FORMULATIONS COMPRISING ECTEINASCIDIN AND A DISACCHARIDE

The present invention relates to formulations. More particularly it relates to compositions and formulations of ecteinascidins, such as ecteinascidin 743.

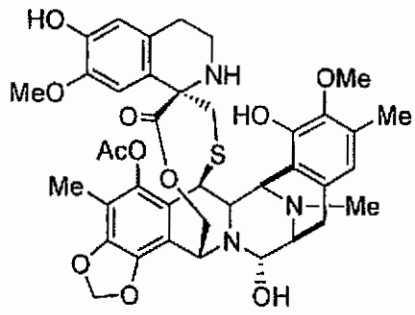
BACKGROUND OF THE INVENTION

Ecteinascidins have been identified, structurally characterized and synthetic methods for making them have been described. See for example, R. Sakai, *et al.*, 1992, *Prco. Natl. Acad. Sci. USA* 89, pages 11456-11460, "Additional antitumor ecteinascidins from Caribbean tunicate: Crystal structures and activities *in vivo*"; R. Menchaca, *et al.*, 2003, *J. Org. Chem.* 68(23), pages 8859-8866, "Synthesis of natural ecteinascidins (ET-729, ET-745, ET-759B, ET-736, ET-637, ET-594) from cyanosafracin B"; and I. Manzanares, *et al.*, 2001, *Curr. Med. Chem. – Anti-Cancer Agents*, 1, pages 257-276, "Advances in the Chemistry and Pharmacology of Ecteinascidins, A Promising New Class of Anticancer Agents"; and references therein. These references describe ecteinascidins. Examples of ecteinascidins are provided by ET-743, ET-729, ET-745, ET-759A, ET-759B, ET-759C, ET-770, ET-815, ET-731, ET-745B, ET-722, ET-736, ET-738, ET-808, ET-752, ET-594, ET-552, ET-637, ET-652, ET-583, ET-597, ET-596, ET-639, ET-641, and derivatives thereof, such as acetylated forms, formylated forms, methylated forms, and oxide forms, such as N-oxide forms.

The structural characterizations of such ecteinascidins are not given again explicitly herein because from the detailed description provided in such references and citations therein; any person of ordinary skill in this technology is capable of obtaining such information directly from the sources cited here and related sources.

At least one of the ecteinascidin compounds, ET-743, has been extensively studied, and it will be referred to specifically herein to illustrate features of this invention.

Ecteinascidin 743 (ET-743) is a tetrahydroisoquinoline alkaloid isolated from the marine tunicate *Ecteinascidia turbinata* and has the following structure:



ET-743

A pharmaceutical composition comprising ET-743 in combination with a pharmaceutically acceptable carrier, diluent or excipient is claimed in US 5,256,663.

A recent review of ET-743, its chemistry, mechanism of action and preclinical and clinical development can be found in van Kesteren, Ch. *et al.*, 2003, *Anti-Cancer Drugs*, 14 (7), pages 487-502: "Yondelis (trabectedin, ET-743): the development of an anticancer agent of marine origin", and references therein.

ET-743 possesses potent antineoplastic activity against a variety of human tumour xenografts grown in athymic mice, including melanoma and ovarian and breast carcinoma.

In clinical phase I studies of ET-743, promising responses were observed in patients with sarcoma and breast and ovarian carcinoma.

Therefore this new drug is currently under intense investigation in several phase II clinical trials in cancer patients with a variety of neoplastic diseases.

As it is explained in WO 0069441, incorporated in full by reference, ET-743 is supplied and stored as a sterile lyophilised product, having ET-743, mannitol and a phosphate buffer. A preferred formulation is one obtained from 0.9% sodium chloride or other suitable infusion vehicle, 250 µg of ET-743 with 250 mg of mannitol, 34 mg of monopotassium phosphate, and phosphoric acid to adjust the pH. This formulation is then reconstituted and diluted for intravenous injection.

ET-743 is a complex chemical entity, as revealed by its structural features. In addition, ET-743 exhibits limited aqueous solubility, and its stability, particularly in biocompatible forms and formulations, is difficult to predict and achieve. These characteristics challenge the ordinary skills and conventional methodologies in this technology, particularly when it comes to the preparation of ET-743 formulations that are to be readily used for medical purposes. Such uses preferably rely on formulations whose characteristics include one or more of the following: biocompatibility, stability under ambient conditions, or under conditions that are as near to ambient conditions as possible, with a shelf life that is as long as possible, and easy reconstitutability to form reconstituted solutions that are as stable under ambient, or near ambient conditions, for as long as possible.

However, conventional formulations and methodologies for preparing such formulations do not provide desirable features and characteristics such as those referred to above. For example, the cited review of 2003 by van Kesteren Ch. *et al.* reports that

> ET 743 has limited aqueous solubility. However, by adjustment of the pH to 4, adequate concentrations of ET 743 could be reached. Instability of ET 743 in aqueous solution necessitated lyophilization in order to increase the storage stability of the pharmaceutical

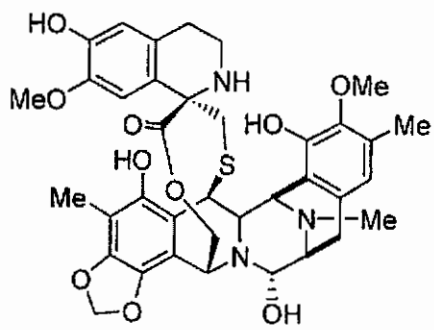
product. ET-743 is currently formulated as a sterile lyophilized product containing 250 µg active substance per dosage unit, 250 mg mannitol as a bulking agent and 0.05 M phosphate buffer at pH 4 in order to solubilize ET-743. This formulation is unstable with long-term storage at refrigerated and room temperature, and should therefore be stored between -15 and -25°C, protected from light. Reconstitution is performed by adding 5 ml Water for Injection, with subsequent dilution in normal saline before i.v. infusion. The reconstituted solution is stable at ambient temperature for up to 24 h.

In practice this product containing 250 µg of ET-743 is manufactured by freeze-drying 5 ml of solution containing ET-743, mannitol, phosphate buffer and water in a moulded vial. Moulded vials containing 1 mg ET-743 are also manufactured by freeze-drying 20 ml of the solution.

Freeze-drying typically involves freezing the solution, reducing the pressure for a period of primary drying to remove water vapour from the frozen material by sublimation and give a semi-dried mass, and increasing the temperature for a period of secondary drying to remove residual water from the semi-dried mass. The vials are then sealed.

The above-described conventional ET-743 formulation suffers from several disadvantages. One of them is that the lyophilised ET-743 formulation has to be stored at about -20°C to prevent decomposition of the ET-743 in order to achieve a shelf life of at least 18 months.

In addition, ET-743 formulations face the problem of formation of relatively large amounts of ET-701 as impurity. ET-701 is the main impurity produced during the lyophilisation process and during storage of the ET-743 formulation. It comes from the hydrolysis of ET-743 and has the following structure:



ET-701

Formation of impurities, however, diminishes or even forestalls the ability to standardize formulations. It is consequently desirable to provide formulations and methods for making the same that provide embodiments whose composition does not readily and unpredictably change by the uncontrolled formation of impurities.

Furthermore, another disadvantage of the above-described conventional ET-743 formulation methodology is that in order to obtain the lyophilised formulation it is necessary to freeze-dry a relatively large amount of solution with fill volumes in the order of 5 to 20 ml. In contrast, it would be desirable to develop a manufacturing methodology for formulations with compounds as complex as ET-743 that permits the making of formulations with higher active substance concentrations, so that the volumes to be handled are consequently reduced. Time and energy are needed in conventional methods for the step of freeze-drying, in view of the relatively high fill volumes of 5 or 20 ml. Along with the time and energy, there is also the risk of decomposition of the ET-743, particularly in the secondary drying.

In view of the potential of ET-743 formulations as antitumoral agents, there is a need to provide a formulation that can solve problems that conventional formulations and manufacturing methodologies do not address or do not completely solve. These problems include the problem of stability of ET-743. Embodiments of ET-743 formulations should preferably exhibit favourable freeze-drying properties, should preferably be susceptible of ready

reconstitution, and they should preferably exhibit dilution properties, such as upon dilution with infusion fluid, while presenting as many as the desirable characteristics of formulations for medical use as referred to herein. As indicated above, embodiments of ET-743 formulations should be stable during long term storage. In addition, the formulation and its manufacturing methodology should satisfy biocompatibility standards and should thus allow for the effective use of a formulation vehicle that is non-toxic, at least at the concentrations used for infusion.

A general review of excipient-drug interactions in parental formulations is provided by Akers, MJ, in Journal of Pharmaceutical Sciences, 91, 2002, 2283-2300. This reference provides, *inter alia*, a section on bulking agents and lyoprotectants, including this subject matter in the context of lyophilisation.

It is envisaged that the methodologies and formulations developed in the context of this invention are applicable to other ecteinascidins, in addition to ET-743.

OBJECTS OF THE INVENTION

It is an object of this invention to provide stable formulations of ecteinascidins, and methods of making such formulations.

It is a specific object of this invention to provide a new stable formulation of ET-743. In particular, a formulation is needed which has greater storage stability. There is especially a need to avoid the formation of impurities. In particular, it is desirable to provide embodiments of formulations that are substantially free of ET-701.

Furthermore, other objects of this invention concern the development of manufacturing methodologies that permit the preparation of ET-743 formulations with ET-743 concentrations that are higher than those achieved by conventional means. Additional objects concern the development of processes for improving the solubility of chemical entities as complex as ET-743, eventually increasing the ET-743 concentration in the solution for lyophilising, and thus reducing the fill volume in the vials before lyophilising the formulation.

SUMMARY OF THE INVENTION

According to the present invention there is provided ET-743 compositions which comprise ET-743 and a disaccharide, and methods for preparing such compositions. Preferred embodiments of such compositions are of pharmaceutical purity.

Other embodiments of this invention are provided by compositions that comprise an ecteinascidin and a disaccharide.

Some embodiments of such compositions are provided by lyophilised formulations which comprise an ecteinascidin such as ET 743 and a disaccharide. Methods for preparing such formulations are provided.

The invention provides methods of reducing, or even substantially eliminating, the formation of impurities in ET-743 formulations. Some embodiments include methodology for reducing, or even substantially eliminating, ET-701 formation in ET-743 formulations.

The invention also provides methodology for more effectively handling formulations of an ecteinascidin such as ET-743, including methods for

making higher concentration formulations and methods for reducing the fill volume of a vial when producing a lyophilised formulation.

The present invention also provides methods of solubilising complex chemical entities, such as ecteinascidins, including but not limited to ET-743. Such methods allow for the manufacturing of a more concentrated solution of ET-743 in bulk solution for lyophilising, leading to reduced fill volumes.

DETAILS OF THE INVENTION

We have found in the context of this invention that disaccharides stabilize ecteinascidin formulations. Ecteinascidins, including ET-743, are complex chemical entities whose behaviour in formulations is not predictable in terms of the behaviour of other unrelated chemical substances. Such behaviour is even more difficult to predict when at least one ecteinascidin is included as the active substance in a formulation that is to satisfy biocompatibility standards, including medical standards. We have further found in this regard that the use of disaccharides as bulking agents can drastically reduce the formation of impurities during the lyophilisation process and storage of ET-743 compositions.

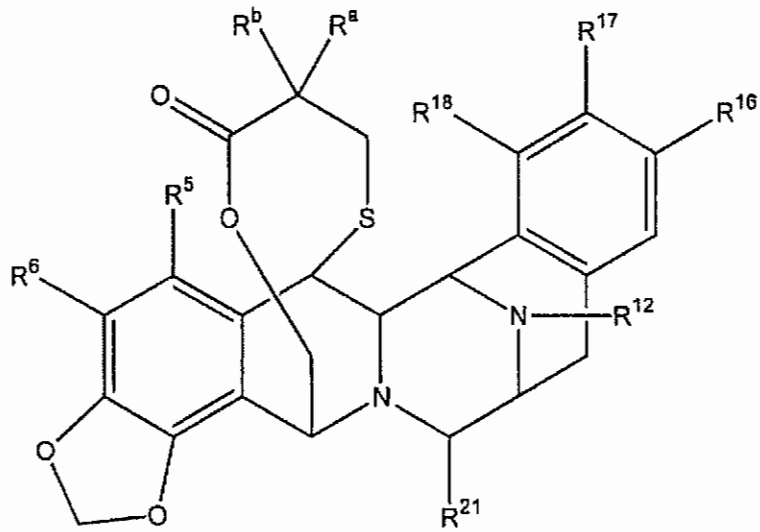
When embodiments of this invention are to provide ET-743 formulations that are substantially free of other ecteinascidins such as ET-701, or at least with a content of ET-701 as low as possible, then ET-701 is regarded as an impurity whose presence in the formulation is to be at least reduced.

In addition, the use of disaccharides also improves the storage conditions allowing long term storage of the lyophilised formulation in a wide

temperature range, including refrigeration conditions and room temperature. The term "stable" as used herein in, for example the expression "a stable ET-743 formulation", refers to a formulation that satisfies stability characteristics as reported herein and equivalents thereof, that are not possessed by conventional formulations and that are not achieved when the formulation is prepared by conventional manufacturing methodologies.

Examples of embodiments of the present invention are provided by novel pharmaceutically acceptable compositions comprising an ecteinascidin such as ET-743 and a disaccharide.

As noted in the introduction, ecteinascidins have been widely described. They may have the following general formula (I):



wherein:

R^5 is OH, alkoxy or alkanoyloxy;

R^6 is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R^{12} is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R^{16} is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R^{17} is OH, alkoxy or alkanoyloxy;

R^{18} is OH, alkoxy or alkanoyloxy;

R^{21} is H, OH, CN or another nucleophilic group; and
R^a is hydrogen and R^b is optionally substituted amino, or
R^a with R^b form a carbonyl function =O, or
R^a, R^b and the carbon to which they are attached form a tetrahydroisoquinoline group.

In these compounds the substituents can be selected in accordance with the following guidance:

Alkyl and alkoxy groups preferably have from 1 to 12 carbon atoms. One more preferred class of alkyl and alkoxy groups has from 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups in the compounds of the present invention. Methoxy, ethoxy and propoxy including isopropoxy are particularly preferred alkyl groups in the compounds of the present invention. Another more preferred class of alkyl and alkoxy groups has from 4 to about 12 carbon atoms, yet more preferably from 5 to about 8 carbon atoms, and most preferably 5, 6, 7 or 8 carbon atoms. As used herein, the term alkyl, unless otherwise modified, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members.

Preferred alkenyl and alkynyl groups in the compounds of the present invention have one or more unsaturated linkages and from 2 to about 12 carbon atoms. One more preferred class of alkenyl or alkynyl groups has from 2 to about 6 carbon atoms, and most preferably 2, 3 or 4 carbon atoms. Another more preferred class of alkenyl or alkynyl groups has from 4 to about 12 carbon atoms, yet more preferably from 5 to about 8 carbon atoms, and most preferably 5, 6, 7 or 8 carbon atoms. The terms alkenyl and alkynyl as used herein refer to both cyclic and noncyclic groups.

Suitable aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical aryl groups contain from 1 to 3 separated or fused rings and from 6 to about 18 carbon ring atoms. Specially preferred aryl groups include substituted or unsubstituted phenyl, naphthyl, biphenyl, phenanthryl and anthracyl.

Suitable alkanoyloxy and alkanoyl groups have from 2 to about 20 carbon atoms, more preferably from 2 to about 8 carbon atoms, still more preferably from 2 to about 6 carbon atoms, even more preferably 2 carbon atoms. Another preferred class of alkanoyloxy groups has from 12 to about 20 carbon, yet more preferably from 14 to about 18 carbon atoms, and most preferably 15, 16, 17 or 18 carbon atoms.

The groups above mentioned may be substituted at one or more available positions by one or more suitable groups such as OR', =O, SR', SOR', SO_2R', NO_2, NHR', $N(R')_2$, =N-R', NHCOR', $N(COR')_2$, $NHSO_2R'$, CN, halogen, C(=O)R', CO_2R', OC(=O)R' wherein each of the R' groups is independently selected from the group consisting of H, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, $C(=O)CH_3$, CO_2H, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl and substituted or unsubstituted aryl. Suitable halogen substituents in the compounds of the present invention include F, Cl, Br and I.

Preferred compounds of the invention are those of general formula (I) wherein one or more of the following definitions will apply:

R^5 is an alkanoyloxy;

R^6 is methyl;

R^{12} is methyl;

R^{16} is methyl;

R^{17} is methoxy;

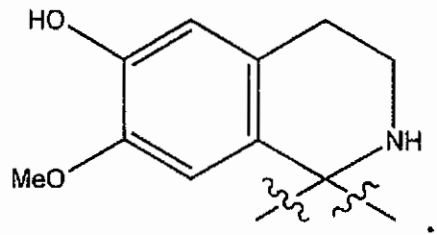
R^{18} is OH;

R^{21} is H, OH or CN; and

R^a is hydrogen and R^b is an amido group, or

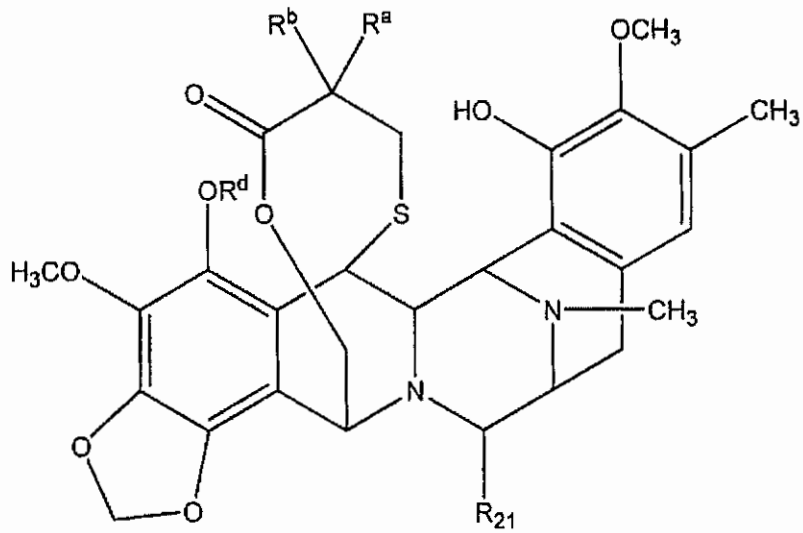
R^a with R^b form =O, or

R^a, R^b and the carbon to which they are attached form a group of formula (II):



Examples of compounds for the present invention include natural ecteinascidins, such as ecteinascidin 743 and other 1,4 bridged fused ecteinascidin compounds disclosed for example in US 5,089,273, US 5,478,932, US 5,654,426, US 5,721,362, US 6,124,293, US 5,149,804, US 09/546,877, US 5,985,876 and WO 01/77115.

Compounds of the following formula (III) are particularly preferred:

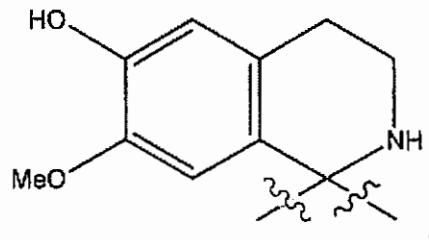


where

R^a is hydrogen and R^b is amido of formula $-NHR^f-$ where R^f is alkanoyl, or

R^a with R^b form =O, or

R^a, R^b and the carbon to which they are attached form a group of formula (II):



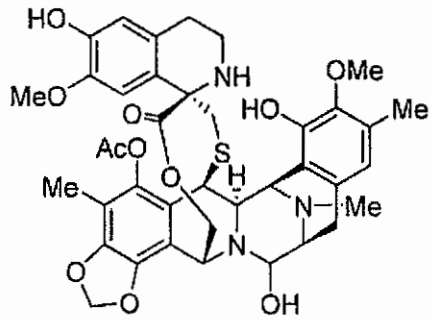
;

R^d is alkanoyl; and

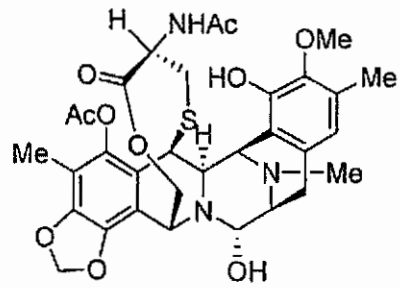
R^{21} is H, OH or CN.

The alkanoyl groups can be acetyl or higher, for example up to C_{20}.

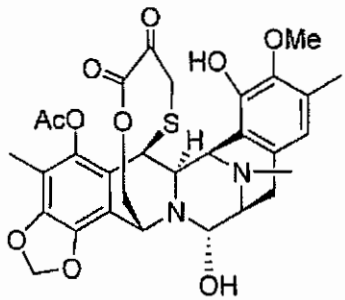
Thus, preferred compounds of this invention include:



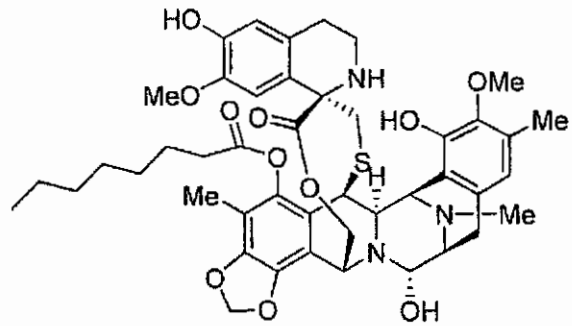
ET743



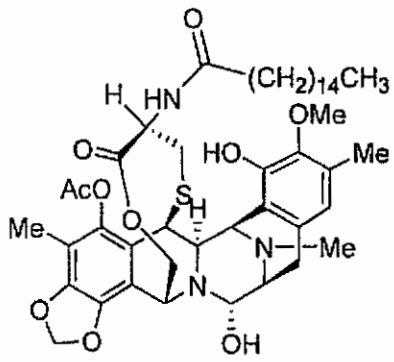
ET637 Derivative A



ET594

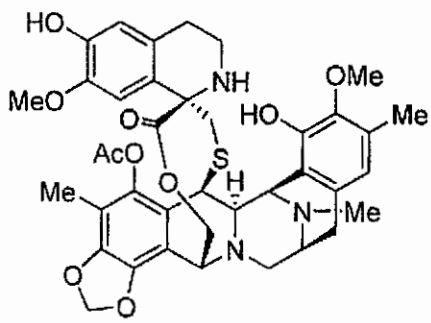


ET743 Derivative A



ET637 Derivative B

or



ET745 ,

and related compounds with different acyl groups.

Ecteinascidin 743, also known as ET743 or ecteinascidin 743 is particularly preferred.

Examples of suitable disaccharides for the compositions of this invention include lactose, trehalose, sucrose, and combinations thereof. Additional examples of disaccharides that can be used in some embodiments of this invention include at least one of maltose, isomaltose, cellobiose, isosaccharose, isotrehalose, sorbose, turanose, melibiose, gentiobiose, and mixtures thereof. Sucrose is currently preferred.

In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a lactose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a trehalose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a sucrose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a maltose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and an isomaltose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a cellobiose-free

disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and an isosaccharose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and an isotrehalose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a sorbose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a turanose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a melibiose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a gentiobiose-free disaccharide.

Thus, in some embodiments, the composition of this invention contains less than 2% or less than 1% or less than 0.5% or less than 0.2% or less than 0.1% by weight of at least one of, preferably each of, lactose, trehalose, sucrose, maltose, isomaltose, cellobiose, isosaccharose, isotrehalose, sorbose, turanose, melibiose, and gentiobiose.

The terms "mixtures thereof" and "combinations thereof" as used herein refer to at least two entities that provide the antecedent basis for the terms "mixtures thereof" or "combinations thereof". By way of illustration, but not as a limitation, the terms "product comprising at least one of A, B, C, and mixtures thereof" refer to embodiments of the product for which any one of the following is satisfied: A is in the product; B is in the product; C is in the product; A and B are in the product; A and C are in the product; B and C are in the product; and A, B and C are in the product.

Furthermore, it is understood that terms such as "reacting", "forming", and related terms, applied to a chemical entity herein refer to any one of: (a) the chemical entity as such, and (b) the chemical entity in the

form in which such entity is present in the reaction medium. Analogously, to name a chemical entity or to give its formula in the context of an operation or reaction step, or to name it or give its formula as being in a medium, whether solid or liquid, including products, formulations, and combinations, refers herein to any one of: (a) the entity as such, and (b) the entity in the form in which such entity is present in the medium. For example, naming an acidic chemical entity herein refers to whichever form or forms such entity is present in the context in which it is named. By way of illustration, but not as a limitation, naming the chemical entity "sodium chloride" or providing its chemical formula refers herein to the entity NaCl as such diatomic molecule, if such is the form in which sodium chloride is present in the relevant medium; it also refers to the collection of undissociated and/or dissociated chemical species if sodium chloride in the relevant medium is entirely or partially dissociated, including species in such medium that are solvated, part of cages, associated with other species, etc.

Any compound referred to herein is intended to represent such specific compound as well as certain variations or forms. In particular, compounds referred to herein may have asymmetric centers and therefore exist in different enantiomeric forms. All optical isomers and stereoisomers of the compounds referred to herein, and mixtures thereof, are considered within the scope of the formulations and methodologies of this invention. Thus any given compound referred to herein is intended to represent any one of a racemate, one or more enantiomeric forms, one or more diastereomeric forms, one or more atropisomeric forms, and mixtures thereof.

Furthermore, compounds referred to herein may exist as geometric isomers (i.e., *cis* and *trans* isomers), as tautomers, or as atropisomers. Additionally, any compound referred to herein is intended to represent hydrates, solvates, and polymorphs, and mixtures thereof when such forms

exist in the medium. In addition, compounds referred to herein may exist in isotopically-labelled forms. All geometric isomers, tautomers, atropisomers, hydrates, solvates, polymorphs, and isotopically labelled forms of the compounds referred to herein, and mixtures thereof, are considered within the scope of the formulations and methodologies of this invention.

To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "about". It is understood that, whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value.

The active substance or substances in the context of this invention can be of natural, semisynthetic or synthetic origin, including combinations of origins. In embodiments where the active substance is an ecteinascidin such as ET-743, the ET-743 can be of natural origin, isolated for example from a tunicate of the genus *Ecteinascidia*, preferably the species *Ecteinascidia turbinata*. The ET-743 can be of synthetic or semisynthetic origin. Reference is made for example to WO 0069862 and WO 0187895, both of which are incorporated in full by reference.

The ratio of the active substance to the bulking agent in embodiments of this invention is determined according to the solubility of the bulking agent and, when the formulation is freeze dried, also according to the freeze-dryability of the bulking agent. It is envisaged that this ratio (w/w) can be about 1:1 in some embodiments, about 1:5 in other embodiments, about 1:10 in still other embodiments, while other embodiments illustrate ratios in the range from about 1:10 to about 1:1. It is envisaged that other

embodiments have such ratios in the range from about 1:10 to about 1:100, and still further embodiments have such ratios in the range from about 1:100 to about 1:1500. When the active compound is ET-743, the ratio (w/w) of ET-743 to bulking agent is typically from about 1:100 to about 1:1500, preferably from about 1:200 to about 1:800, more preferably from about 1:250 to about 1:600, and even more preferably about 1:400.

The lyophilised material is usually presented in a vial which contains a specified amount of ecteinascidin or active compound. When the active compound is ET-743, active amounts are illustrated by 250 μg and 1 mg.

The present invention is not limited by specific container forms or designs, as long as the container is acceptable for its intended use and standards therefore. Embodiments of this invention are provided with a formulation contained in vials, preferably tubing vials.

The lyophilised formulations of this invention can be reconstituted and diluted to give a composition of this invention in the form of a solution ready for intravenous injection. The actual amounts of reconstituting fluid are not limiting features of embodiments of this invention. By way of illustrations, but not as limitations, embodiments of lyophilised formulations according to this invention are reconstituted with a volume of water. Most of such volumes do not exceed about 20 ml, with preferred volumes being in the range from about 1 ml to about 15 ml, more preferably in the range from about 1 ml to about 10 ml, and even more preferably in the range from about 1 ml to about 4 ml. When the active substance is embodied by ET-743, the reconstituted solution in such embodiments contains a concentration of ET-743 up to 500 μg/ml, with concentrations of about 50 μg/ml, about 100 μg/ml, and about 250 μg/ml being preferred.

Reconstituted embodiments of the present invention can further be diluted if so desired, with this further dilution not being a limitation of the present invention. This further dilution is preferably carried out with an aqueous system which is usually 0.9% sodium chloride or 5% glucose. The reconstituted solution will be diluted depending on the concentration in the reconstituted solution and the desired concentration in the diluted solution.

Embodiments of ET-743 formulations according to this invention can be used in the treatment of a variety of cancers, including the treatment of any one of sarcoma, leiomyosarcoma, liposarcoma, osteosarcoma, ovarian cancer, breast cancer, melanoma, colorectal cancer, mesothelioma, renal cancer, endometrial cancer and lung cancer, and conditions with a plurality of such forms of cancer. It is understood that "treatment" in this context refers to an action that leads to an amelioration of the cancer condition(s). Embodiments of ET-743 formulations according to this invention can also be used in the treatment of refractory cancer conditions that have not responded favourably to other treatments. Furthermore, embodiments of formulations according to this invention can be used in the trials with laboratory tissues, including but not limited to clinical trials, analytical trials, and modelling assays.

Embodiments of this invention that comprise an ecteinascidin such as ET-743 are preferably administered by infusion. The infusing step is typically repeated on a cyclic basis, which may be repeated as appropriate over for instance 1 to 20 cycles. The cycle includes a phase of infusing ET-743 formulation, and usually also a phase of not infusing ET-743. Typically the cycle is worked out in weeks, and thus the cycle normally comprises one or more weeks of an ET-743 infusion phase, and one or more weeks to complete the cycle. A cycle of 3 weeks is preferred, but alternatively it can be from 1 to 6 weeks. The infusion phase can itself be a single administration in each cycle of say 1 to 72 hours, more usually of about 1, 3

or 24 hours; or an infusion on a daily basis in the infusion phase of the cycle for preferably 1 to 5 hours, especially 1 or 3 hours; or an infusion on a weekly basis in the infusion phase of the cycle for preferably 1 to 3 hours, especially 2 or 3 hours. A single administration at the start of each cycle is preferred. Preferably the infusion time is about 1, 3 or 24 hours.

The reconstituted and diluted solutions exemplify embodiments of this invention. A formulation that is reconstituted and diluted can be administered intra-venously using the available protocols. The dose will be selected according to the dosing schedule, having regard to the existing data on Dose Limiting Toxicity, on which see for example WO 0069441, WO 0236135 and WO 0339571, and van Kesteren, Ch. *et al.*, 2003, Anti-Cancer Drugs, 14 (7), 487-502. These three WO patent specifications and this van Kesteren article are incorporated by specific reference.

Preferred dosing protocols include:

a) about 1.5 mg/m^2 body surface area, administered as an intravenous infusion over 24 hours with a three week interval between cycles;

b) about 1.3 mg/m^2 body surface area, administered as an intravenous infusion over 3 hours with a three week interval between cycles;

c) about 0.580 mg/m^2 body surface area, administered weekly as an intravenous infusion over 3 hours during 3 weeks and one week rest.

An ecteinascidin such as ET-743 can be used in combination with another drug. For example, it can be administered with another anti-tumour drug. The reader is referred to the list in WO 0069441 and WO 0236135, both of which are incorporated herein by specific reference. Examples of such other drugs include doxorubicin, cisplatin, paclitaxel, carboplatin, pegylated liposomal doxorubicin, docetaxel, capecitabine, and gemcitabine. Drugs with other modes of action can be used, including

dexamethasone. Administration of the other drug can be before, during or after administration of the ecteinascidin such as ET-743.

Embodiments of formulations of this invention that contain an ecteinascidin such as ET-743 can be made by freeze-drying a composition of this invention in the form of a bulk solution including the ecteinascidin and disaccharide. Usually the bulk solution will be buffered, for example to a pH of about 4. Suitable buffering agents include phosphate buffer and citrate buffer. Other possible buffers can be used, such as phosphate/citrate buffer (a mixture of phosphate buffer and citrate buffer), lactate buffer, ascorbate buffer, tartaric/citrate buffer, bicarbonate/hydrochloric acid buffer, acetate buffer, succinate buffer and glycine/hydrochloric acid buffer. Mixtures of buffers can be used. Biocompatible buffers that permit the control of pH at a desired value provide additional embodiments of this invention.

Other components can be included in the bulk solution, for example surface-active agents such as polyoxyethylene 20 sorbitan monooleate or polyoxyl 40 stearate. Other possible surface-active agents include phospholipids, such as a lecithin; polyoxyethylene-polyoxypropylene copolymers, such as a Pluronic surfactant; polyoxyethylene esters of 12-hydroxysteraric acid, such as a Solutol surfactant; ethoxylates of cholesterol, such as diacyl glycerol, dialkyl glycerol; bile salts, such as sodium cholate, sodium deoxycholate; sucrose esters, such as sucrose monolaurate, sucrose monooleate; polyvinyl pyrrolidone (PVP); or polyvinyl alcohol (PVA).

The formulation is normally supplied as a vial containing the lyophilised product. This supply form, however, is not a limitation of the present invention. To provide a vial containing the lyophilised product, the bulk solution is added to a vial and freeze-dried. As mentioned herein, another object of the present invention is to provide a process for improving

the solubility of an ecteinascidin such as ET-743 in order to increase the ecteinascidin concentration in the solution and reduce the fill volume in the vials before proceeding with the lyophilisation process. This methodology developed in the context of the present invention allows for the manufacturing of embodiments of bulk solution with active substance concentration that is higher than that obtained according to conventional methodologies. Reduced fill volumes in comparison with the conventional formulation with mannitol are therefore obtained with the present methodology. This reduction of fill volumes allows savings in time and energy during the freeze-drying step. In addition, there is also a decrease in the risk of decomposition of ET-743, particularly in the secondary drying.

As noted hereinabove, ET 743 has limited aqueous solubility, see for example van Kesteren, Ch., *et al.*, 2003, *Anti-Cancer Drugs*, 14 (7), pages 487-502. Conventional methodologies provide for the adjustment of the medium pH to 4 with buffer, to solubilise ET 743. This pH control is conventionally achieved with a 0.05 M phosphate buffer at pH 4. It was found in the context of this invention that ET-743 solubility is improved in the bulk solution by forming a pre-solution of the ET-743 in an acid. With this pre-dissolution the ET-743 concentration in the bulk solution and the vial can be increased and the fill volume in the vials can be reduced. In these embodiments of the present invention, the fill volume is usually reduced by about 80% with respect to that of the conventional fill volume. By way of illustration, but not as limitations, embodiments of this invention provide a fill volume of 1 ml for a vial containing 0.25 mg ET-743, and 4 ml for a vial containing 1 mg ET-743. The fill volume can optionally reduced further in other embodiments of this invention by increasing the ET-743 concentration.

Conventional methodology comprised the dissolution of ET-743, mannitol and 0.05 M phosphate buffer at pH 4 together with water for

injection; the solubility of the bulk solution was limited due to the low solubility of ET-743 in this medium. It was found in the context of the present invention that pre-treatment of ET-743 in an acid solution improves the ET-743 solubility and allows to have bulking solutions with higher concentrations of ET-743. Thus, the present invention provides processes useful for improving the solubility of ET-743 in the bulking solution that comprise dissolving ET-743 in an acidic medium, mixing the medium with ET-743 with other components of the bulking solution, and, optionally, adjusting the pH. In some illustrative, but not limiting, embodiments of this invention, pH adjustment is accomplished with a phosphate buffer. The acidic medium suitably contains no or substantially no buffering components, and usually consists of aqueous acid.

Illustrative embodiments of bulk solution for freeze drying according to the present invention are provided by a solution of ET-743 buffered at pH 4 with potassium dihydrogen phosphate and phosphoric acid with sucrose as bulking agent.

An illustrative embodiment of the methodology according to this invention provides as follows: ET-743 is dissolved in 0.1N phosphoric acid. Then water for injection ("WFI"), potassium dihydrogen phosphate, sucrose and ET-743 (pre-dissolved in 0.1N phosphoric acid) are mixed. Dissolution is visually checked before continuing, and dissolution is considered complete when it is so appreciated visually The pH of the solution is checked and adjusted to a value in the range from about 1 to about 5, more preferably in the range from about 2 to about 4.5, even more preferably in the rane from about 3 to about 4.5, and most preferably to a pH of about 4.0 by slow addition of a suitable acid. A preferred embodiment of such acid is phosphoric acid, in which case a preferred concentration is about 0.1N. A suitable base is optionally added for pH control. A preferred embodiment of such base is potassium hydroxide, preferably in solution, in which case a

preferred concentration is about 0.1N. The volume is finally adjusted by addition of a suitable, biocompatible fluid, preferably WFI. The bulk solution is then filled in vials according to the desired dose.

The freeze-drying is carried out in some embodiments of this invention by using reduced secondary drying times. A preferred protocol involves cooling to a temperature of about -40°C, primary drying at 40 to 80 μbar for 10 to 50 hours, and secondary drying at a lower pressure and at above 0°C for 10 to 50 hours. In other protocols in the context of this invention cooling to temperatures below -40°C is performed.

Embodiments of this invention comprise lyophilization by cooling product below –40°C. The primary drying is performed at a temperature from about –20°C to about –26°C and a pressure of about 60 μbar for approximately 15 to 40 hours. The secondary drying is carried out at a temperature from about 20°C to about 30°C and a pressure of about 100 μbar for approximately 20 to 40 hours.

Embodiments of lyophilised formulations of this invention are suitable for storage at temperatures significantly higher than conventional formulation storage temperatures. Examples of storage temperatures for formulations according to this invention are around +5°C. These temperatures are readily provided by ordinary refrigerators.

DRAWINGS OF THE INVENTION

Figure 1. Comparative stability study. ET-743 purity evaluation after 6 months storage at 5°C.

Figure 2. Comparative stability study. ET-743 purity evaluation after 12 months at 5°C.

Figure 3. ET-701 impurity production in different formulations stored during 9 months at 5°C.

Figure 4. Comparative ET-743 % purity evolution of the new formulations and 3 batches of reference formulation, stored during 3 months at 5°C.

Figure 5. ET-701 impurity production in different formulations stored during 3 months at 5°C.

Figure 6. Comparative ET-743 % purity evolution of the new formulations and 3 batches of reference formulation, stored during 3 months at 25°C/65% RH.

Figure 7. ET-701 impurity production in different formulations, stored during 3 months at 25°C/65% RH.

Figure 8. Comparative ET-743 % purity evolution of the new formulations stored at 40°C/70% RH during 3 months.

Figure 9. Comparative ET-743 % purity evolution of the new formulations stored at 40°C/70% RH during 3 months.

EXAMPLES

Example 1

This example discloses a comparative stability study of 8 new formulations with the conventional ET-743 formulation (with mannitol). Lactose and sucrose were used to illustrate the present invention. A reference formulation was carried out using mannitol. Other known bulking agents such as dextran (Dextran 40) and povidone (Kollidon 12, PVP) were tried for comparison. Surface-active agents polyoxyl 40 stearate (Myrj 45) or polyoxyethylene 20 sorbitan monooleate (Polysorbate 80) were used in some formulations, and buffer was omitted in some formulations.

Bulk solutions were prepared and freeze-dried by a standardised procedure. A volume of 150 ml of each formulation was prepared:
The amount of potassium phosphate for the final solution (1.02 g) was weighed and dissolved in 90% of final volume (135 ml) of water. Then, the pH was adjusted to pH 4.0 with 0.1N phosphoric acid.
7.85 mg of ET-743 were added to a compounding glass vessel and dissolved by magnetical stirring in 2/3 volume (90 ml) of the potassium phosphate solution for approximately 1 h (dissolution was checked visually).
The amount of bulking agent and surfactant were added and dissolved in 1/3 volume of the potassium phosphate solution. Then, the solution was added to the ET-743 solution and the agitation was maintained for 1 additional hour.
The solution was brought to final weight with water (a density of 1.019 g/cc was adopted for all formulations).
The solution was filtered through a 0.22 μm cellulose filter.
The solution was filled into 25 ml glass vials at 5 ml/vial and maintained at -20°C until lyophilization process.
Lyophilization was performed according to the following table I:

Table I

Freezing time to -48°C:	4 h
Primary drying:	48 h
Secondary drying:	44 h

After freeze-drying, the vials were sealed. The vials were transferred to a refrigerated area (-20°C).

The composition for each vial was as follows (Table II), noting that the water evaporates during the freeze-drying procedure.

Table II

	Reference	Sucrose	Dextran	PVP	Lactose
ET-743	0.250 mg	0.250 mg	0.250 mg	0.250 mg	0.250 mg
Mannitol	250 mg				
Sucrose		500 mg			
Dextran 40			500 mg		
Kollidon 12				375 mg	
Lactose					500 mg
Potassium dihydrogen phosphate	34 mg	34 mg	34 mg	34 mg	34 mg
Phosphoric acid	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for injection	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml

Table II (cont.)

	ET-poly80	ET-myrj	ET-poly80sacc	ET-p80saccunbuffer
ET-743	0.250 mg	0.250 mg	0.250 mg	0.250 mg
Mannitol	250 mg	250 mg		
Sucrose			500 mg	500 mg
Polysorbate 80	50 mg		50 mg	50 mg
Myrj 45		50 mg		
Potassium dihydrogen phosphate	34 mg	34 mg	34 mg	
Phosphoric acid	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	
Water for injection	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml

Stability testing was carried at a temperature of 5 ± 3°C.

The purity evaluation of the nine formulations at 5°C during 3 months is shown in table III and figure 1. In the case of the formulations displaying a higher stability (Reference, Sucrose, Lactose and ET-poly80sacc), the

stability testing was prolonged until 9 months, and in the case of Lactose and Sucrose formulations the stability testing was even prolonged until 12 months due to their high stability. Data in table III and figure 2 show that formulations containing sucrose and lactose displayed an improved stability with a purity decrease of only 2 %. This decrease is significantly lower than the decrease observed in the other assayed formulations.

Table III

	ET-743 purity (%)			
	Reference	Sucrose	Lactose	ET-poly80sacc
Before freeze-drying	98.04	98.34	98.06	97.96
t = 0	97.67	98.15	97.21	97.85
1 month	96.06	96.89	96.35	97.72
3 months	94.37	96.64	95.33	97.58
6 months	92.81	97.01	94.07	95.98
9 months	88.17	95.36	94.83	96.01
12 months		96.31	95.22	

Table III (cont.)

	ET-743 purity (%)				
	Dextran	PVP	ET-poly80	ET-myrj	ET-p80saccunbuffer
Before freeze-drying	96.18	97.62	96.45	97.46	97.45
t = 0	94.64	93.72	92.46	96.36	95.80
1 month	93.42	91.96	89.38	94.36	91.84
3 months	84.90	90.40	81.18	85.31	88.46

As shown in table IV and figure 3, the main degradation product of the reference formulation, ET-701, was dramatically reduced when ET-743 was formulated in the presence of sucrose or lactose.

Table IV

	ET-701 (%)			
	Reference	Sucrose	Lactose	ET-poly80sacc
t = 0	0.188	0.066	0.060	0.050

1 month	0.533	0.063	0.076	0.060
3 months	0.890	0.057	0.054	0.050
6 months	1.732	0.050	0.062	0.050
9 months	3.225	0.050	0.050	0.050

It was found in the context of this invention that disaccharides improve the stability of ET-743 in comparison with mannitol. Embodiments of such disaccharides include lactose, sucrose and mixtures thereof. The stability of the formulations comprising disaccharides is also improved in comparison with other formulations containing other conventional bulking agents such as dextran and povidone. Embodiments of disaccharide formulations according to this invention were determined to be stable for at least 12 months at 5°C. Embodiments of formulations according to this invention have impurity content that is significantly reduced with respect to that of conventional formulations. Presence of ET-701 is accordingly reduced. Embodiments of this invention comprise at least one surface-active agent, such as Polysorbate 80. These embodiments exhibited favorable ET-743 solubility properties and stabilization characteristics. The presence of at least one surface-active agent, however, is not a limiting feature of this invention, and other embodiments do not comprise such agent(s).

Example 2

The purpose of this study was to compare the stability of the standard formulation of ET743 with five new formulations. This study evaluated the stability of the formulations at +5°C.

The composition of tested formulations were the following (Table V), noting that the water evaporates during the freeze-drying procedure:

Table V

	Reference	ETtreal	ETP80treal	ETP80treal 250	ETP80sacc 250	ETP80trealgly 250
ET-743	0.250 mg	0.250 mg	0.250 mg	0.250 mg	0.250 mg	0.250 mg
Mannitol	250 mg					
Trehalose		500 mg	100 mg	100 mg		200 mg
Sucrose					100 mg	
Polysorbate 80			10 mg	10 mg	10 mg	150 mg
Glycine						15 mg
Potassium dihydrogen phosphate	34 mg	34 mg	34 mg	6.8 mg	6.8 mg	
Phosphoric acid	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	
Hydrochloric acid						q.s. pH 4.5
Water for injection	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 1 ml	q.s. 1 ml	q.s. 1 ml

Bulk solutions were prepared and freeze-dried using the following particular protocols:

Formulations ETtreal, ETP80treal and Reference

A weight of 100 g of each formulation was prepared as follows:
The amount of potassium phosphate for the final solution was weighed and dissolved in 90% of final volume (90 ml) of water. Then, the pH was adjusted to pH 4.0 with 0.1N phosphoric acid.
The amount of ET-743 (5.0 mg) was added to a compounding glass vessel and dissolved by magnetical stirring in 2/3 volume (60 ml) of the potassium phosphate solution for approximately 1 h (dissolution was checked visually).
The amount of bulking agent and surfactant were added and dissolved in 1/3 volume of the potassium phosphate solution. Then, the solution was added to the ET-743 solution and the agitation was maintained for 1 additional hour.

The solution was brought to final weight with water.
The solution was filtered through a 0.22 μm cellulose filter, taking an aliquot before filtration for IPC.
The solution was filled into 25 ml vials at 5 ml/vial and maintained at -20°C until the lyophilization process.

Formulations ETP80sacc250, ETP80treal250, ETP80trealgly250

A weight of 30 g of each formulation was prepared as follows:
The amount of potassium phosphate or glycine for the final solution was weighed and dissolved in 90% of final volume (27 ml) of water. Then, the pH was adjusted to pH 4.0 with 0.1N phosphoric acid or 0.1N HCl.
The amount of polysorbate 80 was weighed and added to 1/3 volume of the buffer solution.
The amount of ET-743 (7.5 mg) was added to a compounding glass vessel and dissolved by magnetical stirring in 2/3 volume (60 ml) of the potassium phosphate solution for approximately 1 h (dissolution was checked visually).
The amount of bulking agent was added and dissolved in 2/3 volume of the buffer solution. Then, the solution was added to the ET-743 solution and the agitation was maintained for 10 min.
The solution was brought to final weight with water.
The solution was filtered through a 0.22 μm cellulose filter.
The solution was filled into 10 ml vials at 1 ml/vial and maintained at -20°C until lyophilization process.

Lyophilization process in all six formulations was performed according to the following table VI:

Table VI

Freezing time to -48°C:	2h 30min
Primary drying:	29 h

Secondary drying:	36 h

After freeze-drying, the vials were sealed. The vials were transferred to a refrigerated area (-20°C).

Stability testing was carried at a temperature of 5 ± 3°C.

All the formulations were more stable at 5°C than the reference formulation. No major differences were noted between the new formulations. Table VII discloses the ET-743 chromatographic purity of the formulations under study:

Table VII

	ET-743 purity (%)					
	Reference	ETtreal	ETP80treal	ETP80treal 250	ETP80sacc 250	ETP80trealgly 250
Before filtration	98.63	98.30	97.10	97.67	97.22	98.3
After filtration	98.62	98.46	97.27	97.67	97.06	98.46
After freeze-dried	97.96	98.18	97.65	97.37	97.79	98.18
1 month	97.35	98.22	97.78	96.39	97.40	98.22
3 months	97.65	98.53	98.25	96.61	96.66	98.53
6 months	95.28	97.38	96.93	97.01	97.06	97.38

Example 3

Six formulations ET-NF A, ET-NF B, ET-NF C, ET-NF D, ET-NF E and ET-NF F were manufactured and used for further study of stability at different temperatures.

Sucrose and lactose were selected as bulking agent. Two different buffers were used: sodium citrate buffer 0.1M pH 4 and potassium phosphate 0.05M buffer pH 4. Two different ET-743 concentrations in the bulk solution were tested: 0.250 mg/ml and 0.100 mg/ml. Two different

freeze-dried cycles were used depending on the filling volume (4 ml *vs* 10 ml). A batch of at least 125 vials was manufactured for each formulation.

For each vial the composition of the bulk solution was as follows (Table VIII), noting that the water evaporates during the freeze-drying procedure:

Table VIII

Ingredient	ET-NF A	ET-NF B	ET-NF C	ET-NF D	ET-NF E	ET-NF F
ET-743	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg
Sucrose	400 mg	---	---	1000 mg	1000 mg	---
Lactose	---	400 mg	1000 mg	---	---	200 mg
Citric acid monohydrate	43.6 mg	43.6 mg	109 mg	109 mg	---	43.6 mg
Sodium citrate dihydrate	45.12 mg	45.12 mg	112.8 mg	112.8 mg	---	45.12 mg
Potassium dihydrogen phosphate	---	---	---	---	68 mg	---
Phosphoric acid	---	---	---	---	q.s pH 4	---
Water for injection	4 ml	4 ml	10 ml	10 ml	10 ml	4 ml

Bulk solutions were prepared and freeze-dried using the following particular protocols:

Formulations ET-NF A, ET-NF B and ET-NF F

Preparation of 2L of citric acid approximately 0.2M: 76.96 g of citric acid were dissolved in a volumetric flask of 2L and the solution was brought to the final volume with water for injection. The final molarity of the solution of citric acid was 0.183M.

Preparation of 2L of sodium citrate approximately 0.2M: 117.64 g of sodium citrate were dissolved in a volumetric flask of 2L and the solution was

brought to the final volume with water for injection. The final molarity of the solution of sodium citrate was 0.175M.

Preparation of 4L of citrate buffer pH 4 approximately 0.1M: 1125 mL of citric acid solution 0.2M were mixed with 875 mL of sodium citrate solution 0.2M in a volumetric flask of 4L. The solution was brought to the final volume with water for injection. pH of the solution was checked and adjusted to pH 4. The final molarity of the solution of citrate buffer was 0.089M.

143.83 mg of ET-743 was added to a compounding glass vessel and dissolved by magnetical stirring in approximately 80% of required total volume of citrate buffer 0.1M for approximately 1h (dissolution was checked visually).

Then, the amount of sucrose or lactose (55 g sucrose formulation A and B, and 27.5 g lactose formulation F) was added and the mixture was stirred for an additional period of approximately 1h until dissolution.

After checking the pH, the solution was brought to the final volume by adding citrate buffer 0.1 M pH 4. Re-adjusting to pH 4 with citric acid was needed for formulation A. Density of the final solution: 1.04 g/l. Final weight 572 mg.

The solution was filtered through a 0.22 μm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 4ml. The fill volume was checked at regular intervals (each 15 vials), and fill volume adjusted if required.

After filling lyophilization stoppers were placed and the vials were loaded in the lyophiliser at 5°C.

Lyophilization process was performed according to the following table IX:

Table IX

Cycle	Step	Pressure	Setpoint T (°C)	Slope (min)	Holding time

Loading	Shelves T°	atm	5°C		
Freezing	Freeze 1	atm	-50°C	1 h 50 min 0.5°C/min	
	Freeze 2	atm	-50°C		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	-27°C	45 min 0.5°C/min	
	1° drying	0.080 mb	-27°C		58 h
2nd drying	2° drying	<0.020mb	25°C	3 h 30 min 0.25°C/min	
	2° drying	<0.020mb	25°C		40 h
	stoppering	0.010 mb	25°C		

The vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (-20°C).

Formulations ET-NF C and ET-NF D

Preparation of 1L of citric acid approximately 0.2M: 38.48 g of citric acid were dissolved in a volumetric flask of 1L and the solution was brought to the final volume with water for injection. The final molarity of the solution of citric acid was 0.183 M.

Preparation of 1L of sodium citrate approximately 0.2M: 58.82 g of sodium citrate were dissolved in a volumetric flask of 1L and the solution was brought to the final volume with water for injection. The final molarity of the solution of sodium citrate was 0.175 M.

Preparation of 2L of citrate buffer pH 4 approximately 0.1M: 850 ml of citric acid solution 0.2M were mixed with 650 ml of sodium citrate solution 0.2M in a volumetric flask of 2L. The solution was brought to the final volume with water for injection. pH of the solution was checked and adjusted to pH 4. The final molarity of the solution of citrate buffer was 0.089 M.

141.21 mg of ET-743 was added to a compounding glass vessel and dissolved by magnetical stirring in approximately 80% of total volume of citrate buffer 0.1M for approximately 1h (dissolution was checked visually).
The amount of sucrose or lactose (135 g) was added and the mixture was stirred for an additional period of approximately 1h until dissolution.
After checking the pH, the solution was brought to the final volume by adding citrate buffer 0.1 M pH 4. No re-adjusting of pH was needed.
Density of the final solution: 1.04 g/l. Final weight 1404 mg.
The solution was filtered through a 0.45 μm PVDF filter.
The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 10ml.
The fill volume was checked at regular intervals (each 15 vials), and fill volume adjusted if required.
After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5°C.
Lyophilization process was performed as before for Formulations ET-NF A, ET-NF B and ET-NF F (Table IX).
Due to the large volume in vials, the cycle proposed failed to give an adequate liophilization and collapse was produced. To avoid a new manufacture, all the vials were reconstituted with 10 ml of purified water, purity profile of some reconstituted solutions was checked, stoppers were replaced for two hole stoppers and the following new cycle was used (Table X):

Table X

Cycle	Step	Pressure	Setpoint T (°C)	Slope (min)	Holding time
	Freeze 2	atm	-50°C		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	-23°C	45 min 0.5°C/min	

	1° drying	0.080 mb	-23°C		80 h
2nd drying	2° drying	<0.020 mb	0°C	10h 0.045°C/min	
	2° drying		25°C	62 h 0.006 °C/min	
	stoppering	0.010 mb	25°C		

After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (-20°C).

Formulation ET-NF E

141.21 mg of ET-743 was added to a compounding glass vessel and dissolved by magnetical stirring in 1080 ml wfi + 3,240 mL phosphoric acid 1N for approximately 1 h (dissolution was checked visually).

The amount of sucrose (135 g) and potassium phosphate (9.18 g) was added and the mixture was stirred for an additional period of approximately 1h until total dissolution of the molecule.

After checking the pH and re-adjusting to pH 4 with phosphoric acid 1N, the solution was brought to the final volume by adding water for injection. Density of the final solution: 1.04 g/l. Final weight 1404 mg.

The solution was filtered through a 0.45µm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 10ml. The fill volume was checked at regular intervals (each 15 vials), and fill volume adjusted if required.

After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5°C.

Lyophilization process was performed as before for Formulations ET-NF A, ET-NF B and ET-NF F (Table IX).

Due to the large volume in vials, the cycle proposed failed to give an adequate liophilization and collapse was produced. To avoid a new manufacture, all the vials were reconstituted with 10 ml of purified water, purity profile of some reconstituted solutions was checked, stoppers were replaced for two hole stoppers and a new cycle was used as in the case of formulations ET-NF C and ET-NF D (Table X).
After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (-20°C).

The desired ET-743 concentration was reached in all cases and the impurity profile was similar between formulations. No differences in ET-743 concentration, and impurity profiles were observed during manufacture (before and after filtration, or after filling). The colour of the bulk solution was slight yellowish in those formulations containing lactose.

Formulations with 4 ml filling or 10 ml filling were initially freeze-dried following the indicated protocol. Whereas formulations with 4 ml filling were correctly lyophilised, formulations with 10 ml filling collapsed. A pressure variation in secondary desiccation indicated collapse and boiling of the freeze-dried cake. Vials of formulations ET-NF C, ET-NF D and ET-NF E were reconstituted with 10 ml of purified water. Purity profile of formulations was checked. As no modification in the purity profile was observed in compare with bulk solutions, it was decided to re-lyophilise the vials using the indicated revised freeze-dried cycle. Batches lyophilised as described, resulted in good aspect without collapse but some bottom contraction.

The ET-743 content of vials was within specifications (95% -105%). Impurities profiles showed similarity between formulations and those profiles are comparable with impurities of bulk solutions. Residual water content

was lower or equal than 2% with the largest values being those of formulations of 10 ml filling.

pH of the reconstituted solutions were between pH 4 and pH 4.2 in all cases. Solutions were clear and colourless without visible foreign matter or precipitation. Reconstitution time was similar for all formulations and less than 30 s.

Example 4

The purpose of the study was to investigate the stability of ET-743 in the different formulations ET-NF A, ET-NF B, ET-NF C, ET-NF D, ET-NF E, and ET-NF F at 1 mg/vial under different temperature conditions.

A batch of 130 vials of each formulation ET-NF A, ET-NF B, ET-NF C, ET-NF D, ET-NF E and ET-NF F, 1 mg ET-743/vial, was manufactured according to example 3.

Stability testing was carried at a temperature of 5°C, 25°C/65% RH and 40°C/70% RH.

Figure 4 and table XI show the ET-743 purity evolution of the new formulations during storage at 5°C, in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

Table XI

	ET-743 purity (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	97.70	96.90	97.70	99.06	99.00
1 month	94.70	94.60	94.00	99.28	98.86
2 month	93.80	92.70	93.70	99.21	98.97

3 month	92.50	91.00	91.20	99.22	98.88

Table XI (cont.)

	ET-743 purity (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	98.83	98.98	99.15	98.88
1 month	98.53	99.03	99.14	98.71
2 month	98.88	99.15	99.21	98.80
3 month	98.86	99.12	99.16	98.92

Figure 6 and table XII show the ET-743 purity evolution of the new formulations during storage at 25°C/65% RH, in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

Table XII

	ET-743 purity (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	97.70	96.90	97.70	99.06	99.00
15 days	84.70	84.50	89.50	98.97	98.09
1 month	74.50	72.40	81.90	99.08	98.13
2 month				99.10	98.38
3 month				98.98	98.15

Table XII (cont.)

	ET-743 purity (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	98.83	98.98	99.15	98.88
15 days	98.35	98.90	99.11	98.31
1 month	98.01	99.03	99.13	98.32

2 month	98.35	98.99	99.10	98.28
3 month	98.33	98.93	99.09	98.14

Figure 8 and table XIII show the ET-743 purity evolution of the new formulations during storage at 40°C/70% RH, in comparison with a conventional formulation (containing ET-743, mannitol and phosphate buffer).

Table XIII

	ET-743 purity (%)						
	Reference 4	ET-NF A	ET-NF B	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	94.92	99.06	99.00	98.83	98.98	99.15	98.88
2 days	87.17	99.02	98.10	98.48	98.95	99.12	97.53
7 days	82.04	98.68	97.08	97.86	98.63	98.89	97.20
15 days	76.33	98.75	97.33	97.77	98.55	98.71	96.66
1.5 month		98.52	96.99	97.53	97.54	98.47	95.79
3 month		98.24	95.92	96.67	94.79	97.84	95.91

In addition, figure 5 and table XIV show the ET-701 impurity production of the new formulations during storage at 5°C, in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

Table XIV

	ET-701 (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	0.70	0.84	0.53		0.11
1 month	2.56	3.09	1.73		0.12
2 month	3.45	3.54	2.69	0.12	0.13
3 month	4.61	5.04	4.28	0.13	0.13

Table XIV (cont.)

	ET-701 (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	0.15	0.16	0.15	0.11
1 month	0.12	0.12	0.14	0.11
2 month	0.16	0.14	0.15	0.12
3 month	0.16	0.15	0.15	0.12

Figure 7 and table XV show the ET-701 impurity production of the new formulations during storage at 25°C/65% RH, in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

Table XV

	ET-701 (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	0.70	0.84	0.53	0.09	0.11
15 days	7.99	8.20	4.22	0.14	0.12
1 month	10.56	14.18	6.86	0.11	0.12
2 month				0.15	0.16
3 month				0.17	0.17

Table XV (cont.)

	ET-701 (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	0.15	0.16	0.15	0.11
15 days	0.18	0.17	0.16	0.13
1 month		0.18	0.18	
2 month	0.18	0.19	0.24	0.15
3 month	0.22	0.23	0.24	0.18

As shown in these figures, the stability of the new formulations was higher than the stability of the conventional formulations. In addition, the main degradation product of the conventional formulations, ET-701, was dramatically reduced in the new formulations.

Example 5

Three new formulations based on sucrose as bulking agent were used for further stability studies. These new formulations differ in the phosphate buffer molarity (0.05M vs 0.1M) and the filling volume or, in other words, in the ET-743 concentration in the bulk solution (10 ml vs 4 ml).

The manufacture of a batch of at least 50 vials of each of these formulations is described. A summary of formula description per vial is as follows (Table XVI), noting that the water evaporates during the freeze-drying procedure:

Table XVI

	ET-NF G		ET-NF H		ET-NF I	
	Concentration (mg/ml)	Final composition	Concentration (mg/ml)	Final composition	Concentration (mg/ml)	Final composition
Ingredient						
ET-743	0.250	1 mg	0.100	1 mg	0.250	1 mg
Sucrose	100	400 mg	100	1 g	100	400 mg
Potassium dihydrogen phosphate	6.8	27.2 mg	13.6	136 mg	13.6	54.4 mg
Phosphoric acid	q.s to pH 4.0	q.s pH 4.0	q.s to pH 4.0	q.s pH 4.0	q.s to pH 4.0	q.s pH 4.0
Water for injection	q.s. to 1 ml	4 ml	q.s. to 1 ml	10 ml	q.s. to 1 ml	4 ml

A volume of 240 ml for formulations ET-NF-G and ET-NF-I was prepared:

62.76 mg of ET-743 were added to a compounding glass vessel and dissolved by magnetical stirring in a solution of 192 ml wfi + 1N phosphoric acid (576 µl for NF G, 816 µl for NF I) for approximately 1 h (dissolution is checked visually).

The amounts of sucrose (24 g) and potassium phosphate (1.63 g for NF G; 3.26 g for NF I) were added and the mixture was stirred for an additional period of approximately 1h until dissolution.

After checking the pH and re-adjusting to pH 4.00 if necessary with 1N phosphoric acid, the solution was brought to the final volume by adding water for injection. Density of the final solution: 1.04 g/l. Final weight 249.6 g.The solution was filtered through a 0.45 µm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 4 ml. The fill volume was checked at regular intervals (each 15 vials), and adjusted if required.

After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5°C.

Lyophilization process was performed according to the following parameters (Table XVII):

Table XVII

Cycle	Step	Pressure	Setpoint T (°C)	Slope (min)	Holding time
	Shelves T[a]	atm	5°C		
Freezing	Freeze 1	atm	-50°C	1 h 50 min 0.5°C/min	
	Freeze 2	atm	-50°C		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	-27°C	45 min 0.5°C/min	
	1° drying	0.080 mb	-27°C		58 h

2nd drying	2° drying	<0.020 mb	25°C	3h30min 0.25°C/min	
	2° drying		25°C		40 h
	end	0.010 mb	25°C		

After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (-20°C).

A volume of 600 ml of the formulation ET-NF-H was prepared as follows:

62.76 mg of ET-743 were added to a compounding glass vessel and dissolved by magnetical stirring in a solution of 480 ml wfi + 1.44 ml of 1N phosphoric acid for approximately 1 h (dissolution was checked visually).

The amounts of sucrose (60 g) and potassium phosphate (8.16 g) were added and the mixture was stirred for an additional period of approximately 1h until dissolution.

After checking the pH and re-adjusting to pH 4.00 if necessary with 1N phosphoric acid, the solution was brought to the final volume by adding water for injection. Density of the final solution: 1.04 g/l. Final weight 624 g.

The solution was filtered through a 0.45 μm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 10 ml. The fill volume was checked at regular intervals (each 15 vials), and adjusted if required.

After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5°C.

Lyophilization process was performed according to the following parameters (Table XVIII):

Table XVIII

Cycle	Step	Pressure	Setpoint T (°C)	Slope (min)	Holding time

	Shelves T[a]	atm	5°C		
Freezing	Freeze 1	atm	-50°C	1 h 50 min 0.5°C/min	
	Freeze 2	atm	-50°C		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	-27°C	45 min 0.5°C/min	
	1° drying	0.080 mb	-27°C		62 h
2nd drying	2° drying	<0.020 mb	25°C	3h30min 0.25°C/min	
	2° drying		25°C		50 h
	end	0.010 mb	25°C		

After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (-20°C).

The ET-743 content was within specifications (95%-105%). Impurities profiles showed similarity for all the formulations and comparable with impurities of bulk solutions. Residual water content was lower than 2%, being the highest value for the formulation of 10 ml filling.

The pH of the reconstituted solutions was between pH 4 and pH 4.28 in all cases. Solutions were clear and colourless without visible foreign matter or precipitation. Reconstitution time was similar for all formulations and less than 30 s.

Stability testing was carried during 3 months at a storage temperature of 40°C/70% RH.

Figure 9 and table XIX show the ET-743 purity evolution of the new formulations during storage at 40°C/70% RH.

Table XIX

	ET-743 purity (%)		
	ET-NF G	ET-NF H	ET-NF I
t = 0	99.18	99.23	99.00
7 days	99.04	98.68	
15 days	98.97	98.86	98.79
1.5 month	98.72	98.61	98.40
2 month	98.29		
3 month			97.71

In addition, in figure 8 the stability data is shown in comparison with a conventional formulation (containing ET-743, mannitol and phosphate buffer).

The results obtained in Examples 4 and 5 indicate that all the formulations comprising a disaccharide as bulking agent are more stable than conventional formulations containing mannitol as bulking agent. Formulation ET-NF-G is a preferred formulation.

Embodiments of formulations according to this invention were tested after storage under a plurality of storage conditions (including temperatures of -20°C, 4°C, and 25°C/60% RH) at various storage times (including storage times of 3 months, 6 months, and 9 months). The assay results indicated that at least 99.5% of ET-743 remained after 9 months of storage at -20°C, at least 99% of ET-743 remained after 9 months of storage at 4°C, and at least 97% of ET-743 remained after 9 months of storage at 25°C/60% RH. Total impurities, including ET-701, ET-745, and other impurities did not exceed 1.66% after 9 months of storage at 25°C/60% RH. In addition, the level of ET-701 impurity did not exceed 0.21% after 9 months of said storage conditions.

All the references cited herein are incorporated by reference in their entirety. The features and advantages of this invention are apparent in light of the disclosure provided herein. Based on this disclosure, modifications and adaptations to various conditions and usages can be made, thus generating embodiments within the scope of this invention.

Claims

1. A composition that comprises an ecteinascidin and a disaccharide.

2. A composition according to claim 1, wherein the ecteinascidin comprises ET-743.

3. A composition according to claim 1 or 2, wherein said disaccharide is selected from the group consisting of lactose, trehalose, sucrose, and mixtures thereof.

4. A composition according to claim 3, wherein said disaccharide is sucrose.

5. A composition according to any preceding claim, wherein the ratio (w/w) of ET-743 to disaccharide is from about 1:100 to about 1:1500.

6. A composition according to claim 5, wherein the ratio (w/w) of ET-743 to disaccharide is from about 1:250 to about 1:600.

7. A composition according to claim 6, wherein the ratio (w/w) of ET-743 to disaccharide is about 1:400.

8. A composition according to any preceding claim, which further comprises a buffering agent.

9. A composition according to claim 8, wherein said buffering agent is selected from phosphate buffer, citrate buffer, glycine/hydrochloric acid buffer, and mixtures thereof.

10. A composition according to any preceding claim, which further comprises a surface-active agent.

11. A composition according to claim 10, wherein the surface-active agent is selected from polyoxyethylene 20 sorbitan monooleate, polyoxyl 40 stearate, and mixtures thereof.

12. A composition according to any preceding claim, wherein the composition is in the form of a lyophilised formulation.

13. A lyophilised formulation according claim 12, wherein the lyophilised formulation is in a vial and includes an amount of ET-743.

14. A lyophilised formulation according to claim 13, wherein said amount of ET-743 is about 250 μg.

15. A lyophilised formulation according to claim 14, wherein said vial contains a formulation comprising: about 0.25 mg ET-743, about 100 mg sucrose, and about 6.8 mg phosphate, wherein said 6.8 mg phosphate is calculated as potassium dihydrogen phosphate.

16. A lyophilised formulation according to claim 13, wherein said amount of ET-743 is about 1 mg.

17. A lyophilised formulation according to claim 16, wherein said vial contains a formulation comprising: about 1.0 mg ET-743, about 400 mg sucrose, and about 27.2 mg phosphate, wherein said 27.2 mg phosphate is calculated as potassium dihydrogen phosphate.

18. A method of making a vial containing a lyophilised formulation of an ecteinascidin, comprising freeze-drying a bulk solution that comprises the ecteinascidin and a disaccharide.

19. A method according to claim 18, wherein the ecteinascidin is ET-743.

20. A method of reducing the formation of ET-701 in a formulation of ET-743, comprising freeze-drying a bulk solution that comprises ET-743 and a disaccharide.

21. A method of preparing a lyophilizing formulation as defined in any of claims 12 to 17, comprising dissolving an ecteinascidin in an acidic medium, mixing the pre-dissolved ecteinascidin with the other components of the bulking solution and, optionally, adjusting the pH of the final solution.

22. A method according to claim 21, wherein the ecteinascidin is ET-743.

23. A method of preparing a solution for intravenous infusion, comprising: providing a vial that comprises lyophilised ecteinascidin and a disaccharide, adding water to form a reconstituted solution, and diluting said reconstituted solution with an aqueous system.

24. A method according to claim 23, wherein the ecteinascidin is ET-743.

25. The use of a solution prepared according to claim 23 for the manufacture of a medicament for the treatment of cancer, wherein the solution is administered by intravenous infusion.