically this represents one to two percent of the lipid component of the food product and is

typically as low as a few hundreds of a percent to as much as one percent of the final food product[1]. Erythritol distearate will provide the identical list of functions and, as is the case for monoglycerides and diglycerides, at a level required to achieve the technical effect. The single provision is the limitation of the requirement of a higher melting point; i.e. erythritol distearate will replace or supplement the monoglyceride and diglyceride when high melting point is critical and necessary to achieving the technical effect. The following examples give an indication of when and where erythritol distearate can be used to replace monoglycerides and diglycerides in food applications. Many of these examples are about organoleptic qualities but some are more technical in nature such as the specific case of lipid micro-encapsulation.

Because the distinguishing characteristic associated with the use of erythritol distearate is specifically the melting point, its use will be limited in food applications. For example, encapsulation compositions using monoglycerides and diglycerides would not be completely displaced by erythritol distearate. The use of erythritol distearate is limited to only a few very specific cases where the higher melting point becomes a requirement; for example in the case of protecting a micronutrient in a baking application where the microcapsule is not intended to melt during processing. Other examples would include improving the stability of a food emulsion where the crystalline nature of erythritol distearate gives an improvement to the monoglycerides and diglycerides in the formulation. Generally speaking, monoglycerides and diglycerides are very efficient emulsifiers and erythritol distearate is not a direct replacement for this chemistry. For this reason, erythritol distearate will not find a niche in general food products but is intended as in ingredient for functional or technical foods.

The 1977 Survey on Use of Food Additives as conducted by NAS/NRC reported some information on the use of glycerol monostearate, a similar material. Those use level values were given as[2]:

Grain products/pasta with meat sauce: 0.17%
Vegetable/juices with potatoes-dried: 0.03%
Chocolate covered candy: 0.17%
Baby food cereals and all cereals: 0.072%

The SCOGS committee in 1975, tentative report, Table V, gave the level of addition of glycerides to food by Food Category; weighted mean percent of glycerol monostearate in 18 foods, with most reporting less than 0.5%. Only nuts and nut products had level of addition of more than 1%. The level of all mono- & diglycerides in 29 food categories was greater than glycerol monostearate, but only 4 food categories had concentrations greater than 1%[3]. Erythritol distearate will not exceed these levels of use to achieve a technical effect driven by the consideration of melting point.

Consider also that the route to making erythritol distearate requires multiple processing steps, the isolation of stearic acid from natural food sources, the manufacture of erythritol, and the subsequent esterification of the two. Monoglycerides and diglycerides are made by a single processing step of interesterification of the naturally occurring fat

with glycerin and are far less expensive to produce. On this bases, erythritol distearate is only economic in food products where the technical advantages outweigh the cost of manufacture. Of the applications identified in this notification, all of which are examples of potential uses and not intended to be exclusive or to limit the imagination of cleaver practitioners of the art, the greatest use level would occur where erythritol distearate is used as an emulsifier to enhance the performance of monoglycerides and diglycerides.

Taken on the whole, the technical advantages with the economic disadvantages, the total market niche for erythritol distearate cannot amount to more than a few percentage points of the total market for glycerides. If we take the optimistic estimate of 10% of this market, the estimated percentage of utility in foods in general can only reach about 0.05% of daily food intake. On the basis of a 3-kilogram diet, this amounts to a potential 1.5 gram intake of erythritol distearate if and only if the hypothetical person consumes exclusively processed foods. Our assessment therefore concludes that the maximum daily intake for erythritol distearate is on the order of about 1.5 grams.

Component of encapsulation

As the lipid component for a food microcapsule, dierythritide is more effective than diglyceride because of its higher melting point. When a lipid is used to encapsulate leavening agents (i.e. baking powder) the release point in the oven is governed by its melting range, and it is at this point that the active is freed from the hydrophobic matrix and can mingle with steam and water in the food product to give its full effect. Fully hardened fat (typically referred to as tristearin) has a melting point in the range of 60 – 65°C, glycerol distearate is in the range of 65 – 70°C and has therefore been a preferred fat to provide leavening encapsulation. Erythritol distearate, with its 80°C melting point, gives maximum protection to the leavening agent by releasing it at the optimum time to give the greatest effect in the oven.

There are other examples of advantages for encapsulation with erythritol distearate. The incorporation of ω – 3 and ω - 6 fatty acids in functional foods requires protecting the highly unsaturated fatty acid moiety from oxidative degradation. An oil or fat containing these functional lipids can be commingled with erythritol distearate and prilled to make a capsule. These capsules have greater integrity than similar capsules made from hardened fat or diglycerides because of the higher melting point of the dierythritide. When used in functional foods, the capsules can withstand greater processing temperatures and higher levels of shear than normal fat capsules. The end result is less premature release of the ω – fatty acid and therefore improved stability of the food to oxidative degradation. Vitamins and other nutrients are also lipid encapsulated to protect them from oxidative degradation and extend their stability and shelf life. Further, vitamins and minerals can often contribute off-flavors to functional food products[4]. By replacing hardened fat with erythritol distearate to improve the integrity of the microcapsule the vitamin or mineral can be released in the stomach at the time of digesting, whereas a softer and lower melting lipid would have a tendency to release the off-flavor in the cooking or eating stage of the process. All of these are advantageous uses of erythritol distearate over hardened fat or monoglycerides and diglycerides.

Erythritol distearate can be used to protect beta-carotene from the negative effects of ultra-violet exposure and at the same time eliminate the coloring properties. Its higher melting point acts to retard color release during processing steps. Erythritol distearate can be used to deliver fat-soluble vitamins to a food product due to its ampholitic nature, in this case acting as both a solvent for the vitamin and an emulsifier to carry it into the food system. As an encapsulent carrier for the vitamins, it can retard oxidative degradation in processing and storage of the food product. As a carrying agent for flavors, it can be used to dilute the flavor oil to a useful level for incorporation while acting as a fixative to hold the flavor oil to the food. When compared to monoglycerides and diglycerides, the higher melting point makes it a superior fixative, flavor oils being more volatile from a liquid lipid than a solid one. The type of coatings where this technology is especially applicable includes but is not limited to: Pan Coating, Fluid Bed Encapsulation, Annular Jet, and Spray Chilling or Congealing. All of these techniques are used in the food industry today and all benefit from the improvement in shell integrity due to the higher melting point of erythritol distearate.

Crystal Modification

Modification of fat crystalline form is a common practice in food processing. Erythritol distearate, with its high melting point and rapid solidification tendencies, is indicative of a β' tending lipid. As such, it is an ideal additive for margarine and shortening as a crystal promoter to prevent the formation of large grainy crystals. It can be used as a tempering aid in chocolate and to prevent bloom. In both applications, the ampholitic nature of erythritol distearate contributes to emulsion or dispersion stability by acting as a low HLB emulsifier. Erythritol distearate has dual functionality in these types of applications, acting as an emulsifier and crystal modifier simultaneously.

Component of Shortening

To control staling in bakery products, ampholitic materials are added to the dough to complex the starch and/or soften the crumb. Erythritol distearate can be added to shortening to function in this fashion as both a dough conditioner and shelf life extender. As a component of shortening for baking, erythritol distearate will complex with starch and gluten to provide dough strength and elasticity in the leavening process. As a component of shortening in baking, it can provide stability as part of the emulsifier package for air bubble incorporation, such as is important in cakes and confections. The characteristic that brings about these qualities is its ampholitic nature; just as is the case with monoglycerides and diglycerides, part of the molecule is hydrophilic, part is lipophilic, and it has an HLB of 2.9.

Emulsifier

The chemical structure of erythritol distearate gives it a degree of surface activity comparable to monoglycerides and diglycerides; HLB range for monoglycerides and diglycerides is 2 – 4 and erythritol distearate is about in the middle of this range at 2.9.

Emulsifiers play a key role in food products[5] and are too numerous to completely review in this document. However, in brief, emulsions play a pivotal role in the development of palatable foods. They contribute to desirable mouth feel, are key to the formation of structures such as foams in whipped toppings, provide stability to mixtures of fats and oils, and contribute to creaminess in complex products such as ice cream. Several of the applications listed above for erythritol distearate take advantage of its functionality as an emulsifier: its surface-active properties. When compared to monoglycerides and diglycerides, it is a hard waxy substance and not a plastic lipid as is the case for the latter. As an emulsifier, it can be used to harden the interfacial region when compared to monoglycerides and diglycerides, and this may be important in food products such as whipped sugar toppings or to control the plasticity of batters.

Conclusion

Erythritol distearate is structurally similar to monoglycerides and diglycerides and in so far as the similarity goes, it contributes to food products in ways not dissimilar to monoglycerides and diglycerides. The metabolism pathways are analogous to lipids in general but most specifically to fatty acid esters. This makes it safe for use as a general food component and it should be considered GRAS for use across a broad range of food applications. What distinguishes erythritol distearate from the ubiquitous monoglycerides and diglycerides is the unique high melting point and hard crystalline nature of erythritol distearate. As outlined above, there are several food applications that can take advantage of these characteristics to make the food more palatable, improve the shelf life, or enhance the nutritional characteristics. For these reasons, erythritol distearate should be allowed in foods up to and including the amount necessary to achieve the technical effect.

Safety Evaluation

Stepan Company has made the determination that erythritol distearate is generally recognized as safe based upon scientific procedures, utilizing publicly available reviews, reports, and prior GRAS determinations.

The substance, as identified in Attachment 1 of this notification, is the result of the partial esterification of erythritol with fatty acids. Erythritol is considered GRAS and was documented as GRAS under the GRAS Affirmation Petition 7G0422, with the petition being accepted for filing in the Federal Register of January 15, 1997. This petition was based upon evaluation of the safety of erythritol, the documentation being summarized and presented in the petition, which is fully available to the public at the Dockets Management Branch, US Food and Drug Administration, Docket No. 97G-0063.

A comprehensive review of erythritol, including biochemical, metabolic, toxicological, and clinical data was published in Food and Chemical Toxicology by Munro, et al.

Erythritol was also evaluated for authorizing a health claim on sugar alcohols and dental caries to include the sugar alcohol erythritol. This did result in the inclusion of erythritol in the regulation relating to dietary sugar alcohols and dental caries, 21 CFR 101.80. This GRN is not intended to document health benefits of erythritol distearate.

In addition, GRAS Notification, GRN 76, for erythritol, submitted by Cerestar Holdings B. V., April 30, 2001, received FDA/CFSAN response September 11, 2001. This GRN is effective and permits the use of erythritol as a flavor enhancer, formulation aid, humectent, nutritive sweetener, stabilizer and thickener, sequesterant, and texturizer in foods, with use levels in beverages being 3.5%, and in other foods at levels of 10-100%.

The fatty acid moiety in the erythritol ester is termed stearic acid and is consistent with the affirmed GRAS substance, stearic acid, 21 CFR 184.1090. Note, the stearic acid meets the stearic acid as further defined in the Food Chemical Codex, with reference to the 3rd edition, 1981, which is updated through the 5th edition of Food Chemical Codex, 2004, page 450.

Stearic acid, while specifically defined in the Food Chemical Codex, is organic acids contained from fats chiefly of stearic acid and palmitic acid. Thus, the fatty acids that are esterified with erythritol are considered GRAS for use in foods generally, with no restrictions except to be consistent with current good manufacturing practices.

The in-vivo cleavage of erythritol fatty acid esters to the constituent moieties has been studied by Mattson, et al., in a series of studies published in the Journal of Nutrition and Journal of Lipid Research. It was shown that the efficiency of the cleavage of the ester linkage is variable depending upon the numbers of the ester linkages. There is evidence

that with compounds containing ester groups greater than 3, there is a decrease in ester cleavage and resulting absorption.

With the erythritol diesters having an average of 2 ester linkages, the cleavage is relatively efficient and results in the immediate digestive products of erythritol and fatty acids or fatty acid salts (stearic and palmitic).

Erythritol has been shown in animals and in the human to be rapidly absorbed intact, and excreted virtually quantitatively through the urine. The no effect level for erythritol based upon physiological effects, i.e., laxation-osmotic diuresis, from human clinical experience is greater than 1 gram/kg body weight. (60 grams per 60 kg person)

The estimated maximum intake of erythritol distearate, 1.5 grams/person/day or 25 mg/kg bw. The intake of erythritol equivalent (18% of the erythritol distearate intake) would be an estimated maximum of 270 milligrams per day or 4.5 mg/kg bw. There is 220 fold margin between the expected daily intake and the level which has been shown to not produce any laxation or osmotic diuresis.

Conclusion

Stepan Company, after critical review and analysis of the available information on erythritol distearate, has concluded that the substance is Generally Recognized As Safe for use as a surface active agent or micro-encapsulant as is described in this notification.

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ATTACHMENT No. 6

Acid Value and Free Fatty Acid

Stepan Company Analytical Method
Northfield, Illinois 60093
(847) 446-7500

SM 514-O

SCOPE: This method determines the acid value and free fatty acid of a sample. The sample can be titrated colorimetrically or in the case of a dark sample solution, the method can be done potentiometrically when a phenolphthalein endpoint is not clearly visible. This method is applicable to Stepan esters as well as other surfactants.

SUMMARY: A weighed sample is dissolved in neutral isopropyl alcohol and titrated with standardized sodium or potassium hydroxide. The acid value and/or free fatty acid can be calculated from the titration results.

SAFETY: ISOPROPYL ALCOHOL is FLAMMABLE. Avoid open flames and sparks. Work in a hood or well-ventilated area. Wear proper personal protective equipment.

SODIUM and POTASSIUM HYDROXIDE are CORROSIVE. Avoid eye and skin contact. Wear proper personal protective equipment.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

DEFINITION(S): The acid value is the number of milligrams of potassium hydroxide necessary to neutralize the free fatty or rosin acids in 1g of sample.

APPARATUS:

1. Container, suitable size
2. Magnetic stirrer and stir bars
3. Autotitrator or buret, suitable size
4. Analytical balance, +/- 0.01g

REAGENTS:

1. Sodium or potassium hydroxide, 0.1N, 0.5N, or 0.05N, standardized
2. Isopropyl alcohol (IPA), 99%, neutralized, ethanol, or equivalent
3. Phenolphthalein indicator (see Remark 1)

PROCEDURE:

1. Using the sample size guide below, accurately weigh an appropriate amount of sample into a suitable size container (see Remark 2).

Expected Acid Value	Sample Weight, g
0 - 0.1	55
0.1 - 1.0	20
1 - 4	10
4 - 15	2
15 - 75	0.5
75 - 200	0.25
200 - 500	0.15

2. Add 50-75 mL of neutralized IPA and several drops of phenolphthalein indicator. Mix well until the sample is dissolved. Mild heating on a hot plate may be necessary to completely solubilize some solid samples.

3. Titrate colorimetrically or potentiometrically with standardized sodium or potassium hydroxide until the first permanent pink color appears and persists for 30 seconds (see Remark 3).

4. Calculate the acid value and free fatty acid content as stated in Equations 1 and 2, CALCULATIONS section.

CALCULATIONS:

$$1.\quad \text{Acid Value} = \frac{(\text{NaOH, mL})\ (\text{N NaOH})\ (56.1)}{\text{Sample Weight, g}}$$

$$2.\quad \text{Free Fatty Acid, \%} = \frac{\text{Acid Value x Factor}}{561}$$

ESTER FACTORS

Acetic	060
Adipic	146 (dibasic)
Capric	182
Caprylic	144
Caprate/Caprylic	153
Caproic	116
Lauric	200
Myristic	228
Oleic	282
Palmitic	256
Propionic	074
Ricinoleic	298
Salicylic	138
Stearic	284
Erucic	338

PRECISION and ACCURACY:

PRECISION:

For Acid Values of 100 or Greater:

The standard deviation of the method for between laboratory application is 1.1 acid value units absolute.

The standard deviation of the method for within laboratory application is 0.28 acid value units absolute. Duplicate analyses by a single operator must agree within 0.73 acid value units absolute to be considered acceptable.

REMARKS:

1. For phosphate acid number analysis weigh approximately 0.7g of sample and titrate with 0.1N NaOH.
2. For acid values less than 1.0 titrate with 0.05N NaOH or KOH.

REFERENCES:

1. Annual Book of ASTM Standards, Volume 15.05 (1988), Designation E 301-83.

Saponification Value

Stepan Company Analytical Method
Northfield, Illinois 60093
(847) 446-7500

SM 516-O

SCOPE:

This method determines the saponification value of all normal fats and oils, and organic esters.

Alternatively, the saponification value can be determined potentiometrically using an autotitrator equipped with a glass combination pH electrode.

SUMMARY:

The sample is reacted with alcoholic potassium hydroxide solution and the amount of potassium hydroxide that reacts is determined by acid titration.

SAFETY:

ISOPROPANOL, METHANOL and 3A ALCOHOL are FLAMMABLE. Avoid open flames and sparks. Work in a hood or well- ventilated area. Wear proper personal protective equipment.

POTASSIUM HYDROXIDE is CORROSIVE. Avoid eye and skin contact. Wear proper personal protective equipment.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

DEFINITION(S):

The saponification value (SV) is the amount of alkali necessary to saponify a given quantity of sample. It is expressed as the number of milligrams of potassium hydroxide required to hydrolyze 1 gram of sample.

APPARATUS:

1. Saponification flasks, or round bottom flasks, suitable size
2. Water condenser
3. Hot plate
4. Buret, 50 mL
5. Pipet, volumetric, 25 mL

REAGENTS:

1. Alcoholic potassium hydroxide, 0.5N or 1N
2. Hydrochloric acid, 0.5N, standardized
3. Phenolphthalein indicator solution
4. 3A Alcohol
5. Methanol

6. Potassium hydroxide, pellets

PROCEDURE:

A. PREPARATION of 0.5N ALCOHOLIC KOH:

1. To a suitable size container add 2000 mL of isopropyl alcohol, 1000 mL of methanol and 98.0g, +/-0.1g, of potassium hydroxide pellets.

2. Let this solution stand over night to dissolve the KOH.

3. Filter this 0.5N alcoholic KOH and store in a tightly closed, light resistant container.

B. SAMPLE ANALYSIS:

1. Into a suitable size flat bottom, single neck flask, accurately weigh, in duplicate, a suitable size sample to the nearest mg (see Remark 1).

2. Pipet 25 mL of 0.5N alcoholic KOH and allow the pipet to drain for 30 seconds. If a white precipitate appears, add 25 mL of distilled water (see Remark 2).

3. Prepare 25 mL blank solutions simultaneously with sample analyses.

4. Connect the water condensers to the flasks using a small amount of silicon grease for the ground glass joint.

5. Place the flasks on a hot plate and boil gently, at reflux temperature, until the sample is completely saponified. This usually takes about one hour. Be careful not to allow the vapor ring in the condenser to rise to the top of the condenser or there will be some loss (see Remark 3).

6. After saponification is complete, wash down the inside of the condenser with a small amount of distilled water or alcohol (see Remark 4).

7. Disconnect the condenser. Add several drops of phenolphthalein indicator and titrate with 0.5N HCl to the disappearance of the pink color (see Remark 5).

8. Calculate the saponification value as stated in the CALCULATIONS section.

CALCULATIONS:

$$\text{Saponification Value} = \frac{(\text{mL Blank} - \text{mL Sample})\ (\text{N HCl})\ (56.11)}{\text{Sample Weight, g}}$$

PRECISION and ACCURACY:

These error statements are based on an AOCS study conducted on tallow, coconut, soybean, and linseed oils (see Reference 1).

PRECISION:

The standard deviation of the method for between laboratory application is 0.85 Sap Value percent relative.

The standard deviation of the method for within laboratory application is 0.80 Sap Value percent relative. Duplicate analyses by a single operator must agree within 3.2 Sap Value percent relative to be considered acceptable.

REMARKS:

1. Sample Size Guide

Sap Value	Estimated Sample Wt., g
0 - 25	10
25 - 60	5
60 - 90	3
90 - 130	2
130 - 200	1.5 - 2.0
200 - 300	1
300 - 400	0.75
500 +	0.5 - 0.3

2. Analysis should be run in duplicate. Checks should agree within one Sap Value unit per 100 Sap units.

3. Some samples, particularly those difficult to saponify, may require more than 1 hour. This can only be determined by trial. Clarity and homogeneity of the test solution are partial indicators of complete saponification, but they are not necessarily absolute criteria (see Reference 1). Typically, products with expected Sap Values greater than 250 need to be refluxed for 1.5 hours.

4. When analyzing ethoxylated hydrogenated castor oil, 3A alcohol must be used to rinse the condenser.

5. If sample gels on cooling, it should be warmed slightly before titrating.

REFERENCES:

1. Official Methods and Recommended Practices of the American Oil Chemist's Society, Fourth Edition, Method TI 1a 64 (Reapproved 1989).

Hydroxyl Value Determination by Acetylation

Stepan Company Analytical Method
Northfield, Illinois 60093
(847) 446-7500

SM 053-O

SCOPE:

This method determines the hydroxyl groups in nonionics and polymer glycols, esters, and ethers. It may be applied to the determination of the hydroxyl function in many other substances provided the material contains primary hydroxyl groups.

Primary and secondary amines along with higher fatty acids react with the acetylating reagent to form stable compounds. These interferences will affect the accuracy of the analysis.

Alternatively, the OHV can be determined potentiometrically using an autotitrator equipped with a glass combination pH electrode.

SUMMARY:

The hydroxyl group is acetylated with a solution of acetic anhydride in pyridine. The excess reagent is decomposed with water and the acetic acid which is formed is titrated with standardized sodium hydroxide solution.

SAFETY:

ACETIC ANHYDRIDE is CORROSIVE, FLAMMABLE, TOXIC, and an IRRITANT. Avoid eye and skin contact. Avoid open flames and sparks. Work in a hood or well ventilated area. Wear proper personal protective equipment.

PYRIDINE is FLAMMABLE, TOXIC, and an IRRITANT. Avoid eye and skin contact. Avoid open flames and sparks. Work in a hood or well ventilated area. Wear proper personal protective equipment.

SODIUM and POTASSIUM HYDROXIDE are CORROSIVE. Avoid eye and skin contact. Wear proper personal protective equipment.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

DEFINITION(S):

Hydroxyl value is the number of milligrams of KOH equivalent to the hydroxyl content in 1 gram of sample.

APPARATUS:

1. Flask, with ground-glass joints, 250 mL

2. Condensers
3. Hot plate
4. Buret, suitable size

REAGENTS:

1. Pyridine
2. Acetic anhydride, reagent grade
3. Sodium or potassium hydroxide (NaOH or KOH), 2N or 1N, standardized, (1N KOH is used for OHV determination of some Maywood products)
4. Phenolphthalein indicator

PROCEDURE:

A. PREPARATION of ACETYLATING REAGENT:

1. Add exactly 3.5 mL of water to 1 liter of pyridine and mix thoroughly. Add 140 mL of acetic anhydride and mix thoroughly (see Remark 1).

2. Store the acetylating reagent in a brown glass bottle in the dark. The bottle must be labeled with the date of reagent preparation. This reagent has an EXPIRATION DATE and must not be used if more than 2 WEEKS old.

B. HYDROXYL VALUE ANALYSIS:

1. Weigh a sufficient sample to give approximately three-fourths the titration value of the blank into a 250 mL flask having a standard ground-glass joint. See Equation 1, CALCULATIONS section for sample size determination (see Remark 2).

2. Pipet 25 mL of the acetylating reagent to the flask. Grease the ground-glass joints and attach the condenser to the flask. Prepare a blank by pipetting 25 mL of acetylating reagent to another flask not containing any sample (see Remark 3).

3. Reflux the sample and blank solutions for 1 hour on a hot plate (see Remark 4). With the condenser attached, remove the flasks from the heat and rinse the condensers with 30-50 mL of water. Allow the flasks to cool to room temperature.

4. Remove the condensers and rinse the glass joints with a few mL of water. Add a few drops of phenolphthalein indicator and titrate with sodium or potassium hydroxide to the appearance of the pink endpoint which is permanent for at least 15 seconds or the inflection point using a potentiometric titrator.

CALCULATIONS:

$$1.\ \text{Sample Size, g} = \frac{(7.5)\ (\text{NaOH or KOH N})\ (56.1)}{\text{OHV}}$$

OHV is the expected hydroxyl value. The sample size should not exceed 15g.

$$2.\ \text{Uncorrected Hydroxyl Value} = \frac{(B - S) \times N \times 56.1}{\text{Sample Weight, g}}$$

Where: B = Titrant mL for the Blank

S = Titrant mL for the Sample

N = Titrant Normality

The true hydroxyl value is calculated from the uncorrected hydroxyl value by adding the acid value or by subtracting the base value.

PRECISION and ACCURACY:

PRECISION:

Ten replicate determinations were performed on 5 samples by 2 operators over a 2 week period in 1 laboratory.

Sample	OHV Average	Std. Dev, %	RSD, %
WECOBEE® FS	3.21	0.25	7.79
WECOBEE® M	3.78	0.19	5.03
NEOBEE® M-20	2.44	0.37	15.16
NEOBEE® M-5	0.90	0.17	18.89
Ethylene Glycol Monostearate (EGMS)	119.69	0.96	0.80

REMARKS:

1. It is important that both the amount of water and the order of addition shall be strictly adhered to, otherwise low results are likely to be obtained. The presence of the water inhibits the formation of dark colored resins caused by a reaction between dry pyridine and acetic anhydride during refluxing.

2. This analysis must be done in duplicate along with duplicate blanks for qualifying the method.

3. For samples with hydroxyl values under 150, reflux the sample for 2 hours to complete the acetylation.

REFERENCES:

1. Method originator, G.T.Battaglini

2. Snell and Biffen, Commercial Methods of Analysis, New York, Chemical Publishing Company, Inc., 1964, p. 439.

Carbon Chain Distribution of Esters by Gas Chromatography

Stepan Company Analytical Method
Northfield, Illinois 60093
(847) 446-7500

SM 499- I

SCOPE: This method determines the carbon chain distribution of C6, C8, C10, C12, C16 and C18 esters.

SUMMARY: The sample is hydrolyzed with 1N methanolic sodium hydroxide, neutralized to the phenolphthalein endpoint with 1:1 methanol:hydrochloric acid solution, then esterified with boron trifluoride (BF3) in methanol. The methyl esters are extracted with petroleum ether. Saturated sodium chloride is added to aid the separation. A naphthalene internal standard is added to an aliquot of the petroleum ether layer. The gas chromatographic results are reported as mole percent or weight percent of the total fatty acid methyl esters.

SAFETY: METHANOL is FLAMMABLE and a POISON. Avoid eye and skin contact. Avoid open flames and sparks. Wear proper personal protective equipment. Work in a hood or well ventilated area.

PETROLEUM ETHER is extremely FLAMMABLE. Avoid open flames and sparks. Wear proper personal protective equipment. Work in a hood or well ventilated area.

BORON TRIFLUORIDE is an IRRITANT and a POISON. Avoid eye and skin contact. Wear proper personal protective equipment. Work in a hood or well ventilated area.

HYDROCHLORIC ACID is CORROSIVE. Avoid eye and skin contact. Wear proper personal protective equipment. Work in a hood or well ventilated area.

NAPHTHALENE is a POISON. Avoid eye and skin contact. Wear proper personal protective equipment. Work in a hood or well ventilated area.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

APPARATUS:

1. Gas chromatograph or HP Chemstation, equipped with an FID and integrator

2. Capillary column, DB-Wax, 30m x 0.32mm ID, 0.25um film thickness, or equivalent
3. Syringe, 10 uL
4. Container, suitable size
5. Hot plate
6. Pipet, volumetric, 1 mL
7. Flask, volumetric, 100 mL
8. Analytical balance, +/- 0.01g

REAGENTS:

1. Sodium hydroxide (NaOH), 1N, in methanol
2. Phenolphthalein indicator
3. Methanol:HCl, 1:1, vol:vol.
4. Boron trifluoride (BF3), in methanol
5. Petroleum ether
6. Sodium chloride (NaCl) solution, aqueous, saturated
7. Naphthalene, 0.200M, in petroleum ether

PROCEDURE:

A. STANDARD PREPARATION:

C6, C8, C10, and C12 ESTERS:

1. Accurately weigh 0.20g methyl caprylate standard and 0.10g methyl caprate standard, +/- 0.01g, into a 100 mL volumetric flask.

2. In a separate 100 mL volumetric flask, accurately weigh 0.60g methyl caproate standard and 1.0g of methyl laurate standard, +/- 0.01g.

3. Dilute the second flask to volume with petroleum ether. Stopper the flask and mix by inverting.

4. Pipet 1 mL of the second solution to the first volumetric flask and pipet 1 mL of 0.200M naphthalene in petroleum ether. Dilute to volume with petroleum ether.

C16 and C18 ESTERS:

1. Accurately weigh 0.15g each of methyl stearate and methyl palmitate, +/- 0.01g, into a 100 mL volumetric flask.

2. Pipet 1 mL of 0.200M naphthalene in petroleum ether. Dilute to volume with petroleum ether.

B. SAMPLE PREPARATION:

1. Weigh 0.30g sample into a suitable size container. Add 25 mL of 1N methanolic NaOH and boil for 2 minutes.

2. Add several drops of phenolphthalein indicator and neutralize with 1:1 methanol:HCl.

3. Add 25 mL BF3 in methanol and boil for 2 minutes.

4. Let cool and add 100 mL of petroleum ether and 25 mL saturated NaCl. Mix thoroughly and allow to settle.

5. Transfer 5 mL of the petroleum ether (top) layer to a vial and add 50 uL of 0.200M naphthalene in petroleum ether.

C. SAMPLE ANALYSIS:

1. Inject 1 uL of the standard and sample using the gas chromatographic conditions stated below. Calculate component concentrations as stated in Equations 1, 2, 3, 4, and 5, CALCULATIONS section (see Appendix 1).

Gas Chromatographic Conditions:

Detector Temp	250 °C
Injection Temp	245 °C
Temp 1	50 °C
Time 1	2 minutes
Rate	5 °C/minute
Temp 2	245 °C
Time 2	0 minutes
Helium	2 mL/minute
Hydrogen	35 mL/minute
Air	300 mL/minute

Integrator Parameters:

Zero	0
Attenuation	3
Chart Speed	0.5
Threshold	0
Area Reject	5
Peak Width	0.04

Time Table:

Integrator 9	0
Integrator -9	2.5
C6-C12 Stop Time	30 minutes
C16-C18 Stop Time	45 minutes

CALCULATIONS:

1. Component Concentrations in Standard Mixture for NEOBEE® M-5(C6, C8, C10, C12) and GMS Pure (C16 and C18):

$$\text{Molarity} = \frac{\text{Wt. of Component in Std., g}}{\text{Molecular Wt. x 0.005 lts.}}$$

$$\text{Mole \%} = \frac{\text{Molarity of Component in Std. (C6, C8, C10, C12) or (C16 and C18)}}{\text{Sum of Concentrations of Components in Std. (C6+C8+C10+C12) or (C16 + C18)}} \times 100$$

Molecular Weight for C6, C8, C10, and C12 Standard:

C6 Methyl Caproate = 130.19
C8 Methyl Caprylate = 158.24
C10 Methyl Caprate = 186.30
C12 Methyl Laurate = 214.35

Molecular Weight for C16 and C18 Standard:

C16 Methyl Palmitate = 270.46
C18 Methyl Stearate = 298.51

2. Component Concentrations in Sample (C6, C8, C10, C12) or (C16 and C18)

$$\text{Mole \%} = \text{Conc. of Comp. in Std.} \times \frac{\text{Comp. Area in Sample}}{\text{Comp. Area in Std.}}$$

3. Normalization to 100 Percent:

$$\frac{\text{Comp. Mole \% (C6, C8, C10, C12) or (C16 and C18)}}{\text{Total Mole \% (C6+C8+C10+C12) or (C16+C18)}} \times 100$$

4. Component Response Factor in Standard, RF =

$$\frac{\text{Comp. Area in Std.}}{\text{Comp. Weight in Std, g}}$$

Calculate the RF for each component in the standard.

5. Component Weight in Sample = $\frac{\text{Area of Comp. in Sample}}{\text{Component RF in Standard}}$

Component Weight Percent in Sample = $\frac{\text{Component Weight in Sample}}{\text{Total Weight}} \times 100$

Calculate the weight percent for each component in sample.

PRECISION and ACCURACY:

PRECISION:

Replicate injections were made of a series of different concentrations (0.02M, 0.01M, and 0.005M for NEOBEE® M-5 and 0.01M, 0.005M, and 0.002M for GMS Pure), five injections of each concentration were made by one operator over a 5 day period in one lab. The data below represents precision for this method.

CARBON CHAIN LENGTH:

NEOBEE® M-5:

Mole %	C6	C8	C10	C12
Mean RT	6.907	12.399	17.846	22.744
Std. Dev.	0.048	0.037	0.36	0.046
RSD, %	0.7	0.3	0.2	0.2

GMS PURE:

Mole %	C16	C18
Mean RT	29.26	32.89
Std. Dev.	0.001	0.001
RSD, %	0.003	0.003

ACCURACY:

The method was performed on a known mixture of fatty acids formulated to reflect concentration in the actual product, an internal standard of 0.2M naphthalene in petroleum ether was added to the standard and the final product. The following recoveries were observed.

NEOBEE® M-5:

Fatty Acid	Mole% Prepared	Mole% Recovered	Difference
C6	2.67	2.31	+0.36
C8	68.86	70.18	-1.37
C10	25.87	24.99	+0.89
C12	2.58	2.51	+0.07

GMS PURE:

Fatty Acid	Mole% Prepared	Mole% Recovered	Difference
C16	48.34	48.60	-0.26
C18	51.66	51.40	+0.26

Residual Methanol in Various Matrices by Headspace Gas Chromatography

Stepan Company Analytical Method
Northfield, Illinois 60093
(847) 446-7500

SM 149-M

SCOPE:

This headspace gas chromatographic (HSGC) method determines methanol (MeOH) in various matrices, aqueous and non-aqueous alike, at 1-300 ppm levels. The results are expressed in ppm (ugrams/gram). The procedure is a standard addition approach and can be applied for MeOH determination in HCG/CG Base, methyl esters (various cuts, from C6 to C22), esterquats, esteramines intermediates and for any other matrix as long there is no chromatographic interference towards methanol or the internal standard (ISTD). Iso-butyl alcohol and n-propanol can be used as an internal standard, Remark 1.

SUMMARY:

A 2-gram sample of material is weighed out in triplicate; two vials are spiked at 100 (25 uL), and 200 ppm (50 uL) with 1% MeOH in IPA. An internal standard solution is added to each vial. The MeOH content is calculated using linear regression analysis for all three runs, one un-spiked and two spiked. The estimated detection and quantitation limits are 0.5 and 1.0 ppm respectively (see Remark 2).

The amount of added MeOH by "spike" is based on the expected methanol level in a sample and can be modified accordingly. The 25 and 50 uL spikes with 1% MeOH solution are suitable for MeOH content up to 300 ppm; for materials with higher MeOH content use, a more concentrated MeOH solution but do not exceed the the spike volume of 50 uL.

The methanol partition between the vapor and liquid phases in headspace procedures is matrix sensitive. In some cases, when analyzing a series of samples of the same matrix, e.g. methyl esters, a 3-point external calibration curve can be derived for one sample (one un-spiked and two spiked) and used to determine MeOH in other samples from one un-spiked run.

SAFETY:

METHANOL, ISOPROPANOL and iso-BUTANOL are FLAMMABLE and POISONOUS. Avoid eye and skin contact. Avoid open flames and sparks. Wear proper personal protective equipment. Work in a hood or well ventilated area.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

APPARATUS:

1. Gas Chromatograph, Agilent 6890 GC or equivalent equipped with an FID, a volatile interface (as an inlet) and electronic pressure control (EPC) and a computer workstation
2. Programs on computer workstation, HP GC software, version A.03.03 or higher; Microsoft Windows, version 4.0 of higher; Microsoft Excel, version 5.0 or higher
3. Headspace analyzer, HP 7694 or HP 19395 or equivalent, with a 1 mL sample loop
4. Column, DB-WAXETR, 30 m x 0.25 mm, df = 0.25 microns
5. Flask, volumetric, 10 mL
6. Pipet, positive displacement, 25 uL, 1 mL and 5 mL (25 uL pipet must have a glass tip)
7. Analytical balance, +/- 0.0001g
8. Headspace vials, 20 mL, Teflon/butyl septa, crimp caps, open top, vial crimper and decrimper

REAGENTS:

1. Methanol, HPLC grade
2. Internal Standard (ISTD), iso-butyl alcohol (2-Methyl-1-propanol), purity > 99% or n-Propanol, HPLC grade
3. Isopropanol, HPLC grade
4. Citric Acid, 50% aqueous solution (only for samples containing free amines such as betaines intermediates CG or CDO Base)

PROCEDURE:

A. INSTRUMENT CONDITIONS for COMPUTER CONTROLLED 6890 GC (with EPC):

1. Install the capillary DB-WAXETR into the inlet and detector of the GC oven according to the column and instrument manufacturer's recommendations.

2. Program the 6890 GC using the following pages of Instrument Edit screen as follows:

Select Injection Source: Manual and enter the location of transfer line (e.g. Back).

Edit Parameters:

Columns (Carrier Flows): Install electronically an appropriate column (e.g. Back or "2")

a. Mode: Ramp Flow
Select the inlet and detector in use.

b. Inlet (Injection Port, Volatile Interface):

Temperature	220 °C
Split Ratio	30:1

c. Column (Carrier Flows):
Mode: Ramp Flow (Helium)

Flow 1	1 mL/minute 11.6 psi @ 40 °C

Time 1 (Hold)	0.05 minutes
Ramp 1	5.0 mL/minute/minute
Flow 2	3 mL/minute
Time 2 (Hold)	0.1 minutes
Ramp 2	10.0 mL/minute/minute
Flow 3	1 mL/minute
Time 3 (Hold)	9 minutes
Ramp 3	10.0 mL/minute/minute
Flow 4	3 mL/minute
Time 4 (Hold)	5.0 minutes

d. Oven:

Initial Temperature	40 °C
Hold Time	6 minute
Ramp 1	10 °C/minute
Temperature 2	70 °C
Time 2	1.0 minutes
Ramp 2	60 °C/minute
Temperature 3	250 °C
Time 3	1.0 minutes
Run Time	14 minutes

e. Signal:
Select Save Data for the FID in use
Select Rate: 10 Hz

f. FID Detector:

Temperature	250 °C
Air Flow	350 mL/minute
Hydrogen Flow	30 mL/minute
Nitrogen Flow (make-up gas)	20 mL/minute

g. Aux:
Select Aux Channel designated for vial pressure
Initial: 15 psi, Hold 3.0 minutes
Ramp 1: 15 psi to 5 psi, Hold 10.0 minutes

Options:
Select psi units pressure for Aux Channel

3. Program the HP 7694 headspace sampler conditions as follows:

Sample oven:	85 °C
Sample valve:	140 °C
Transfer line:	150 °C

Timing (sampling) in minutes for headspace autosampler:

GC Cycle:	20.0
Sample Equilibration:	10.0
Vial Pressurization:	0.20
Loop Fill:	0.20
Loop Equilibration:	0.05
Sample Inject:	1
Oven Stabilization:	1.0
Agitation:	High
Injections per Vial:	1

B. STANDARD AND SAMPLE PREPARATION:

INTERNAL STANDARD SOLUTION (ISTD solution):

1. In an appropriate container (e.g. 20 mL scintillation vial) mix well 50 uL (use 25 uL x 2 or 50 uL positive displacement micro pipet) of iso-butyl alcohol (or n-propanol) and 5 mL (positive displacement pipette) of HPLC grade isopropanol. This is the internal standard solution.

SAMPLE AND CALIBRATION STANDARD PREPARATION:

Methanol, Spiking Solution (1%, 10 ug/uL):

1. Accurately weigh 0.08 - 0.10g, +/- 0.0001g, of methanol into a 10 mL volumetric flask. Dilute to volume with IPA and mix well. This is the methanol spiking solution. It can be stored in a refrigerator up to 2 weeks.

2. Accurately weigh 2.0 +/- 0.0050 gram aliquots of the sample for methanol analysis into three separate headspace vials.

3. Prepare the "spiked vials" by adding the methanol spiking solution in 25 uL increments using a positive displacement micropipet:

Level 1	un-spiked ("as is")
Level 2	25 uL (100 ppm)
Level 3	50 uL (200 ppm)

4. Add 25 uL of the ISTD solution to each vial using positive displacement micropipet.

5. For samples containing free amines such as HCG or CG Base, add by pipet 1mL of the 50% citric acid solution to each vial.

6. Close the vial and shake vigorously (or use Vortex mixer) for 10 seconds. These are the Methanol Calibration Solutions.

C. STANDARD and SAMPLE ANALYSIS:

ChemStation/Calculation Abstract:

Cal. Table, methanol concentration: ppm (ugrams/gram)
Internal standard concentration, amount: "1"

Sample weight: not entered, all calculations are based on 2-gram sample size

Calculated Report: ISTD, results are reported in ppm (ugrams/gram

Multiplier/Dilution factor: 1

1. Program the GC as stated in PROCEDURE A, INSTRUMENT CONDITIONS, using GC manual or on-line help. Select the ISTD Report from Data Analysis. Save the procedure as a Method under the specific name.

2. Inject the following Methanol Calibration Solutions (3 vials)

Level 1	un-spiked
Level 2	25 uL
Level 3	50 uL

3. Calculate the amount of methanol added, in ppm, to each calibration solution (Level 2 and 3) as stated in the CALCULATIONS section.

4. Using Data Analysis/Graphics features, identify the methanol and an internal standard peak (iso-butyl or n-propanol alcohol), according to the attached GC profile of Appendix 1 or 2.

5. Set up the integration events so only these two peaks are integrated, MeOH and ISTD (iso-BuOH or n-PrOH).

6. Using the Data Analysis/Cal. Table, determine the initial amount of methanol (in ppm) in a sample as follows:

Construct a 3-level calibration table for MeOH with iso-BuOH (or n-PrOH) as an internal standard. Enter methanol amounts, 0.001, for Level 1 and the calculated methanol amounts (in ppm) added to vials for Level 2 and 3 (spiked vials); for ISTD the amount is "1" . Make sure the integration was done correctly for both components at each level. The initial content of methanol (in the sample) is calculated using linear regression capability of Microsoft Excel (by exporting the calibration table into a Microsoft Excel spreadsheet) or a hand calculator.

7. When analysing a series of samples of the same matrix e.g. methyl esters, the calibration curve derived in Step 6, can be re-constructed as an external calibration and applied as follows:

a. Enter the initial MeOH content (determined in Step 6) for Level 1("as is" sample) and for Level 2 and 3 enter the calculated MeOH amounts for each level plus the initial amount.

b. Select Data Acquisition and Data Analysis from the method's Run Time Checklist; select the ISTD option for calculated quantitative results and Printer as a Report destination from Report menu.

c. Inject the sample solution according to Run Time Checklist of the method. The quantitative results, in ppm (ugram/gram), will be printed automatically upon completion of the run.

CALCULATIONS: The calculations listed below can be performed using Microsoft Excel template (see Appendix 3).

Calculate the Methanol (MeOH) concentration in the spiking solution(10 mL vol. flask):

1. MeOH in SS = WT(MeOH) x P(MeOH) x 100

Where:

MeOH in SS = MeOH Concentration in mg/mL or ug/uL in the Spiking Solution

WT(MeOH) = Weight, g of MeOH in the Spiking Solution

P(MeOH) = Purity of MeOH Reagent, Expressed as a Decimal Fraction ("1" for MeOH >99.99%)

100 = Dilution/Unit Conversion Factor

2. Calculate the amount of MeOH added to the calibration standards (added via spikes)to a 2-gram sample in ppm (ug/gram):

MeOH(Level 2) = MeOH in SS x 25 /2

MeOH(Level 3) = MeOH in SS x 50 /2

Where:

MeOH in SS = MeOH Concentration in the Spiking Solution (ug/uL)

25 and 50 = Amount of Spiking Solution Added to Each Spiked Vial in uL

2 = Sample Weight, g

3. Set-up a linear regression curve (use Excel template or hand calculator) and calculate the peak area ratio of MeOH/ISTD vs. amount ratio for all three levels. The ISTD amount is entered as "1", therefore, the curve is area ratio (y's) vs. MeOH amount (x's)

Initial Amount = Intercept/Slope

4. For Section C, Step 7: re-construct calibration curve as follows:
enter the initial MeOH amount for Level 1, for Level 2 and 3 the added amount (by spike) plus the initial comment.
The curve should include the origin.

The amount (ppm) of MeOH in a sample is read from the curve using area ratio (MeOH/ISTD from the sample run).

Steps 1 through 3 can be performed automatically using "Methanol.xls" spreadsheet (see Appendix 3).

PRECISION and ACCURACY:

The procedure is based on a standard addition approach and the spread of response factors over the investigated range for individual samples was used to determine indirectly the accuracy and precision of the method. The RSD for the response factors derived for soybean methyl esters was found to be less than 5% at methanol levels 5 -750 ppm using 100 and 200 ppm spikes, ref. #2614-66.

The precision of multiple determinations using standard addition approach was not determined.

REMARKS:

1. n-Propanol and iso-butyl alcohol can be used as internal standards. n-Propanol is not suitable for esterquats that contain percent levels of isopropyl alcohol.

2. The limit of detection (LOD) and limit of quantitation (LOQ) of MeOH is somewhat matrix dependent. The LOD and LOQ are roughly estimated from the area counts of un-spiked materials; for methyl esters, they are 1 and 5 ppm respectively. None of the examined samples had methanol content less than 4 ppm.

Fatty Acid Methyl Esters by Gas Chromatography

Stepan Company Analytical Method
Northfield, Illinois 60093
(847) 446-7500

SM 103-G

SCOPE:

This automated gas chromatographic method determines the composition of fatty acid methyl esters from C6 to C20 as weight percent at 0.1 to 95% level.

This method is also applicable to any methyl ester (C6 to C20) mixture provided all the peaks are identified and the amount of components with three double bonds does not exceed 5%. The procedure was not verified for mixtures with components of more than three double bonds.

SUMMARY:

An ester sample is dissolved in petroleum ether or heptane at approximately 15 mg/mL and injected into the gas chromatograph (GC). The peak area of each component is adjusted using relative response factors (see Appendix 2). The adjusted area percent is an estimate of the weight percent. Peak assignment is made from a calibration run of a methyl ester standard of known composition.

SAFETY:

ESTER SAMPLES, HEPTANE and PETROLEUM ETHER are FLAMMABLE and POISONOUS. Avoid eye and skin contact. Avoid open flames and sparks. Wear proper personal protective equipment. Work in a hood or well ventilated area.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

APPARATUS:

1. Gas Chromatograph, Agilent 6890 or equivalent equipped with an electronic pressure control an FID detector; autosampler and a computer workstation
2. Programs on computer workstation, HP GC software, version A.08.03 or higher; Microsoft Office, version 98 or NT with Microsoft Excel, version 5.0 or higher
3. Column, DB-WAXETR, 30m x 0.25mm, df = 0.25 microns
4. Syringe, 5 uL or autosampler capable of injecting 0.1 uL
5. Flask, volumetric, 25 mL
6. Autosampler vials, 2 mL, Teflon lined caps, vials and crimper

REAGENTS:

1. Heptane or petroleum ether, reagent grade
2. Methyl ester reference standards, code #GLC 4B and 65 from Nu Check Prep, 100mg ampoule of each

PROCEDURE:

A. INSTRUMENT CONDITIONS for 6890 GC EQUIPPED with SPLIT/SPLITLESS INLET, ELECTRONIC PRESSURE CONTROL (EPC) for all GASES, and AUTOSAMPLER:

1. Install the capillary DB-WAXETR column into the inlet and the detector of the GC oven according to the column and instrument manufacturer's recommendations.

2. Program the GC acquisition parameters from the Instrument menu as follows:

Select Injection Source: GC Injector (e.g. front)

Edit Parameters:
Injector : Select an appropriate Injection Source (e.g. front)
Injection Volume: 0.1 uL
Select "4" "PostInj" washes with each solvent (A and B) and 3 sample washes

Columns (Carrier Flows): Install electronically an appropriate column (e.g. front or "1")

Mode: Ramp Flow

Select the inlet and detector in use

Flows:

Initial: 1 mL/minute, hold 14.0 minutes
Ramp 1: 10 mL/minute/minute to 3 mL/minute, hold 2.0 minutes

Inlets (Injection Port) :
Mode: Split
Gas: Helium
Split Ratio: 30:1
Heater: ON, 250 °C

Pressure (column head pressure) and Total Flow ON, the calculated values should be approximately 12.5 psi and 33 mL/minute respectively.

Oven:
Initial: 100 °C, hold 1.5 minutes

Ramp 1: 30 °C/minute to 200 °C, hold 0.0 minute
Ramp 2: 5 °C/minute to 245 °C, hold 0.0 minute
Ramp 3: 50 °C/minute to 270 °C, hold 1.0 minute (total run time = 15.3 minutes)

Oven Equilibration: 1 minute

Detectors (FID detector) :

Heater: ON, 250 °C
Hydrogen Flow: ON, 30 mL/minute
Air Flow: ON, 350 mL/minute

Makeup Flow: ON, 20 mL/minute
Flame: ON

Signals:
Select Save Data: ON, and All for Detector in use

Data Rate: 10 Hz

B. STANDARD PREPARATION:

1. Quantitatively dissolve 100 mg of each, GLC 4B and 65, methyl ester standards, into a 25 mL volumetric flask with heptane (See Appendix 1).

2. Dilute to volume with heptane and mix thoroughly.

3. Divide the standard solution evenly into autosampler vials. Seal the vials and store them in a refrigerator. Refrigerated solutions can be stored up to 2 years.

C. SAMPLE PREPARATION:

1. Add 1 drop (15-20mg) of ester sample into an autosampler vial. Add approximately 1.4-1.5 mL of heptane or petroleum ether (about three fourths of the vial volume).

2. Seal the vial and mix thoroughly.

D. STANDARD and SAMPLE ANALYSIS:

For routine analysis in a QC lab, skip Steps 1-6.

1. Program the GC as outlined PROCEDURE A, INSTRUMENT CONDITIONS, using GC manual or on-line help.

2. Select the Area % Report from Data Analysis.

3. Save the program as a method, e.g. "ESTER.M".

4. Install the "c65pdata.mac" , "c65data.mac", "endexcel.mac" and "c65temp.xls" in the core directory of the ChemStation (see Remark 1). The Excel template and macros are in Graphics section of the method.

5. Inject 0.1 uL of the standard solution. Assign peaks according to the attached GC profile (see Appendix 3). The peak assignment is done in the Calibration Table, for the component's amount enter the weight % provided in Appendix 1.

6. At the Run Time Checklist, enter "macro c65pdata.mac, go" as a Customized Data Analysis Macro and "macro endexcel.mac, go" as the Post-Run Command/Macro. Save the procedure.

7. Inject 0.1 uL of the sample solution using a procedure e.g. "ESTER.M". The quantitative results will be printed automatically. The ChemStation report will give area% while the Excel report provide area %, estimated weight % Sap Value and Iodine Value.

CALCULATIONS:

All calculations are performed automatically via Excel template

The template was set-up as follows:
1. The area count of each component is multiplied by relative
responsive factor (Rel.RF), (see Appendix 2).

2. Area % is calculated on the "adjusted" area. The adjusted Area %
is Weight % estimate.

3. Milliequivalent contribution of each component "i" in the ester
mixture is calculated as follows:

milli_i = {[(weight percent/100]_i / MW_i} x 1000

Where: MW_i = molecular weight of component "i".

4. SAP Value:

SAP Value = Total milli_i x 56.1

Where: Total milli_i = sum of the individual component contributions

present in the mixture

5. Iodine Value:

Iodine Value = Total Unsaturated milli x 12.69 x 2

Where:

Total Unsaturated milli = sum of the contributions from unsaturated

(with double bonds) components present in the mixture.

The individual millis contributions are also multiplied:

by "1.0" for unsaturated with one double bond

by "2.0" for unsaturated with two double bond

by "3.0" for unsaturated with three double bond

PRECISION and ACCURACY:
PRECISION:

The detection limit is 0.1%. In the calibration run, the recovery for the major components (at concentration > 2%) should be at 90-110%.

Stepan

Stepan Company

Northfield, Illinois 60093
Telephone 847 446 7500

RECEIVED
JUN 5 2006
OFFICE OF
FOOD ADDITIVE SAFETY

June 1, 2006

Dr. Edmundo Garcia
Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Garcia:

SUBJECT: GRAS Notification Amendment for Erythritol Distearate

This submission is being made on behalf of Stepan Company with respect to the subject GRAS substance, Erythritol Distearate for use in food as a surface active agent or micro-encapsulant, that was submitted on May 24, 2006. The enclosed page has been provided to further characterize the use of Erythritol Distearate in food.

We would appreciate if you could place the enclosed information in the files of the GRAS Notification.

Sincerely,

Tracy Strilich
Senior Regulatory Chemist
Product Safety and Compliance Department

Enclosures:
Food Use of Erythritol Distearate

Cover Letter to FDA 6-1-06.doc

Printed on Recycled Paper

Food Use of Erythritol Distearate

As a surface active agent, Erythritol Distearate is intended to be used in all food types except meat, poultry, fish, and produce. The substance is intended to be used in applications similar to those of mono- and di- fatty acid glycerol esters as are listed in the SCOGS Evaluation of Health Aspects of Glycerin and Glycerides as Food Ingredient, 1975. Specifically, Table V of Part B – Glycerides gives the listing of foods in which glycerol fatty acid esters are employed.

Submission End

consultants to the regulated industry

Center For Regulatory Services, Inc.

5200 Wolf Run Shoals Road
Woodbridge, VA 22192-5755
703 590 7337 *Fax 703 580 8637
cfrsrv@aol.com

November 15, 2006

AM

Fax 301 436 2964

Dr. Paullette Gaynor
FDA/CFSAN, HFS-255
Office of Food Additive Safety
5100 Paint Branch Parkway
College Park, MD 20740

Subject: GRN 000203, Withdrawal

Dear Dr. Gaynor.

The additional information that you requested in your e-mail of 8/22/06 regarding the subject GRN is being assembled and new analysis being conducted.

Since the information was not available in a timely matter, we hereby request that the subject GRN be withdrawn without prejudice to resubmission.

The comments that you forwarded will be addressed in the resubmission.

Sincerely:

(b)(6)

William A. Olson, Ph.D.
Regulatory Representative
Stepan Company

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192-5755
Telephone 703 590 7337 Faxline 703 580 8637

cc: T. Strilich, Stepan Company

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