ETOPOSIDE TONIRIBATE FORMULATION

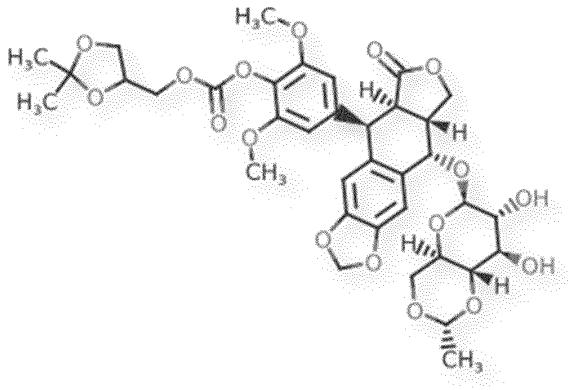
FIELD

The present invention relates to new stable pharmaceutical formulations of etoposide toniribate, infusion solutions of etoposide toniribate and methods of treating cancer using said formulations and infusion solutions.

BACKGROUND

Etoposide is a chemotherapeutic drug derived from podophyllotoxin and acts as an inhibitor of topoisomerase II. The enzyme topoisomerase II induces transient DNA double strand breaks to enable modifications of DNA tertiary structure. Etoposide acts as a topoisomerase II poison leading to a stabilization of the cleavable complex, resulting in multiple non- repairable double strand breaks.

Etoposide toniribate is an analogue and/or pro-drug of etoposide, differing from etoposide in that a water-soluble ester group is attached to etoposide. Etoposide toniribate corresponds to a compound according to formula I:



(Formula I)

Etoposide toniribate (also known as Cap7.1 or EDO-S7.1) is effective to treat cancer, including metastatic tumours, tumours reducing an organ function, and/or in patients having advanced, progressive cancer or an end-stage cancer. The efficacy of etoposide toniribate in the treatment of cancer is reported in, for example: Keilholz U *et al. First-in-man dose escalation and pharmacokinetic study of CAP7.1, a novel prodrug of etoposide, in adults*

with refractory solid tumours. Eur J Cancer. 2017 Jul;80:14-25; and Pape UF *et al*. *Randomized, multicenter phase II trial of CAP7.1 in patients with advanced biliary tract cancers*. J. Clin. Oncol; 2016, 34:4_suppl, 441-441, each of which is incorporated herein by reference.

While etoposide toniribate is an effective anti-cancer therapy, to date it has proven to be difficult to formulate. Currently, etoposide toniribate is formulated as a powder that must be stored at -70 Celsius. The powder must be solubilised in polyethoxylated castor oil (e.g. Cremophor EL®) and ethanol (50:50 oil to ethanol) in glass vials, and then subsequently diluted in sodium chloride (0.9%) for infusion. This is inappropriate for large scale production of an etoposide toniribate formulation, and the reconstitution process is cumbersome and time-consuming. There is therefore a need for new stable formulations of etoposide toniribate.

Amongst the advantages of the present invention is that it provides a solution to this problem in the art.

SUMMARY OF INVENTION

The present invention provides a new liquid formulation of etoposide toniribate that is stable at refrigerator temperature (2-8°C), and room temperature (20-25°C) for at least 3 months, and is still stable even at higher temperatures. The pharmaceutical formulations of the invention can be directly diluted to form an infusion solution suitable for administration to a patient. Infusion solutions prepared from pharmaceutical formulations according to the invention are also stable at room temperature. The invention thus provides a formulation that is easier to store and handle in comparison to known formulations of etoposide toniribate.

Accordingly, in a first aspect the invention provides a liquid pharmaceutical formulation comprising:
etoposide toniribate;
a polysorbate; and
ethanol.

In another aspect the invention provides a liquid pharmaceutical formulation comprising:

Etoposide toniribate;
PEG;
a polysorbate;
Ethanol;
Benzyl alcohol.

In certain preferred embodiments, the formulation comprises etoposide toniribate at a concentration in the range of from 10 mg/ml to 20 mg/ml.

In certain preferred embodiments, the formulation comprises etoposide toniribate at a concentration in the range of from 40 mg/ml to 100 mg/ml, preferably 50 mg/ml to 100 mg/ml. In certain preferred embodiments, the formulation comprises etoposide toniribate at a concentration of 50 mg/ml or 91 mg/ml.

In certain preferred embodiments, the PEG is PEG 200-600. In certain preferred embodiments, the PEG is PEG 200-400. In preferred embodiments, the PEG is selected from PEG 200, PEG 300, and PEG 400. In certain preferred embodiments, the PEG in the formulation is PEG 300.

In certain embodiments, the formulation further comprises citric acid.

In certain preferred embodiments, the polysorbate is polysorbate 80.

In a preferred embodiment, the formulation is non-aqueous.

In certain embodiments, the formulation has a pH in the range of from pH 3 to 5, preferably pH 3 to 4. In certain embodiments the formulation has a pH in the range of from pH 3.5 to 4.0. In certain embodiments, the formulation has a pH of 3.7.

In certain embodiments, the formulation has a pH in the range of from pH 5 to 8, preferably from pH 5.0 to 7.8. In certain embodiments, the formulation has a pH in the range of from 5.0 to 7.0, optionally from pH 5.0 to 6.0, or from pH 6.0 to 7.0. In certain alternative preferred embodiments, the formulation has a pH in the range of from 7.3 to 7.8.

In a further aspect is provided a method of preparing an infusion solution comprising diluting a pharmaceutical formulation according to the invention in a diluent. In a further aspect is provided an infusion solution prepared according to such a method.

In preferred embodiments, the diluent is selected from water for injection; 5% glucose solution; 0.45% NaCl saline and 0.9% NaCl saline. In preferred embodiments, the diluent is water for injection.In preferred embodiments, the infusion solution has a concentration of etoposide toniribate in the range of from of from 1 mg/ml to 10 mg/ml, preferably in the range of from 1 mg/ml to 5 mg/ml. Preferably the concentration of etoposide toniribate is 3.1 mg/ml.

In another preferred embodiment, the infusion solution has a concentration of etoposide toniribate in the range of from of from 0.5 mg/ml to 0.8 mg/ml. Preferably the concentration of etoposide toniribate is 0.5 mg/ml or 0.8 mg/ml.

In a further aspect is provided a kit comprising: a pharmaceutical formulation according to the invention; and a diluent. In preferred embodiments, the diluent is selected from water for injection; 5% glucose solution; 0.45% NaCl saline and 0.9% NaCl saline. In preferred embodiments, the diluent is water for injection.

In another aspect is provided a kit comprising: a pharmaceutical formulation according to the invention; and a diluent. In preferred embodiments, the diluent is selected from water for injection; 5% glucose solution; and 0.9% NaCl saline. In preferred embodiments, the diluent is water for injection.

In a further aspect is provided a pharmaceutical formulation or an infusion solution according to the invention, for use in therapy.

In a further aspect is provided a pharmaceutical formulation or an infusion solution according to the invention, for use in a method of treating cancer.

In a further aspect is provided a pharmaceutical formulation or an infusion solution according to the invention, for use in a method of treating biliary tract cancer

In a further aspect is provided a method of treating cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical formulation or an infusion solution according to the invention.

In preferred embodiments of all aspects of the invention, the pharmaceutical formulation comprises:

50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml ethanol.

In preferred embodiments of all aspects of the invention, the pharmaceutical formulation comprises:

91 mg/ml Etoposide toniribate;
631 mg/ml Polysorbate 80; and
252 mg/ml ethanol.

In preferred embodiments of all aspects of the invention, the pharmaceutical formulation comprises:

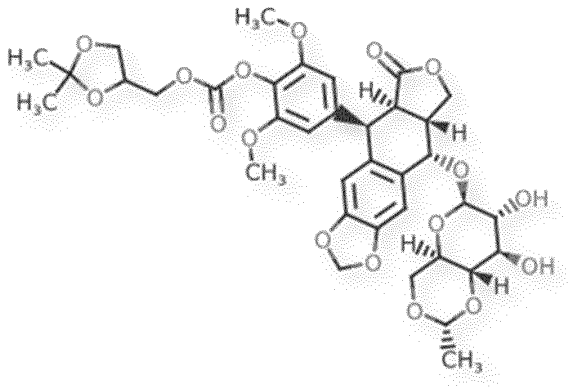
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml ethanol; and
31 mg/ml Benzyl alcohol.

In preferred embodiments of all aspects of the invention, the pharmaceutical formulation comprises:

20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml ethanol; and
31 mg/ml Benzyl alcohol.

DETAILED DESCRIPTION

As used herein, “Etoposide toniribate” and “Cap7.1” are used interchangeably to refer to a compound according to formula I:



(Formula I)

Chemotherapeutic treatment of cancer with etoposide toniribate requires appropriate formulation of the compound. To date, etoposide toniribate has been formulated as a dry powder, which must be stored at -70°C, thereby placing significant demands for maintenance of a reliable storage and distribution cold-chain.

For administration, the powder is dissolved and reconstituted in polyethoxylated castor oil (e.g. Cremophor EL®) and ethanol (at a ratio of 50:50 oil to ethanol), and then subsequently diluted in sodium chloride (0.9%) for infusion.

The present invention provides new formulations of etoposide toniribate such that the drug can be effectively and stably stored and can be efficiently prepared for administration to patients.

Accordingly, in a first aspect the invention provides a liquid pharmaceutical formulation comprising:

etoposide toniribate;
a polysorbate; and
ethanol.

In another aspect the invention provides a liquid pharmaceutical formulation comprising:

Etoposide toniribate;

PEG 300;

a polysorbate;

Ethanol; and

Benzyl alcohol.

As demonstrated in the accompanying Examples, a formulation comprising these ingredients shows long-term stability at a range of temperatures, from refrigerator temperature to 40°C. The formulation can also easily be diluted to prepare an infusion solution for administration to a patient. Infusion solutions prepared from the pharmaceutical formulations of the invention also show particularly favourable stability at room temperature, as shown in the Examples.

As will be appreciated by the skilled person, “pharmaceutical formulation” in this context refers to a formulation suitable for administration to a patient, and is used interchangeably with “formulation” in this description.

In preferred embodiments the concentration of etoposide toniribate in the formulation is in the range of from 1 mg/ml to 50 mg/ml. Preferably the concentration of etoposide toniribate in the formulation is in the range of from 5 mg/ml to 40 mg/ml, preferably 5 mg/ml to 30 mg/ml. More preferably, the concentration of etoposide toniribate in the formulation is in the range of from 10 mg/ml to 20 mg/ml.

In certain preferred embodiments the concentration of etoposide toniribate in the formulation is 10 mg/ml. In certain preferred embodiments the concentration of etoposide toniribate in the formulation is 20 mg/ml.

In another preferred embodiments the concentration of etoposide toniribate in the formulation is in the range of from 25 mg/ml to 150 mg/ml. Preferably the concentration of etoposide toniribate in the formulation is in the range of from 30 mg/ml to 140 mg/ml, preferably 40 mg/ml to 130 mg/ml, preferably 40 mg/ml to 110 mg/ml. More preferably, the concentration of etoposide toniribate in the formulation is in the range of from 50 mg/ml to 100 mg/ml, preferably 50 mg/ml to 91 mg/ml.

In certain preferred embodiments the concentration of etoposide toniribate in the formulation is 50 mg/ml. In certain preferred embodiments the concentration of etoposide toniribate in the formulation is 91 mg/ml.

The pharmaceutical formulation of the invention comprises a polysorbate. Polysorbates are surfactants derived from PEGylated sorbitan (a derivative of sorbitol) esterified with fatty acids, for example oleic acid. Polysorbates include Polysorbate 80 and Polysorbate 20, also known as Tween 80 and Tween 20, respectively. In certain embodiments the polysorbate in the formulation is selected from polysorbate 80 and polysorbate 20. In preferred embodiments of the pharmaceutical formulation of the present invention the polysorbate is Polysorbate 80 (polyoxyethylene-sorbitan(20)-monooleate).

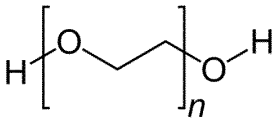
In certain preferred embodiments, the concentration of the polysorbate (preferably Polysorbate 80) in the formulation is in the range of from 500 mg/ml to 1000 mg/ml. Preferably, the polysorbate is at a concentration in the range of from 600 mg/ml to 800 mg/ml, more preferably 630 mg/ml to 800 mg/ml. In a preferred embodiment, the polysorbate (e.g. polysorbate 80) is present in the formulation at a concentration in the range of from 630 mg/ml to 750 mg/ml. Preferably the polysorbate is present at a concentration of 750 mg/ml. Alternatively preferably, the polysorbate is present at a concentration of 631 mg/ml.

In another preferred embodiments, the concentration of the polysorbate (preferably Polysorbate 80) in the formulation is in the range of from 50 mg/ml to 100 mg/ml. Preferably, the polysorbate is at a concentration in the range of from 60 mg/ml to 100mg/ml, more preferably 60 mg/ml to 90 mg/ml. In a preferred embodiment, the polysorbate is present in the formulation at a concentration in the range of from 70 mg/ml to 90 mg/ml, more preferably in the range of from 75 mg/ml to 85 mg/ml. Preferably the polysorbate is present at a concentration of 80 mg/ml.

Most preferably the polysorbate is polysorbate 80 and is present in the formulation at a concentration of 80 mg/ml.

Most preferably the polysorbate is polysorbate 80 and is present in the formulation at a concentration of 750 mg/ml.

The pharmaceutical formulation of the invention comprises PEG 200-600 – that is, polyethylene glycols having an average molecular weight in the range of from 200 to 600 g/mol. The PEG used in the formulations of the invention have the general formula:

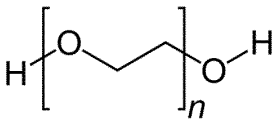


where "n" is in the range of from 4 to 12. For example, "n" may be 4, 5, 6, 7, 8, 9, 10, 11 or 12.

The skilled person will appreciate that for PEG used in the formulations of the invention, the molecular weight or number of repeat units "n" refers to the average for the particular PEG used.

In certain preferred embodiments, the PEG is PEG 200-400. In preferred embodiments, the PEG is selected from PEG 200, PEG 300, and PEG 400.

In certain preferred embodiments, the PEG in the formulation is PEG 300. That is, polyethylene glycols having the general formula:



where "n" is 6. As would be appreciated by the skilled person, PEG 300 is also known as PEG 6, macrogol 300 or polyglycol 300.

In preferred embodiments, the concentration of PEG (e.g. PEG 300) in the formulation is in the range of from 100 mg/ml to 1000 mg/ml. Preferably the concentration of PEG in the formulation is in the range of from 500 mg/ml to 1000 mg/ml. More preferably, the concentration of PEG, preferably PEG 300, in the formulation is in the range of from 500 mg/ml to 700 mg/ml, more preferably 600 mg/ml to 700 mg/ml.

In a most preferred embodiment the concentration of PEG in the pharmaceutical formulation is 650 mg/ml. Preferably the PEG is PEG 300.

The pharmaceutical formulation of the invention comprises ethanol – that is, 100% ethanol. 100% ethanol is also known as dehydrated alcohol, anhydrous ethanol, or absolute ethyl alcohol.

In preferred embodiments, the concentration of dehydrated alcohol in the formulation is in the range of from 10 mg/ml to 1000 mg/ml. Preferably the concentration of dehydrated alcohol in the formulation is in the range of from 50 mg/ml to 500 mg/ml. More preferably, the concentration of dehydrated alcohol in the formulation is in the range of from 100 mg/ml to 400 mg/ml, more preferably 200 mg/ml to 300 mg/ml. In a further preferred embodiment, the concentration of dehydrated alcohol in the formulation is in the range of from 240 mg/ml to 260 mg/ml.

In a most preferred embodiment the concentration of ethanol in the pharmaceutical formulation is 250 mg/ml.

In preferred embodiments, the concentration of ethanol in the formulation is in the range of from 1 % to 50 % (v/v). Preferably the concentration of ethanol in the formulation is in the range of from 5 % to 50 % (v/v). More preferably, the concentration of ethanol in the formulation is in the range of from 10 % to 40 % (v/v), more preferably 20 % to 40 % (v/v). In a further preferred embodiment, the concentration of ethanol in the formulation is in the range of from 25 % to 35 % (v/v). In certain preferred embodiments the concentration of ethanol in the formulation is 31% (v/v).

In preferred embodiments, the concentration of ethanol in the formulation is in the range of from 10 mg/ml to 1000 mg/ml. Preferably the concentration of ethanol in the formulation is in the range of from 50 mg/ml to 500 mg/ml. More preferably, the concentration of ethanol in the formulation is in the range of from 100 mg/ml to 400 mg/ml, more preferably 200 mg/ml to 300 mg/ml. In a further preferred embodiment, the concentration of ethanol in the formulation is in the range of from 240 mg/ml to 250 mg/ml.

In a most preferred embodiment the concentration of ethanol in the pharmaceutical formulation is 242 mg/ml.

In preferred embodiments, the concentration of ethanol in the formulation is in the range of from 1 % to 50 % (v/v). Preferably the concentration of ethanol in the formulation is in the range of from 5 % to 50 % (v/v). More preferably, the concentration of ethanol in the formulation is in the range of from 10 % to 40 % (v/v), more preferably 20 % to 40 % (v/v). In a further preferred embodiment, the concentration of ethanol in the formulation is in the range of from 25 % to 35 % (v/v). In certain preferred embodiments the concentration of ethanol in the formulation is 30% (v/v). In certain preferred embodiments the concentration of ethanol in the formulation is 33% (v/v).

The pharmaceutical formulation of the invention comprises benzyl alcohol (also known as benzenemethanol).

In preferred embodiments, the concentration of benzyl alcohol in the formulation is in the range of from 1 mg/ml to 100 mg/ml. Preferably the concentration of benzyl alcohol in the formulation is in the range of from 5 mg/ml to 50 mg/ml. More preferably, the concentration of benzyl alcohol in the formulation is in the range of from 10 mg/ml to 40 mg/ml, more preferably 20 mg/ml to 40 mg/ml. In a further preferred embodiment, the concentration of benzyl alcohol in the formulation is in the range of from 30 mg/ml to 35 mg/ml.

In a most preferred embodiment the concentration of benzyl alcohol in the pharmaceutical formulation is 31 mg/ml.

In preferred embodiments, the concentration of benzyl alcohol in the formulation is in the range of from 0.1 % to 10 % (v/v). Preferably the concentration of benzyl alcohol in the formulation is in the range of from 0.5 % to 10 % (v/v).

Preferably, the concentration of benzyl alcohol in the formulation is in the range of from 1 % to 4 % (v/v), more preferably 2 % to 4 % (v/v). In a further preferred embodiment, the concentration of benzyl alcohol in the formulation is in the range of from 2.5 % to 3.5 % (v/v). In a further preferred embodiment, the concentration of benzyl alcohol in the formulation is in the range of from 2.8 % to 3.3 % (v/v). In certain preferred embodiments the concentration of benzyl alcohol in the formulation is 3% (v/v).

Alternatively and preferably, the concentration of benzyl alcohol in the formulation is in the range of from 4 % to 10 % (v/v), more preferably 4 % to 8 % (v/v). In a further preferred embodiment, the concentration of benzyl alcohol in the formulation is in the range of from 5 % to 7% (v/v). In a further preferred embodiment, the concentration of benzyl alcohol in the formulation is in the range of from 5.5 % to 6.5 % (v/v). In certain preferred embodiments the concentration of benzyl alcohol in the formulation is 6% (v/v).

In certain embodiments, the formulation has a pH in the range of from pH 3 to 4, preferably from pH 3.5 to 4.0. In certain embodiments, the formulation has a pH of 3.7.

In certain embodiments, the formulation of the invention has a pH in the range of from pH 5 to 8. In certain preferred embodiments, the pharmaceutical formulation has a pH in the range of from pH 5.0 to 7.8.

Preferably, the pharmaceutical formulation has a pH in the range of from pH 7.3 to 7.8.

Alternatively and preferably, the pharmaceutical formulation has a pH in the range of from pH 5.0 to 7.3, preferably a pH in the range of pH 5.0-7.0.

Preferably the pharmaceutical formulation has a pH in the range of from pH 6.0 to 7.0

Alternatively and preferably, the pharmaceutical formulation has a pH in the range of from pH 5.0 to 6.0.

The pH of the formulation can be adjusted by addition of a suitable organic acid, for example citric acid, acetic acid, tartaric acid, malic acid or succinic acid, preferably citric acid.

In certain preferred embodiments the formulation further comprises citric acid.

In a preferred embodiment the formulation comprises citric acid and has a pH of less than 7.3, for example a pH in the range of pH 5.0 to 7.3, preferably a pH in the range of pH 5.0-7.0. Preferably the formulation comprises citric acid and has a pH in the range of from 6.0-

7.0. Alternatively and preferably, the formulation comprises citric acid and has a pH in the range of from 5.0-6.0.

In alternative preferred embodiments the formulation does not include citric acid.

In certain preferred embodiments the pharmaceutical formulation comprises:

50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml Ethanol.

In certain preferred embodiments, the pharmaceutical formulation comprises:

91 mg/ml Etoposide toniribate;
631 mg/ml Polysorbate 80; and
252 mg/ml Ethanol.

In certain preferred embodiments the pharmaceutical formulation consists essentially of:

50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml Ethanol.

In certain preferred embodiments the pharmaceutical formulation consists essentially of:

91 mg/ml Etoposide toniribate;
631 mg/ml Polysorbate 80; and
252 mg/ml Ethanol.

In such embodiments, the formulation may further comprise a suitable acid (e.g. citric acid) such that the formulation has the desired pH, as described above.

In certain preferred embodiments the pharmaceutical formulation consists of:

50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml Ethanol.

In certain preferred embodiments the pharmaceutical formulation consists of:

91 mg/ml Etoposide toniribate;

631 mg/ml Polysorbate 80; and
252 mg/ml Ethanol.

In a preferred aspect is provided a pharmaceutical product comprising a vial filled with 4ml of a pharmaceutical formulation of the invention as described herein. In preferred such embodiments, the pharmaceutical formulation according to the invention comprises:

0.2g of etoposide toniribate,
3g of polysorbate 80, and
1g ethanol.

In certain preferred embodiments the pharmaceutical formulation comprises:
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

In certain preferred embodiments, the pharmaceutical formulation comprises:
20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

In such embodiments, the formulation may further comprise a suitable acid (e.g. citric acid) such that the formulation has the desired pH, as described above.

In certain preferred embodiments the pharmaceutical formulation consists essentially of:
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

In certain preferred embodiments the pharmaceutical formulation consists essentially of:
20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

In such embodiments, the formulation may further comprise a suitable acid (e.g. citric acid) such that the formulation has the desired pH, as described above.

In certain preferred embodiments the pharmaceutical formulation consists of:
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

In certain preferred embodiments the pharmaceutical formulation consists of:
20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

The formulations of the present invention are particularly advantageous due to their stability, as demonstrated in the accompanying Examples. As used herein, a stable pharmaceutical formulation is one in which the etoposide toniribate retains its physical stability and/or chemical stability and/or biological activity upon storage at the intended storage temperature (e.g. 2-8°C or room temperature). For example, a stable pharmaceutical formulation, between the time that it is made and the time that it is used (or reaches the end of its intended shelf-life), does not undergo any changes in its physical, chemical or biological properties which renders it unsafe or ineffective for its intended pharmaceutical use.

Stability of etoposide toniribate formulations can be determined by, for example, assessing physical stability by the level of particulates accumulated in the formulation as a result of precipitation.

In preferred embodiments, formulations according to the invention exhibit no precipitation after storage at 2-8°C for 72 hours.

Stability may also be assessed by measurement of the change in percentage of impurities in the formulation over time. Impurity levels can be determined by methods known to the person skilled in the art, for example chromatography methods.

In certain embodiments, formulations of the invention exhibit an increase in percentage of total impurities in the formulation of less than 0.5% over 3 months at 2-8°C, preferably an increase of total impurities in the formulation of less than 0.4% over 3 months at 2-8°C. In certain embodiments, formulations of the invention exhibit an increase in percentage of total impurities in the formulation of less than 0.5% over 5 months at 2-8°C, preferably an increase of total impurities in the formulation of less than 0.4% over 5 months at 2-8°C. In certain preferred embodiments, formulations of the invention exhibit an increase in percentage of total impurities in the formulation of less than 0.3% over 5 months at 2-8°C, preferably an increase of total impurities in the formulation of less than 0.2% over 5 months at 2-8°C. (Changes in percentage of impurity are given in absolute terms – i.e. an increase of total impurities from 0.1% to 0.3% over the given period is a change of 0.2%.)

In certain embodiments, formulations of the invention exhibit an increase in percentage of the cis-Cap7.1 impurity in the formulation of less than 0.3% over 3 months at 2-8°C. In certain embodiments, formulations of the invention exhibit an increase in percentage of the cis-Cap7.1 impurity in the formulation of less than 0.3% over 5 months at 2-8°C, preferably less than 0.2% over 5 months at 2-8°C.

Stability may also be assessed by measurement of the changes in etoposide toniribate concentration over time.

In certain preferred embodiments, formulations according to the invention exhibit a decrease in concentration of etoposide toniribate of no more than 0.5 mg/ml over 5 months

at 2-8°C. In certain preferred embodiments, formulations according to the invention exhibit a decrease in concentration of etoposide toniribate of no more than 5% of the starting concentration over 5 months at 2-8°C.

Stability may also be determined by assessing chemical stability, indicated by the level of purity of the API over time. Purity levels can be determined by methods known to the person skilled in the art, for example chromatography methods, preferably HPLC.

In preferred embodiments, formulations according to the invention exhibit no visible precipitation after storage at 2-8°C for up to 72 hours, preferably up to 7 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 2-8°C for up to 12 days, optionally up to 19 days, up to 26 days, up to 39 days, up to 49 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 2-8°C for up to 12 weeks.

In preferred embodiments, formulations according to the invention exhibit no visible precipitation after storage at 20-25°C for up to 72 hours, preferably up to 7 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 20-25°C for up to 12 days, optionally up to 14 days, optionally up to 19 days, up to 24 days, up to 26 days, up to 28 days, up to 35 days, up to 39 days, up to 42 days, up to 49 days, up to 56 days, up to 63 days, optionally up to 75 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 20-25°C for up to 12 weeks. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 20-25°C for up to 90 days. In such embodiments, storage at 20-25°C is at 60% relative humidity (RH).

In preferred embodiments, formulations according to the invention exhibit no visible precipitation after storage at 30°C for up to 7 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 30°C for up to 14 days, optionally up to 24 days, up to 28 days, up to 35 days, up to 42 days, up to 49 days, up to 56 days, up to 63 days, optionally up to 75 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 30°C for up to 12 weeks. Preferably, formulations according to the invention exhibit no visible precipitation after

storage at 30°C for up to 90 days. In such embodiments, storage at 30°C is at 65% relative humidity (RH).

In preferred embodiments, formulations according to the invention exhibit no visible precipitation after storage at 40°C for up to 7 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 30°C for up to 14 days, optionally up to 24 days, up to 28 days, up to 35 days, up to 42 days, up to 49 days, up to 56 days, up to 63 days, optionally up to 75 days. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 40°C for up to 12 weeks. Preferably, formulations according to the invention exhibit no visible precipitation after storage at 40°C for up to 90 days. In such embodiments, storage at 40°C is at 75% relative humidity (RH).

In preferred embodiments, formulations according to the invention exhibit greater than 99% API purity after storage at 2-8°C for up to 72 hours, preferably up to 7 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 2-8°C for up to 12 days, optionally up to 19 days, up to 26 days, up to 39 days, up to 49 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 2-8°C for up to 12 weeks.

In preferred embodiments, formulations according to the invention exhibit greater than 99% API purity after storage at 20-25°C for up to 72 hours, preferably up to 7 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 20-25°C for up to 12 days, optionally up to 14 days, optionally up to 19 days, up to 24 days, up to 26 days, up to 28 days, up to 35 days, up to 39 days, up to 42 days, up to 49 days, up to 56 days, up to 63 days, optionally up to 75 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 20-25°C for up to 12 weeks. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 20-25°C for up to 90 days. In such embodiments, storage at 20-25°C is at 60% relative humidity (RH).

In preferred embodiments, formulations according to the invention exhibit greater than 99% API purity after storage at 30°C for up to 7 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 30°C for up to 14 days,

optionally up to 24 days, up to 28 days, up to 35 days, up to 42 days, up to 49 days, up to 56 days, up to 63 days, optionally up to 75 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 30°C for up to 12 weeks. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 30°C for up to 90 days. In such embodiments, storage at 30°C is at 65% relative humidity (RH).

In preferred embodiments, formulations according to the invention exhibit greater than 99% API purity after storage at 40°C for up to 7 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 30°C for up to 14 days, optionally up to 24 days, up to 28 days, up to 35 days, up to 42 days, up to 49 days, up to 56 days, up to 63 days, optionally up to 75 days. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 40°C for up to 12 weeks. Preferably, formulations according to the invention exhibit greater than 99% API purity after storage at 40°C for up to 90 days. In such embodiments, storage at 40°C is at 75% relative humidity (RH).

In a further aspect the invention provides a method of making an etoposide toniribate formulation according to the invention, the method comprising: (a) mixing the polysorbate and ethanol, and stirring the mixture; (b) adding the etoposide toniribate to the mixture obtained in step (a) and stirring the resultant mixture.

In a further aspect the invention provides a method of making an etoposide toniribate formulation according to the invention, the method comprising: (a) mixing the etoposide toniribate, ethanol, and benzyl alcohol and stirring the mixture; (b) adding the PEG 300 and the polysorbate to the mixture obtained in step (a) and stirring to obtain a solution. In certain embodiments the rate of stirring is increased from step (a) to step (b).

In certain embodiments the method further comprises adjusting the pH of the mixture obtained in step (b) through addition of an acid (e.g. citric acid).
In certain embodiments the method further comprises sterile filtering the etoposide toniribate solution obtained in step (b). In certain preferred embodiments, the polysorbate is polysorbate 80 having a pH of less than 7, preferably in the range of from pH 3-7.

In a further aspect the invention provides a kit comprising a pharmaceutical formulation according to the invention. In certain embodiments the kit further comprises instructions for the use of the formulation. In certain embodiments the pharmaceutical formulation is provided in the kit in a vial.

In certain embodiments the pharmaceutical formulation is provided in a vial at a fill volume in the range of from 5 ml to 20 ml. In certain preferred embodiments the pharmaceutical formulation is provided in a vial at a fill volume of 10 ml.

In preferred embodiments the vial is a photo-protective vial, for example an amber vial. In certain embodiments the pharmaceutical formulation is provided in a vial at a fill volume in the range of from 1 ml to 20 ml. In certain preferred embodiments the pharmaceutical formulation is provided in a vial at a fill volume in the range of from 4 ml to 10 ml. In certain preferred embodiments the pharmaceutical formulation is provided in a vial at a fill volume in the range of from 4 ml to 6 ml.

In certain embodiments the kit comprises instructions for preparing an infusion solution according to the invention. In certain embodiments the kit comprises instructions for treating a patient in accordance with the methods of therapy provided herein.

In certain embodiments, the kit further comprises a diluent. In certain such embodiments the diluent is selected from sodium chloride solution (optionally a 0.45% or 0.9% sodium chloride solution), glucose solution (optionally 5% glucose solution), and water for injection.

Intravenous administration of etoposide toniribate to a patient is preferred. To prepare an infusion solution suitable for effective intravenous administration, the pharmaceutical formulations of the invention need to be diluted in a suitable diluent.

As demonstrated herein, the infusion solutions prepared from by diluting the pharmaceutical formulations of the invention have the advantage that they are stable at room temperature (controlled at 20-25°C, 60% RH). As shown in the examples, infusion solutions prepared using any of glucose solution, sodium chloride solution (saline) or water for injection (WFI) exhibited no precipitation when stored for 24 hours at room temperature (20- 25°C, 60% relative humidity (RH).

As shown in the examples, infusion solutions prepared using any of glucose solution, sodium chloride solution (0.45% or 0.9%) or water for injection (WFI) exhibited physical

stability (as shown by lack of precipitation) and chemical stability (>99% purity) when stored for 24 hours at room temperature. This stability makes the infusion solutions convenient to prepare and handle prior to administration, making the infusion solutions particularly advantageous.

Accordingly, in a further aspect the invention provides an infusion solution comprising a pharmaceutical formulation according to the invention and a diluent. The invention also provides a method of preparing an infusion solution, the method comprising diluting a pharmaceutical formulation according to the invention in a diluent. The invention further provides an infusion solution prepared according to such a method.

In certain embodiments of each of these aspects the diluent is selected from sodium chloride solution (optionally 0.45% sodium chloride solution or 0.9% sodium chloride solution), glucose solution (optionally 5% glucose solution), and water for injection. In certain embodiments the diluent is sodium chloride (saline), optionally 0.45% or 0.9% saline. In certain embodiments the diluent is glucose solution, optionally 5% glucose solution. In certain embodiments the diluent is water for injection (WFI). Using WFI as the diluent is particularly preferred due to the beneficial osmolality of the resultant infusion solution being closest to the physiological osmolality of 280-290 mOsm/kg, as shown in the Examples.

In certain embodiments, the etoposide toniribate concentration in the infusion solution is in the range of from 0.1 mg/ml to 5 mg/ml. In certain preferred embodiments, the etoposide toniribate concentration in the infusion solution is in the range of from 0.5 mg/ml to 3 mg/ml.

In certain preferred embodiments, the etoposide toniribate concentration in the infusion solution is in the range of from 0.5 mg/ml to 0.8 mg/ml.

In certain preferred embodiments, the etoposide toniribate concentration in the infusion solution is 0.5 mg/ml or 0.8 mg/ml. In certain alternative preferred embodiments, the etoposide toniribate concentration in the infusion solution is 0.7 mg/ml or 2.9 mg/ml.

In certain embodiments, the etoposide toniribate concentration in the infusion solution is in the range of from 1 mg/ml to 10 mg/ml. In certain preferred embodiments, the etoposide

toniribate concentration in the infusion solution is in the range of from 1 mg/ml to 5 mg/ml, preferably 2 mg/ml to 4 mg/ml. In certain preferred embodiments, the etoposide toniribate concentration in the infusion solution is in the range of from 3 mg/ml to 4 mg/ml, preferably 3.0 mg/ml to 3.5 mg/ml. In certain preferred embodiments, the etoposide toniribate concentration in the infusion solution is 3.1 mg/ml. In certain preferred embodiments, the etoposide toniribate concentration in the infusion solution is 3.2 mg/ml.

The skilled person will appreciate that the concentration of the formulation components in infusion solutions according to the invention reflects the dilution of etoposide toniribate relative to the pharmaceutical formulation from which the solution is prepared. For example, where an infusion solution having a concentration of etoposide toniribate of 3 mg/ml is prepared from a pharmaceutical formulation having an etoposide toniribate concentration of 30 mg/ml, the concentration of the other components in the infusion solution will be 10x more dilute versus the pharmaceutical formulation concentrations.

In a further aspect the invention provides a method of treating cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical formulation according to the invention or an infusion solution according to the invention.

In certain preferred embodiments, the formulation or infusion solution is administered intravenously.

In certain preferred embodiments, the cancer is selected from the group consisting of biliary tract cancer, adenocarcinoma (e.g. colon adenocarcinoma and colorectal adenocarcinoma), hypopharynx cancer (e.g. squamous cell hypopharynx cancer, especially with pulmonary metastases), lung cancer (e.g. lung carcinoma, small cell lung cancer and squamous cell cancer of the lung), diffuse large cell lymphoma, Burkitt's lymphoma, Hodgkin lymphoma, Non-Hodgkin lymphoma, histiocytic lymphoma, lymphatic lymphoma, acute T-cell leukaemia, pre-B-acute lymphoblastic leukaemia, thymus carcinoma (e.g. sarcomatoid thymus carcinoma, especially with pulmonary metastases), urothelium carcinoma, testicular cancer (e.g. testicular germ cell tumour, seminoma and non-seminoma, especially with renal, pulmonary, retroperitoneal, hepatic and/or cerebral metastases), prostate cancer, bladder cancer, AIDS-related Kaposi's sarcoma, Ewing

sarcoma, rhabdomyosarcoma, neuroblastoma (in particular paediatric neuroblastoma, especially advanced paediatric neuroblastoma), ovarian cancer (e.g. ovarian germ cell tumour, or ovarian carcinoma), and breast cancer. In preferred embodiments the method is a method of treating biliary tract cancer.

As used herein, "patient" refers to a subject to be administered therapy, for example for cancer. In preferred embodiments of all aspects of the invention, the patient is a human patient.

As used in the claims, "comprising" is given its conventional construction of meaning that any further component, step or feature can be present in addition to those recited in the claim. "Consisting essentially of" is intended to mean further components may be present provided they do not materially affect the essential characteristics of the formulation. "Consisting of" is given its convention construction of meaning no further component, step or feature is present beyond those recited in the claim.

With the above context, the following consecutively itemized A embodiments provide further specific aspects of the invention:

Item A1: A liquid pharmaceutical formulation comprising:
Etoposide toniribate;
PEG;
a polysorbate;
Ethanol;
Benzyl alcohol.

Item A2: The pharmaceutical formulation of item A1, comprising etoposide toniribate at a concentration in the range of from 10 mg/ml to 20 mg/ml, optionally at a concentration of 10 mg/ml or 20mg/ml.

Item A3: The pharmaceutical formulation of item A1 or item A2, wherein the polysorbate is polysorbate 80.

Item A4: The pharmaceutical formulation of any preceding item A, wherein the PEG is PEG 200-600.

Item A5: The pharmaceutical formulation of any preceding item A, wherein the PEG is PEG 200-400.

Item A6: The pharmaceutical formulation of any preceding item A, wherein the PEG is PEG 300.

Item A7: The pharmaceutical formulation of any preceding item A, wherein the pharmaceutical formulation has a pH in the range of from pH 5.0 to 7.8.

Item A8: The pharmaceutical formulation of any one of items A1-A7, wherein the pharmaceutical formulation has a pH in the range of from 7.3 to 7.8.

Item A9: The pharmaceutical formulation of any one of items A1-A7, wherein the pharmaceutical formulation has a pH in the range of from 6.0 to 7.0.

Item A10: The pharmaceutical formulation of any one of items A1-A7, wherein the pharmaceutical formulation has a pH in the range of from 5.0 to 6.0.

Item A11: The pharmaceutical formulation of any preceding item A, wherein the pharmaceutical formulation further comprises citric acid.

Item A12: The pharmaceutical formulation of any preceding item A, wherein the pharmaceutical formulation comprises:
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

Item A13: The pharmaceutical formulation of any one of items A1-A11, wherein the pharmaceutical formulation comprises:

20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml dehydrated ethanol; and
31 mg/ml Benzyl alcohol.

Item A14: The pharmaceutical formulation of any one of items A1-A10, wherein the pharmaceutical formulation consists of, or consists essentially of:
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml dehydrated ethanol; and
31 mg/ml Benzyl alcohol.

Item A15: The pharmaceutical formulation of any one of items A1-A10, wherein the pharmaceutical formulation consists of, or consists essentially of:
20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

Item A16: A method of preparing an infusion solution comprising diluting a pharmaceutical formulation according to any one of items A1-A15 in a diluent.

Item A17: An infusion solution prepared by diluting a pharmaceutical formulation according to any of items A1-A15 in a diluent.

Item A18: An infusion solution comprising a pharmaceutical formulation according to any one of items A1-A15 and a diluent.

Item A19: A method according to item 16 or an infusion solution according to item A17 or A18, wherein the diluent is selected from: water for injection; 5% glucose solution; and 0.9% NaCl saline.

Item A20: A method or an infusion solution according to item A19, wherein the diluent is water for injection.

Item A21: A method or an infusion solution according to any one of items A16-A20, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 0.1 mg/ml to 1 mg/ml.

Item A22: A method or an infusion solution according to any one of items 16-21, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 0.5 mg/ml to 0.8 mg/ml.

Item A23: A method or an infusion solution according to any one of items A16-A22, wherein the etoposide toniribate concentration in the infusion solution is 0.5 mg/ml or 0.8 mg/ml.

Item A24: A kit comprising: a pharmaceutical formulation of any one of items A1-A15; and a diluent.

Item A25: The kit of item A24, wherein the diluent is selected from: water for injection; 5% glucose solution; and 0.9% NaCl saline.

Item A26: A pharmaceutical formulation according to any one of items A1-A15 or an infusion solution according to any one of items A16-A23, for use in therapy.

Item A27: A pharmaceutical formulation according to any one of items A1-A15 or an infusion solution according to any one of items A16-A23, for use in a method of treating cancer.

Item A28: A pharmaceutical formulation according to any one of items 1-15 or an infusion solution according to any one of items A16-A23, for use in a method of treating biliary tract cancer

Item A29: A method of treating cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical formulation

according to any one of items A1-A15, or an infusion solution according to any one of items A16-A23.

Further preferred embodiments within the scope of the present invention are set out as itemized B embodiments below:

Item B1: A liquid pharmaceutical formulation comprising:
etoposide toniribate;
a polysorbate; and
ethanol.

Item B2: The pharmaceutical formulation of item B1, comprising etoposide toniribate at a concentration in the range of from 50 mg/ml to 100 mg/ml.

Item B3: The pharmaceutical formulation of item B1 or item A2, wherein the polysorbate is polysorbate 80.

Item B4: The pharmaceutical formulation of any preceding item B wherein the polysorbate concentration is in the range of from 600 mg/ml to 800 mg/ml.

Item B5: The pharmaceutical formulation of any preceding item B wherein the polysorbate concentration is 750 mg/ml.

Item B6: The pharmaceutical formulation of any preceding item B wherein the ethanol concentration is in the range of from 200 mg/ml to 300 mg/ml.

Item B7: The pharmaceutical formulation of any preceding item B wherein the ethanol concentration is 250 mg/ml.

Item B8: The pharmaceutical formulation of any preceding item B, wherein the pharmaceutical formulation comprises:
50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml Ethanol.

Item B9: The pharmaceutical formulation of any one of items B1-B7, wherein the pharmaceutical formulation comprises:
91 mg/ml Etoposide toniribate;
631 mg/ml Polysorbate 80; and
252 mg/ml Ethanol.

Item B10: The pharmaceutical formulation of any one of items B1-B7, wherein the pharmaceutical formulation consists, or consists essentially of:
50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml Ethanol.

Item B11: The pharmaceutical formulation of any one of items B1-B7, wherein the pharmaceutical formulation consists, or consists essentially of:
91 mg/ml Etoposide toniribate;
631 mg/ml Polysorbate 80; and
252 mg/ml Ethanol.

Item B12: The pharmaceutical formulation of any preceding item, wherein the pharmaceutical formulation has a pH in the range of from pH 3 to 4.

Item B13: The pharmaceutical formulation of any preceding item, wherein the pharmaceutical formulation has a pH of 3.7.

Item B14: A method of preparing an infusion solution comprising diluting a pharmaceutical formulation according to any one of items B1-B13 in a diluent.

Item B15: An infusion solution prepared by diluting a pharmaceutical formulation according to any of items B1-B13 in a diluent.

Item B16: An infusion solution comprising a pharmaceutical formulation according to any one of items B1-B13 and a diluent.

Item B17: A method according to item B14 or an infusion solution according to item B15 or B16, wherein the diluent is selected from: water for injection; 5% glucose solution; 0.45% NaCl saline, and 0.9% NaCl saline.

Item B18: A method or an infusion solution according to item B17, wherein the diluent is water for injection.

Item B19: A method or an infusion solution according to any one of items B14-B18, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 1 mg/ml to 10 mg/ml.

Item B20: A method or an infusion solution according to any one of items B14-B19, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 1 mg/ml to 5 mg/ml.

Item B21: A method or an infusion solution according to any one of items B14-B20, wherein the etoposide toniribate concentration in the infusion solution is 3.1 mg/ml.

Item B22: A kit comprising: a pharmaceutical formulation of any one of items B1-B13; and a diluent.

Item B23: The kit of item B22, wherein the diluent is selected from: water for injection; 5% glucose solution; 0.45% NaCl saline, and 0.9% NaCl saline.

Item B24: A pharmaceutical formulation according to any one of items B1-B13 or an infusion solution according to any one of items B15-B21, for use in therapy.

Item B25: A pharmaceutical formulation according to any one of items B1-B13 or an infusion solution according to any one of items B15-B21, for use in a method of treating cancer.

Item B26: A pharmaceutical formulation according to any one of items B1-B13 or an infusion solution according to any one of items B15-B21, for use in a method of treating biliary tract cancer

Item B27: A method of treating cancer in a patient in need thereof, the method comprising administering to the patient an effective amount of a pharmaceutical formulation according to any one of items B1-B13, or an infusion solution according to any one of items B15-B21.

Examples

Etoposide toniribate as API was known to be poorly soluble in water, and unstable at acidic and basic pH. To date, the API has been formulated by dissolving it in Cremophor RH40 and ethanol. However, this formulation is cumbersome to use and lacks scalability. Experiments were undertaken to develop a new stable formulation of etoposide toniribate that is stable at refrigerator temperature (2-8°C) and compatible with acceptable infusion diluents, such that the infusion solution is stable for at least 2-3 hours at room temperature (assessed at controlled room temperature of 20-25°C, 60% relative humidity (RH)). It is also desirable for the infusion solution to have an osmolality close to blood osmolality (280-295 mOsm/Kg).

Example 1

Formulation: 25 g Polysorbate 80 and 10 g Ethanol were mixed (polysorbate 80 having an acidic pH was used for all the exemplified formulations). To the resulting mixture of 36 ml 3.6 g CAP 7.1 were added with stirring. The solution was filtered with membrane 0.2µm and 1.1 ml filled into glass vials (5ml), giving 100mg of CAP7.1 per vial. The vials were sealed with 13mm bromobutyl rubber stoppers and Al-Crimp caps. The vials were stored at 5°C ± 3°C and 20-25°C/60% RH as well. The stability was examined by HPLC (method for related substances) over 12 weeks. The in-use stability was proofed in dilution with 0.9% NaCl solution. Results are compiled in table 1A (5°C ± 3°C), table 2A (20-25°C/60% RH) and table 3A (in-use) respectively.

The formulation was found to be stable for at least 12 weeks at 2-8°C and at 20-25°C with 60% relative humidity (Tables 1A and 2A). When an “in use” infusion solution was prepared to approximately 3.3 mg/ml in 0.9% saline, the infusion solution was stable for at least 6 hours at room temperature (20-25°C, 60% RH), with the 20 hour results also showing acceptable stability levels.

Example 2

As the stability of the formulation based on polysorbate 80 and ethanol could be demonstrated in Example 1 a formulation containing 200 mg CAP 7.1 per vial has been

tested. For better handling a bigger Vial (10R) was chosen and the filling volume was increased up to 4 ml (filled CAP7.1 concentration 50 mg/ml).

Formulation of example2

One vial contains	3g Polysorbate 80
	1g Ethanol
	0.2 g CAP 7.1

Filling volume 4 ml per vial
Primary packaging: 10R Vials HKI; Teflon coated rubber stoppers sealed with Al-crimp caps.

The vials were stored at 5°C ± 3°C; 20-25°C/60% RH; 30°C/65% RH and 40°C/75% RH as well. The stability was examined by HPLC (Method for related substances) over 90 days. The results are presented in Table 4A (API purity assay) and Table 5A (related substance HPLC).

Results: Based on the purity assay by area% of Table 4, the formulation is shown to be stable for at least 90 days at 5°C ± 3°C and at 20-25°C/60% RH. At 30°C/65% RH and 40°C/75% RH storage the assay dropped from 99.55% initial to 99.40% and 99.06% respectively. These values still indicate remarkable stability of the formulation.

The related substances data of Table 5A confirm this stability. The only “decomposition” product formed during storage at 30°C/65% RH and 40°C/75% RH was the compound at RRT 0.97. This is a known substance representing an isomer of CAP 7.1. Overall the formulation showed stability under all storage conditions for at least 90 days.

Repeat experiments across at least 4 batches produced across separate laboratories confirmed these results and further showed that the formulation was stable for at least 112 days at 5°C ± 3°C, at 20-25°C/60% RH and at 30°C/65% RH.

Example 3

For administration to patients, the stable formulation needs to be diluted in an aqueous solution. To identify suitable diluents for the infusion solution, the formulation of Example 2

was diluted in various solutions to a concentration of approx. 3.1 mg/ml CAP7.1. and the stability and osmolality assessed. The diluents tested were isotonic (0.9%) sodium chloride solution, 0.45% sodium chloride solution, and 5% Glucose solution.

Infusion solutions were prepared as follows: One vial of the formulated concentrate, containing 200 mg of API, 3g Polysorbate 80 and 1g Ethanol was diluted with 60 ml diluent (water for injection, 0.9% NaCl solution, 0.45% NaCl solution or 5% Glucose solution). The resulting solution for infusion contains about 3.13 mg/ml of API.

Results: The osmolality of the different infusion solutions is shown below:

Diluent	Osmolality [mOsmol/kg]
Water for injection	360
0.45% NaCl solution	526
0.9% NaCl solution	689
5% Glucose solution	719

The infusion solution prepared using water for injection shows an osmolality most-similar to blood osmolality (approx. 275-295 mOsm/kg). It is expected that an isotonic infusion solution could be prepared when about 70 ml WFI is used as diluent.

Stability of the infusion solutions is shown in Table 6A below. In summary, all the tested infusion solutions showed similar stability, each exhibiting chemical and physical stability when stored at room temperature for 24 hours. In particular, over 24 hours for each infusion solution the content of CAP 7.1 decreased about 0.2%. The only decomposition product was Etoposide, which increased in the same range, indicative of hydrolytic decomposition. Physical stability measured by assessing visible and sub-visible particles was found to be highly stable, with very low levels of particles detected.

Example 4

To assess photostability, a formulation was prepared according to Example 2, filled in amber glass vials and exposed to light providing an overall irradiated illumination of more than 1.2 million lux hours and an integrated near ultraviolet energy of nearly 200 watt hours/square metre. Additionally, one sample packed in secondary packaging (cardboard box) and one sample protected by wrapping in aluminium foil were also examined as dark

controls. Comparative tabulated results of assay and chemical purity are provided in Tables 7A and 8A below.

Results: the data show that there was no change in assay or chemical purity following irradiation. It was demonstrated that the selected primary packaging itself (amber glass) is sufficient to provide light protection. Experiments testing photo-stability of the formulation under irradiation in transparent vials found that the formulation decomposed. Photo-protective primary packaging (e.g. amber vials) or secondary packaging (e.g. cardboard) should therefore be used for storage.

Example 5

The possibility of preparing aqueous formulations of CAP7.1 for lyophilisation was investigated. In general it was possible to dissolve CAP 7.1 at sufficient concentrations for preparation of a lyophilised product. However, the chemical stability was poor. After a short storage time hydrolytic decomposition of the API to Etoposide was detected, as shown in Table 9A. These data shows decomposition of about 1 % for the aqueous composition over 17 days at 5°C ± 3°C. The main decomposition products are the stereoisomer of CAP 7.1 and Etoposide which increased from 0.1% to 0.33% during the storage.

Conclusions

Stability data from the batch formulations suggest a very stable medicinal product at refrigerated storage and also at 20-25°C/60% RH, as well as at higher temperatures. The infusion solutions prepared from the formulation meet all requirements for clinical use in terms of physical and chemical properties, in use stability of the infusion solutions, osmolality etc. Furthermore the formulation allows a comfortable use in hospital routine because of easy handling of the medicinal product during preparation of infusions and the in use stability of about 20 hours.

Table 1A

Stability of solution of 100mg CAP 7.1 in 1.1 ml Polysorbate 80 / Ethanol at 5°C ± 3°C (related substances by HPLC)

	Visual	Intensity of peaks by Area%													
Tween 80/ EtOH **100mg/1.1ml**	Rel-/absolute Retention Time	0.22/ 4.4	0.31/ 6.41	0.51/ 10.43	0.54/ 10.59 (Etoposide)	0.93/ 18.6	0.95/ 18.95	0.97/ 19.45	**CAP 7.1** 20.8	1.1/ 22.14	1.11/ 22.36	1.16/ 23.24	1.17/ 23.68	1.19/ 23.89	1.20/ 24.3
initial	clear	0.02%	nd	nd	0.06%	0.02%	0.14%	0.25%	99.28%	0.07%	0.04%	0.09%	nd	0.03%	nd
3 days refrigerator	clear	nd	nd	0.01%	0.05%	0.01%	0.11%	0.2%	99.45%	0.05%	0.03%	0.06%	nd	0.02%	nd
7 days refrigerator	clear	0.01%	0.01%	0.06%	0.02%	0.06%	0.15%	0.25%	99.28%	0.06%	0.03%	0.09%	0.01%	0.03%	nd
12 days refrigerator	clear	nd	nd	nd	0.05%	0.02%	0.14%	0.23%	99.33%	0.05%	0.05%	0.08%	nd	0.04%	nd
19 days refrigerator	clear	nd	nd	nd	0.07%	0.02%	0.15%	0.26%	99.29%	0.06%	0.03%	0.09%	0.01%	0.04%	nd
26 days refrigerator	clear	nd	nd	nd	0.06%	0.02%	0.14%	0.24%	99.36%	0.05%	0.03%	0.07%	nd	0.03%	nd
39 days refrigerator	clear	nd	nd	nd	0.05 %	0.02%	0.12%	0.20%	99.43%	0.05%	0.02%	0.06%	0.00%	0.03%	0.01%
49 days refrigerator	clear	nd	nd	nd	0.04%	0.01%	0.10%	0.17%	99.55%	0.04%	0.02%	0.05%	0.02%	0.01%	nd
12 weeks refrigerator	clear	nd	nd	nd	0.04%	0.01%	0.10%	0.18%	99.52%	0.05%	0.01%	0.06%	0.01%	0.01%	nd

nd= not detected

Table 2A:

Stability of solution of 100mg CAP 7.1 in 1.1 ml Polysorbate 80 / Ethanol at 25°C/60% RH (related substances by HPLC)

	Visual	Intensity of peaks by Area%													
Tween 80/EtOH/	Rel-/ absolute Retention Time	0.22/ 4.4	0.31/ 6.41	0.51/ 10.43	0.54/ 10.59	0.93/ 18.6	0.95/ 18.95	0.97/ 19.45	**CAP 7.1**	1.1/ 22.14	1.11/ 22.36	1.16/ 23.24	1.17/ 23.68	1.19/ 23.89	1.20/ 24.3
100mg/1.1ml					(Etoposide)				20.8						
3 days 25°C/60%rh	clear	nd	nd	0.01%	0.07%	0.02%	0.15%	0.25%	99.29%	0.06%	0.03%	0.08%	0.01%	0.03%	nd
7 days 25°C/60% rh	clear	0.01%	0.01%	nd	0.08%	0.02%	0.16%	0.28%	99.19%	0.05%	0.06%	0.03%	0.08%	0.03%	nd
12 days 25°C/60% rh	clear	nd	nd	nd	0.07%	0.02%	0.14%	0.26%	99.28%	0.06%	0.05%	0.08%	nd	0.04%	nd
19 days 25°C/60% rh	clear	nd	nd	nd	0.08%	0.02%	0.14%	0.31%	99.29%	0.05%	0.01%	0.07%	nd	0.03%	nd
26 days 25°C/60% rh	clear	nd	nd	nd	0.09%	0.02%	0.14%	0.31%	99.27%	0.05%	0.01%	0.08%	nd	0.03%	nd
39 days 25°C/60% rh	clear	nd	nd	nd	0.08%	0.02%	0.13%	0.31%	99.29%	0.05%	0.01%	0.07%	0.01%	0.03%	0.01%
49 days 25°C/60% rh	clear	nd	nd	nd	0.06%	0.01%	0.10%	0.27%	99.45%	0.04%	nd	0.05%	0.00%	0.02%	0.00%
12 weeks 25°C/60% rh	clear	nd	nd	nd	0.08%	0.01%	0.10%	0.37%	99.30%	0.04%	nd	0.06%	0.03%	0.01%	nd

Table 3A:

In-use stability of diluted solutions; about 3.3 mg/ml in 0.9% NaCl solution at room temperature

		Intensity of peaks by Area%							
Tween 80/EtOH/ 100mg/1.1ml diluted with 30 ml NaCl	visual	0.22/4.39	0.28/5.72	0.52/10.42	0.56/11.20	0.94/19.01	0.97/19.55	CAP 7.1 20.8	1.16/23.41
initial	clear	0,06%	0,16%	0,05%	0,03%	0,08%	0,15%	99,42%	0,05%
after 6 hours at ambient temperature	clear	0,08%	0,10%	0,04%	0,18%	0,07%	0,16%	99,34%	0,03%
after20 hours at ambient temperature	clear	0,07%	0,14%	0,05%	0,25%	0,07%	0,17%	99,22%	0,03%

Table 4A a and 4A b:

Table 4A a Stability of solution of 200mg CAP 7.1 in 4.0 ml Polysorbate 80 / Ethanol up to 10 weeks

Batch F347		Date of manufacturing: 02.08.2018						Date of storage: 03.08.2018				
Parameter *(Specification)*	**Initial value**	**Storage conditions** [°C/ % RH]	**1 week**	**2 weeks**	**3 weeks**	**4 weeks**	**5 weeks**	**6 weeks**	**7 weeks**	**8 weeks**	**9 weeks**	**10 weeks**
HPLC Purity Data area%	99.55%	KS	99.63%	99.57%	99.52%	99.55%	99.50%	99.51%	99.53%	99.53%	99.50%	99.52%
		25 / 60	99.57%	99.56%	99.50%	99.45%	99.50%	99.44%	99.50%	99.50%	99.48%	99.47%
		30 / 65	99.55%	99.58%	99.49%	99.50%	99.48%	99.41%	99.46%	99.46%	99.41%	99.42%
		40 / 75	99.56%	99.55%	99.42%	99.54%	99.31%	99.35%	99.23%	99.23%	99.20%	99.19%

Table 4A b **Stability of solution of 200mg CAP 7.1 in 4.0 ml Polysorbate 80 / Ethanol up to 90 days (12 weeks)**

Batch F347			Date of manufacturing: 02.08.2018						Date of storage: 03.08.2018				
Parameter *(Specification)*	**Initial value**	**Storage conditions** [°C/ % RH]	**90 days**										
HPLC Purity Data area%	99.55%	KS	99.53%										
		25 / 60	99.49%										
		30 / 65	99.40%										
		40 / 75	99.06%										

Table 5Aa and 5Ab, 5ac, 5Ad:

Table 5Aa Related substances; Stability at 5°C ± 3°C of solution of 200mg CAP 7.1 in 4.0 ml Polysorbate 80 / Ethanol up to 90 days

	Visual examination	Intensity of peaks by Area%														
#F347	Rel-/ absolute Retention Time	0.22/4.4	0.31/6.41	0.51/10.43	0.54/10.59	0.80/16.6	0.86/17.32	0.93/18.6	0.95/18.95	0.97/19.45	CAP 7.1	1.1/22.14	1.11/22.36	1.16/23.24	1.17/23.68	1.19/23.89
20 g/ 400 g					(Etoposide)						20.8					
initial	clear	nd	nd	nd	0.02%	nd	nd	nd	0.12%	0.22%	99.55%	0.03%	0.01%	0.05%	nd	0.01%
7 days 5 ± 3°C	clear	nd	nd	nd	nd	nd	nd	nd	0.08%	0.21%	99.63%	0.03%	0.01%	0.04%	nd	0.01%
14 days 5 ± 3°C	clear	nd	nd	nd	nd	nd	nd	nd	0.13%	0.22%	99.57%	0.02%	0.01%	0.04%	nd	0.01%
24 days 5 ± 3°C	clear	nd	nd	nd	0.02%	nd	0.01%	nd	0.11%	0.22%	99.52%	0.02%	0.02%	0.05%	0.01%	0.01%
28 days 5 ± 3°C	clear	nd	nd	nd	0.03%	nd	nd	nd	0.11%	0.21%	99.55%	0.03%	nd	0.07%	nd	0.01%
35 days 5 ± 3°C	clear	nd	nd	nd	0.03%	0.01%	nd	nd	0.12%	0.21%	99.50%	0.03%	0.01%	0.05%	0.02%	0.01%
42 days 5 ± 3°C	clear	nd	nd	nd	0.04%	nd	nd	nd	0.12%	0.22%	99.51%	0.03%	0.01%	0.05%	0.01%	0.01%
49 days 5 ± 3°C	clear	nd	nd	nd	0.02%	nd	nd	nd	0.12%	0.22%	99.53%	0.03%	0.01%	0.05%	0.01%	0.02%
56 days 5 ± 3°C	clear	nd	nd	nd	0.01%	nd	nd	nd	0.12%	0.23%	99.53%	0.03%	0.01%	0.05%	0.02%	0.01%
63 days 5 ± 3°C	clear	nd	nd	nd	0.03%	nd	nd	nd	0.12%	0.22%	99.50%	0.03%	0.01%	0.05%	0.02%	0.02%
70 days 5 ± 3°C	clear	nd	nd	nd	0.01%	nd	nd	nd	0.12%	0.23%	99.52%	0.03%	0.01%	0.05%	0.02%	0.02%
90 days 5 ± 3°C	clear	nd	nd	nd	0.01%	0.01%	nd	nd	0.11%	0.21%	99.53%	0.03%	0.01%	0.06%	nd	0.03%

Table 5Ab Related substances; Stability at 25 °C/60%RH of solution of 200mg CAP 7.1 in 4.0 ml Polysorbate 80 / Ethanol up to 90 days

	Visual examination	Intensity of peaks by Area%													
#F347 **20 g/ 400 g**	Rel-/ absolute Retention Time	0.22/4.4	0.31/6.41	0.51/10.43	0.54/10.59 (Etoposide)	0.80/16.6	0.86/17.32	0.93/18.6	0.95/18.95	0.97/19.45	**CAP 7.1** 20.8	1.1/22.14	1.11/22.36	1.16/23.24	1.17/23.68	1.19/23.89
initial	clear	nd	nd	nd	0.02%	nd	nd	nd	0.12%	0.22%	99.55%	0.03%	0.01%	0.05%	nd	0.01%
7 days 25°C/60%rh	clear	nd	nd	nd	nd	nd	nd	nd	0.12%	0.22%	99.57%	0.03%	0.01%	0.05%	nd	0.01%
14 days 25°C/60%rh	clear	nd	nd	nd	nd	nd	nd	nd	0.12%	0.22%	99.56%	0.03%	0.01%	0.05%	nd	0.01%
24 days 25°C/60%rh	clear	nd	nd	nd	0.03%	0.01%	0.01%	nd	0.11%	0.23%	99.50%	0.03%	0.01%	0.05%	0.01%	0.01%
28 days 25°C/60%rh	clear	nd	nd	nd	0.01%	nd	nd	nd	0.10%	0.33%	99.45%	0.03%	0.01%	0.05%	0.01%	0.01%
35 days 25°C/60%rh	clear	nd	nd	nd	0.02%	0.01%	nd	nd	0.11%	0.24%	99.50%	0.03%	0.01%	0.05%	0.02%	0.01%
42 days 25°C/60%rh	clear	nd	nd	nd	0.04%	0.03%	nd	nd	0.12%	0.23%	99.44%	0.03%	0.01%	0.05%	0.04%	0.02%
49 days 25°C/60%rh	clear	nd	nd	nd	0.02%	0.01%	nd	nd	0.11%	0.24%	99.50%	0.03%	0.01%	0.05%	0.01%	0.01%
56 days 25°C/60%rh	clear	nd	nd	nd	0.02%	nd	nd	nd	0.12%	0.25%	99.50%	0.03%	0.01%	0.05%	0.01%	0.01%
63 days 25°C/60%rh	clear	nd	nd	nd	0.04%	0.02%	nd	nd	0.10%	0.25%	99.48%	0.02%	nd	0.05%	0.03%	0.02%
70 days 25°C/60%rh	clear	nd	nd	nd	0.04%	0.01%	nd	nd	0.11%	0.25%	99.47%	0.03%	0.01%	0.05%	0.01%	0.02%
90 days 25°C/60%rh	clear	nd	nd	nd	0.02%	0.02%	nd	nd	0.10%	0.20%	99.49%	0.03%	0.01%	0.05%	nd	0.03%

Table 5Ac Related substances; Stability at 30 °C/65%RH of solution of 200mg CAP 7.1 in 4.0 ml Polysorbate 80 / Ethanol up to 90 days

	Visual examination	Intensity of peaks by Area%														
#F347	Rel-/ absolute Retention Time	0.22/4.4	0.31/6.41	0.51/10.43	0.54/10.59	0.80/16.6	0.86/17.32	0.93/18.6	0.95/18.95	0.97/19.45	CAP 7.1	1.1/22.14	1.11/22.36	1.16/23.24	1.17/23.68	1.19/23.89
20 g/ 400 g					(Etoposide)						20.8					
initial	clear	nd	nd	nd	0.02%	nd	nd	nd	0.12%	0.22%	99.55%	0.03%	0.01%	0.05%	nd	0.01%
7 days 30°C/65%rh	clear	nd	nd	nd	nd	nd	nd	nd	0.12%	0.22%	99.55%	0.03%	0.01%	0.05%	nd	0.02%
14 days 30°C/65%rh	clear	nd	nd	nd	nd	nd	nd	nd	0.08%	0.23%	99.58%	0.03%	0.01%	0.05%	nd	0.01%
24 days 30°C/65%rh	clear	nd	nd	nd	0.03%	0.01%	0.01%	nd	0.11%	0.25%	99.49%	0.03%	0.01%	0.05%	0.01%	0.01%
28 days 30°C/65%rh	clear	nd	nd	nd	0.03%	0.01%	0.01%	nd	0.11%	0.23%	99.50%	0.03%	0.01%	0.05%	0.01%	0.01%
35 days 30°C/65%rh	clear	nd	nd	nd	0.04%	0.01%	0.01%	nd	0.10%	0.26%	99.48%	0.03%	0.01%	0.05%	0.01%	0.01%
42 days 30°C/65%rh	clear	nd	nd	nd	0.05%	0.03%	0.01%	nd	0.12%	0.26%	99.41%	0.03%	nd	0.05%	0.03%	0.03%
49 days 30°C/65%rh	clear	nd	nd	nd	0.04%	0.01%	nd	nd	0.11%	0.27%	99.46%	0.03%	0.01%	0.05%	0.01%	0.01%
56 days 30°C/65%rh	clear	nd	nd	nd	0.03%	0.01%	nd	nd	0.11%	0.27%	99.46%	0.03%	nd	0.05%	0.01%	0.01%
63 days 30°C/65%rh	clear	nd	nd	nd	0.04%	0.03%	nd	nd	0.11%	0.29%	99.41%	0.03%	nd	0.05%	0.02%	0.02%
70 days 30°C/65%rh	clear	nd	nd	nd	0.04%	0.03%	nd	nd	0.11%	0.30%	99.42%	0.03%	0.01%	0.04%	0.01%	0.02%
90 days 30°C/65%rh	clear	nd	nd	nd	0.06%	nd	nd	nd	0.11%	0.29%	99.40%	0.03%	nd	0.05%	nd	0.04%

Table 5A d **Related substances; Stability at 40 °C/75%RH of solution of 200mg CAP 7.1 in 4.0 ml Polysorbate 80 / Ethanol up to 90 days**

	Visual examination	Intensity of peaks by Area%												
#F347 20 g/ 400 g	Rel-/ absolute Retention Time	0.22/4.4	0.31/6.41	0.51/10.43	0.54/10.59 (Etoposide)	0.82/16.35	0.86/17.31	0.93/18.6	0.95/18.95	0.97/19.45	CAP 7.1 20.8	1.1/22.14	1.11/22.36	1.16/23.24	1.17/23.68	1.19/23.89
initial	clear	nd	nd	nd	0.02%	nd	nd	nd	0.12%	0.22%	99.55%	0.03%	0.01%	0.05%	nd	0.01%
7 days 40°C/75%rh	clear	nd	nd	nd	nd	nd	nd	nd	0.11%	0.24%	99.56%	0.03%	0.01%	0.04%	nd	0.01%
14 days 40°C/75%rh	clear	nd	nd	nd	nd	nd	nd	nd	0.08%	0.27%	99.55%	0.03%	0.01%	0.05%	nd	0.01%
24 days 40°C/75%rh	clear	nd	nd	nd	0.04%	0.02%	0.01	nd	0.10%	0.30%	99.42%	0.04%	0.00%	0.04%	0.01%	0.01%
28 days 40°C/75%rh	clear	nd	nd	nd	0.02%	0.00%	0.01%	nd	0.11%	0.18%	99.54%	0.03%	0.01%	0.06%	0.04%	0.01%
35 days 40°C/75%rh	clear	nd	nd	nd	0.07%	0.04%	nd	nd	0.10%	0.35%	99.31%	0.03%	nd	0.06%	0.01%	0.02%
42 days 40°C/75%rh	clear	nd	nd	nd	0.03%	0.05%	nd	nd	0.10%	0.37%	99.35%	0.03%	nd	0.04%	0.01%	0.02%
49 days 40°C/75%rh	clear	nd	nd	nd	0.09%	0.06%	nd	nd	0.10%	0.41%	99.23%	0.03%	nd	0.04%	0.02%	0.04%
56 days 40°C/75%rh	clear	nd	nd	nd	0.05%	0.06%	nd	nd	0.10%	0.44%	99.23%	0.03%	nd	0.05%	0.01%	0.03%
63 days 40°C/75%rh	clear	nd	nd	nd	0.10%	0.03%	nd	nd	0.09%	0.47%	99.20%	0.02%	nd	0.04%	0.02%	0.03%
75 days 40°C/75%rh	clear	nd	nd	nd	0.05%	0.06%	nd	nd	0.07%	0.51%	99.19%	0.03%	nd	0.04%	0.02%	0.03%
90 days 40°C/75%rh	clear	nd	nd	nd	0.09%	0.07%	nd	nd	0.10%	0.56%	99.06%	0.02%	nd	0.05%	nd	0.05%

Table 6A:

Sample dilution	Chemical Stability Rel. area% of CAP 7.1 peak			Physical Stability Sub visible particles Counts/ml [≥ 10µm / ≥ 25µm]	
	Initial	After 5 hours	After 24 hours	initial	After 24 hours
Water for injection	99.48 % Etoposide 0.06 %	99.39 % Etoposide 0.17%	99.30 % Etoposide 0.27%	2/0	2/0
0.45% NaCl solution	99.53 % Etoposide 0.04 %	99.38 % Etoposide 0.19 %	99.27 % Etoposide 0.30 %	2/0	2/0
0.9% NaCl solution	99.58 % Etoposide 0.04 %	99.37 % Etoposide 0.20 %	99.23 % Etoposide 0.33 %	2/0	1/0
5% Glucose solution	99.52 % Etoposide 0.04 %	99.41 % Etoposide 0.15 %	99.30 % Etoposide 0.27 %	3/0	1/0

Table 7A:

Sample description	Storage condition	Initial value Assay [Area%]	Assay after irradiation [Area%]
#1901001	unwrapped	99.20	99.18
#1901001	in cardboard box		99.19
#1901001	Wrapped in aluminium foil		99.18

Table 8A:

	Visual examination	Intensity of peaks [Area%]																
#L1901001	Rel-/ absolute Retention Time	0.25/ 9.25	0.76/ 28.12	0.77/ 28.39	0.89/ 32.75	0.92/ 33.85	0.95/ 35.01	0.96/ 35.16	0.97/ 35.52	0.98/ 36.12	**CAP 7.1 36.8 min**	1.01/ 37.23	1.03/ 37.76	1.04/ 38.41	1.06/ 39.17	1.10/ 40.47	1.12/ 41.19	1.16/ 42.53	1.20/ 44.27
			(Etopos/t)															
initial	clear	nd	0.02%	nd %	0.01%	0.01%	0.01%	0.01%	0.07%	0.23%	99.20%	0.34%	nd	0.03%	0.01%	0.06%	nd	nd	nd
After 1.2 million Luxh unwrapped	clear	nd	0.03%	nd %	nd %	nd %	0.01%	0.02%	0.08%	0.22%	99.18%	0.32%	0.01%	0.03%	0.01%	0.06%	0.01%	nd	0.02%
After 1.2 million Luxh Al-foil wrapped	clear	nd	0.03%	nd %	nd %	nd %	0.01%	0.02%	0.08%	0.22%	99.18%	0.33%	0.01%	0.03%	0.01%	0.05%	0.01%	nd	0.02%
After 1.2 million Luxh Card box	clear	nd	0.02%	nd %	nd %	nd %	0.01%	0.02%	0.08%	0.22%	99.19	0.33%	0.01%	0.03%	0.01%	0.05%	0.01%	nd	0.02%

Table 9A:

Retention Time / Storage time	RRT:0,51 RT:10,43	RRT: 0,54 RT: 10,89 (Etoposide)	RRT: 0,95 RT: 19,13	RRT: 0,97 RT: 19,67	CAP 7.1 RT: 20,25	RRT: 1,10 RT: 22,32	RRT: 1,15 RT: 23,46	RRT: 1,19 RT: 24,11
initial	nd	0,10 %	0,10 %	0,48 %	99,21 %	0,05 %	0,04 %	0,03 %
5 days	0,09 %	0,17 %	0,09 %	0,67 %	98,83 %	0,04 %	0,07 %	0,04 %
12 days	0,09 %	0,22 %	0,09 %	0,87 %	98,58 %	0,03 %	0,07 %	0,04 %
17 days	0,17 %	0,33 %	0,10 %	1,10 %	98,18 %	0,04 %	0,04 %	0,04 %

Example 6

Etoposide toniribate as API was known to be poorly soluble in water, and unstable at acidic and basic pH. To date, the API has been formulated by dissolving it in Cremophor RH40 and ethanol. However, this formulation is cumbersome to use and lacks scalability. Experiments were undertaken to develop a new formulation of etoposide toniribate that is stable at refrigerator temperature (2-8°C) and compatible with acceptable infusion diluents, such that the infusion solution is stable for at least 2-3 hours at room temperature (assessed at controlled room temperature of 20-25°C, 60% relative humidity (RH)). It is also desirable for the infusion solution to have an osmolality close to blood osmolality (280-295 mOsm/Kg).

Test 1

A 5 ml solution was prepared according to the following amounts:

Composition (5 ml)	
CAP 7.1	100 mg
PEG 300	3.250 mg
Polysorbate 80	400 mg
Ethanol	1.51 ml
Benzyl alcohol	0.30 ml

The order of addition of the materials was as follows: CAP 7.1, ethanol, benzyl alcohol, PEG 300 and polysorbate 80. Initially stirred at 700 rpm until the addition of PEG 300 when the speed was increased to 1000 rpm for 1 hour. The formulation was stored in a refrigerator (2-8°C) for 72 hours.

RESULTS: The samples were observed after 72 hours in the refrigerator. No precipitate occurred.

Test 2 (Batch PFD019/01)

The formulation was prepared according to the following:

	Composition/10 ml
CAP 7.1	200 mg
PEG 300	0.800 g
Polysorbate 80	6.500 g
Ethanol	2.42 g
Benzyl alcohol	0.31 g

The order of addition and the manufacturing process were the same as in the previous test: CAP 7.1, ethanol, benzyl alcohol, PEG 300 and polysorbate 80. Initially stirred at 700 rpm until the addition of PEG 300 when the speed was increased to 1000 rpm for 1 hour. The formulation was stored in a refrigerator (2-8°C) for 72 hours.

RESULTS: The samples were observed after 72 hours in the refrigerator. No precipitate occurred. The formulation had a pH of 7.8.

By analysis, a concentration of 20.0 mg/ml (theoretical 20 mg/ml) was obtained after verification of the analytical method, evaluating linearity and recovery in the range 0.1 – 0.0001 mg/ml.

Test 3

The formulation was prepared according to the following:

	Composition/40 ml
CAP 7.1	400 mg
PEG 300	400 mg
Polysorbate 80	3.2 g
Ethanol	9.7 g
Benzyl alcohol	1.25 g

The order of addition of the materials was as follows: CAP 7.1, ethanol, benzyl alcohol, PEG 300 and polysorbate 80. Initially stirred at 700 rpm until the addition of PEG 300 when the speed was increased to 1000 rpm for 1 hour.

Preparation of 0.9% saline solution:

NaCl	4.5g
Water for injection	500 ml

Dissolve NaCl in the water for injection. Stir at 910 rpm until dissolved. Add the previously prepared CAP 7.1 solution to the saline solution. Stirring continued at 910 rpm for 5 minutes.

From analysis of the saline solution, a concentration of 0.82 mg/ml was obtained. A final pH of the solution of 7.23 was obtained.

RESULTS: Below is a summary table describing the appearance of the CAP 7.1 solution in saline stored at 2-8°C.

Day	**Day 1**					**Day 2**	**Day 3**
Time	13:30	15:00	16:00	17:00	18:00	8:00	7:00
Preparation Time	0	1 h 30 min	2 h 30 min	3 h 30 min	4 h 30 min	18 h 30 min	41 h 30 min
Appearance*	C	C	C	C	C	C	C

(*) Appearance clear solution with no particles. It will be Compliant (C) if this criterion is met, or Non-Compliant (NC) if it is not met.

It can be concluded that the appearance of the CAP 7.1 solution was stable in 0.9% saline at least up to 41 hours.

Test 4

The formulation was prepared according to the following:

	Composition/ 10ml
CAP 7.1	100 mg
PEG 300	6.50 g
Polysorbate 80	0.80 g

Dehydrate alcohol	2.42 g
Benzyl alcohol	0.31 g

A batch size of 1,450 ml was made, and vials filled with a volume of 10 ml.

The order of addition of the materials was as follows: CAP 7.1, ethanol, benzyl alcohol, PEG 300 and polysorbate 80. Initially stirred at 700 rpm until the addition of PEG 300 when the speed was increased to 1000 rpm for 1 hour. A final pH of the solution of 7.26 and a density of 1.017 g/ml were obtained.

RESULTS: The results of this study are shown in Tables 1B-3B below. From the results obtained for this pre-stability (I) of CAP 7.1 solution for injection, it was concluded that it was not stable at room temperature (20-25°C/60% RH). The impurity Cis-CAP 7.1 increases at 2 months from 0.18% to 1.14%, while unknown impurities (rrt: 0.28 and rrt: 0.95) remained constant, so it was decided to stop the pre-stabilities at room temperature at the 2M time point.

However, the formulation was stable under refrigerator conditions (2-8ºC). The degradation of the CAP 7.1 solution was lower, since the impurity Cis-CAP 7.1 only increased from 0.18% to 0.65% at the 6M time point.

Table 1B:

ASSAY								
Time	Concentration (mg/mL)							
Bulk (t = 0)	10.02							
	Temperature 2-8 °C/N_2		Temperature 2-8 °C/O_2		Temperature 25 °C/N_2		Temperature 25 °C/O_2	
	V	I	V	I	V	I	V	I
	C (mg/mL) / % RSD	C (mg/mL) / % RSD	C (mg/mL) / % RSD	C (mg/mL) / % RSD	C (mg/mL) / % RSD	C (mg/mL) / % RSD	C (mg/mL) / % RSD	C (mg/mL) / % RSD
1 week	9,95 / 0,4	9,84 / 4,3	9,91 / 0,1	9,91 / 0,5	9,96 / 0,6	9,67 / 2,6	9,94 / 0,9	9,85 / 0,6
2 weeks	9,71 / 0,2	9,70 / 0,9	9,80 / 1,1	9,67 / 0,0	9,67 / 0,4	9,55 / 2,3	9,66 / 0,0	9,71 / 0,3
3 weeks	10,16 / 0,4	10,16 / 0,4	10,03 / 0,8	10,15 / 0,1	10,06 / 1,1	10,03 / 0,8	10,05 / 0,8	10,02 / 0,2
5 weeks	9,96 / 0,3	10,05 / 0,7	10.03 / 0.3	10,00 / 0,2	10,00 / 0,4	9,98 / 0,9	9,88 / 0,2	9,94 / 0,1
1.5 months	10,16 / 0,9	10.13 / 0.2	9.96 / 0.7	10.10 / 0.1	9.97 / 0.3	10.03 / 0.4	9.98 / 0.6	9.95 / 0.7
2 months	9.93 / 0.3	9.89 / 1.7	9.98 / 0.4	9.82 / 1.7	9.59 / -	9.83 / 1.2	9.89 / 0.6	9.76 / 2.1
3 months	10.07 / 0.6	-	10.06 / 0.9	-	-	-	-	-
4 months	-	-	-	9.86 / 0.2	-	-	-	-
6 months	-	9.87 / 0.2	-	-	-	-	-	-

Table 2Ba (SSRR):

Time	Impurity	RRT	% Impurity
Bulk (t = 0)	Impurity Cis-CAP7.1	0.98	0.18
	Unknown impurity	0.28	0.12
	Unknown impurity	0.95	<LOQ
	Totals		0.3

Table 2Bb (SSRR):

		Temperature 2-8 °C/N2						Temperature 2-8 °C/O2					
		V			I			V			I		
		Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
1 week		Impurity Cis-CAP7.1	0.98	0.21	Impurity Cis-CAP7.1	0.98	0.21	Impurity Cis-CAP7.1	0.98	0.22	Impurity Cis-CAP7.1	0.98	0.2
		Unknown impurity	0.29	0.12	Unknown impurity	0.29	0.11	Unknown impurity	0.29	<LOQ	Unknown impurity	0.29	<LOQ
		Unknown impurity	0.95	0.1	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	0.1
		Totals		0.43	Totals		0.32	Totals		0.22	Totals		0.3
		Temperature 25 °C/N2						Temperature 25 °C/O2					
		V			I			V			I		
		Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
		Impurity Cis-CAP7.1	0.98	0.34	Impurity Cis-CAP7.1	0.98	0.36	Impurity Cis-CAP7.1	0.98	0.33	Impurity Cis-CAP7.1	0.98	0.33
		Unknown impurity	0.29	0.11	Unknown impurity	0.29	0.1	Unknown impurity	0.29	0.12	Unknown impurity	0.29	<LOQ
		Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	0.1	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ
		Totals		0.45	Totals		0.56	Totals		0.45	Totals		0.33
2 weeks		Temperature 2-8 °C/N2						Temperature 2-8 °C/O2					
		V			I			V			I		
		Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
		Impurity Cis-CAP7.1	0.98	0.25	Impurity Cis-CAP7.1	0.98	0.27	Impurity Cis-CAP7.1	0.98	0.27	Impurity Cis-CAP7.1	0.98	0.25
		Unknown impurity	0.28	<LOQ	Unknown impurity	0.28	<LOQ	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.1
		Unknown impurity	0.95	0.11	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ
		Totals		0.36	Totals		0.27	Totals		0.39	Totals		0.35

Table 2Bb (cont):

		Temperature 25 °C/N2						Temperature 25 °C/O2					
		V			I			V			I		
		Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
3 weeks		Impurity Cis-CAP7.1	0.98	0.5	Impurity Cis-CAP7.1	0.98	0.49	Impurity Cis-CAP7.1	0.98	0.51	Impurity Cis-CAP7.1	0.98	0.49
		Unknown impurity	0.28	0.1	Unknown impurity	0.28	0.11	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.11
		Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ
	Temperature 2-8 °C/N2							Temperature 2-8 °C/O2					
		V			I			V			I		
		Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
		Impurity Cis-CAP7.1	0.98	0.25	Impurity Cis-CAP7.1	0.98	0.27	Impurity Cis-CAP7.1	0.98	0.27	Impurity Cis-CAP7.1	0.98	0.28
		Unknown impurity	0.28	0.14	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.17	Unknown impurity	0.28	0.14
		Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	0.1	Unknown impurity	0.95	0.12	Unknown impurity	0.95	<LOQ
		Totals		0.39	Totals		0.49	Totals		0.56	Totals		0.42
	Temperature 25 °C/N2							Temperature 25 °C/O2					
		V			I			V			I		
		Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
		Impurity Cis-CAP7.1	0.98	0.61	Impurity Cis-CAP7.1	0.98	0.63	Impurity Cis-CAP7.1	0.98	0.62	Impurity Cis-CAP7.1	0.98	0.61
		Unknown impurity	0.28	0.14	Unknown impurity	0.28	0.15	Unknown impurity	0.28	0.16	Unknown impurity	0.28	0.16
		Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ

Table 2Bb (cont):

5 weeks	Temperature 2-8 °C/N_2						Temperature 2-8 °C/O_2						
	V			I			V			I			
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
	Impurity Cis-CAP7.1	0.98	0.31	Impurity Cis-CAP7.1	0.98	0.3	Impurity Cis-CAP7.1	0.98	0.27	Impurity Cis-CAP7.1	0.98	0.28
	Unknown impurity	0.28	0.13	Unknown impurity	0.28	0.14	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.12
	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	0.1	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ
	Totals		0.44	Totals		0.54	Totals		0.39	Totals		0.4
	Temperature 25 °C/N_2						Temperature 25 °C/O_2						
	V			I			V			I			
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
	Impurity Cis-CAP7.1	0.98	0.85	Impurity Cis-CAP7.1	0.98	0.83	Impurity Cis-CAP7.1	0.98	0.8	Impurity Cis-CAP7.1	0.98	0.81
	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.11	Unknown impurity	0.28	0.13	Unknown impurity	0.28	0.15
	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ
1.5 months	Temperature 2-8 °C/N_2						Temperature 2-8 °C/O_2						
	V			I			V			I			
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
	Impurity Cis-CAP7.1	0.98	0.33	Impurity Cis-CAP7.1	0.98	0.31	Impurity Cis-CAP7.1	0.98	0.32	Impurity Cis-CAP7.1	0.98	0.32
	Unknown impurity	0.28	0.1	Unknown impurity	0.28	0.1	Unknown impurity	0.28	0.13	Unknown impurity	0.28	0.11
	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	0.12	Unknown impurity	0.95	0.1
	Totals		0.43	Totals		0.41	Totals		0.57	Totals		0.53

Table 2Bb (cont):

	Temperature 25 °C/N2						Temperature 25 °C/O2						
	V			I			V			I			
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
	Impurity Cis-CAP7.1	0.98	0.92	Impurity Cis-CAP7.1	0.98	0.90	Impurity Cis-CAP7.1	0.98	0.94	Impurity Cis-CAP7.1	0.98	0.95
	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.11	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.11
	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	<LOQ
2 months	Temperature 2-8 °C/N2						Temperature 2-8 °C/O2						
	V			I			V			I			
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
	Impurity Cis-CAP7.1	0.98	0.33	Impurity Cis-CAP7.1	0.98	0.32	Impurity Cis-CAP7.1	0.98	0.34	Impurity Cis-CAP7.1	0.98	0.35
	Unknown impurity	0.28	0.11	Unknown impurity	0.28	0.11	Unknown impurity	0.28	0.13	Unknown impurity	0.28	0.12
	Unknown impurity	0.95	0.1	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	0.11	Unknown impurity	0.95	<LOQ
	Totals	–	0.54	Totals	–	0.43	Totals	–	0.58	Totals	–	0.47
	Temperature 25 °C/N2						Temperature 25 °C/O2						
	V			I			V			I			
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
	Impurity Cis-CAP7.1	0.98	1.14	Impurity Cis-CAP7.1	0.98	1.19	Impurity Cis-CAP7.1	0.98	1.19	Impurity Cis-CAP7.1	0.98	1.23
	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.13	Unknown impurity	0.28	0.12	Unknown impurity	0.28	0.15
	Unknown impurity	0.95	0.11	Unknown impurity	0.95	LOQ	Unknown impurity	0.95	<LOQ	Unknown impurity	0.95	0.11

Table 2Bb (cont):

	Temperature 2-8 °C/N_2						Temperature 2-8 °C/O_2					
	V			I			V			I		
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
3 months	Impurity Cis-CAP7.1	0.98	0.39	-	-	-	Impurity Cis-CAP7.1	0.98	0.37	-	-	-
	Unknown impurity	0.28	0.13	-	-	-	Unknown impurity	0.28	0.14	-	-	-
	Unknown impurity	0.95	0.1	-	-	-	Unknown impurity	0.95	0.09	-	-	-
	Totals	-	0.63	-	-	-	Totals	-	0.6	-	-	-
	Temperature 2-8 °C/N2						Temperature 2-8 °C/O2					
	V			I			V			I		
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
4 months	Impurity Cis-CAP7.1	-	-	-	-	-	Impurity Cis-CAP7.1	-	-	Impurity Cis-CAP7.1	0.98	0.41
	Unknown impurity	-	-	-	-	-	Unknown impurity	-	-	Unknown impurity	0.28	0.16
	Unknown impurity	-	-	-	-	-	Unknown impurity	-	-	Unknown impurity	0.95	0.11
	Totals	-	-	-	-	-	Totals	-	-	Totals	-	0.68
	Temperature 2-8 °C/N2						Temperature 2-8 °C/O2					
	V			I			V			I		
	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity	Impurity	RRT	% Impurity
6 months	Impurity Cis-CAP7.1	-	-	Impurity Cis-CAP7.1	0.98	0.65	Impurity Cis-CAP7.1	-	-	Impurity Cis-CAP7.1	-	-
	Unknown impurity	-	-	Unknown impurity	0.28	0.17	Unknown impurity	-	-	Unknown impurity	-	-
	Unknown impurity	-	-	Unknown impurity	0.95	0.11	Unknown impurity	-	-	Unknown impurity	-	-
	Totals	-	-	Totals	-	0.93	Totals	-	-	Totals	-	-

Table 3B (pH):

Time	pH									
Bulk (t = 0)	7.26									
	Temperature 2-8 °C/N_2			Temperature 2-8 °C/O_2			Temperature 25 °C/N_2		Temperature 25 °C/O_2	
	V		I	V		I	V	I	V	I
	pH		pH	pH		pH	pH	pH	pH	pH
1 week	7.53		7.54	7.56		7.59	7.32	7.54	7.70	7.48
2 weeks	7.80		7.68	7.81		7.60	7.70	7.68	7.23	7.66
3 weeks	7.70		7.52	7.69		7.72	7.69	7.70	7.79	7.69
5 weeks	8.02		8.03	8.01		8.02	7.90	7.95	8.01	8.01
1 month 1/2	7.63		7.85	7.59		7.60	7.88	7.64	7.65	7.50
2 months	7.86		7.73	7.86		7.78	7.62	7.60	7.98	7.49
3 months	7.46		-	7.88		-	-	-	-	-
4 months	-		-	-		7.99	-	-	-	-
6 months	-		7.77	-		-	-	-	-	-

Test 5

The objective was to determine the stability of the formulation dissolved in 5% glucose solution for perfusion. The formulation solution was prepared with a concentration of 10 mg/ml as set out below and vials filled with a volume of 40 ml/vial. The formulation was subsequently dissolved in 500 ml of 5% glucose solution to obtain an in bag concentration of CAP 7.1 of 0.8 mg/ml.

The formulation was prepared according to the following:

	Composition/ 40ml
CAP 7.1	400 mg
PEG 300	26 g
Polysorbate 80	3.2 g
Ethanol	9.7 g
Benzyl alcohol	1.258 g

The order of addition of the materials was as follows: CAP 7.1, ethanol, benzyl alcohol, PEG 300 and polysorbate 80. The mixture was stirred at 700 rpm until the addition of PEG 300 when the speed was increased to 1000 rpm for 1 hour.

5% glucose serum was prepared from 25g glucose and 500ml water for injection.

The previously prepared CAP 7.1 solution was added to the 5% glucose solution to obtain a CAP7.1 concentration of 0.8 mg/ml. Stirring continued at 910 rpm for 5 minutes.

Once the test was finished, the solution was kept at room temperature (20-25°C/60% RH) and the appearance of the solution checked for 24 hours.

RESULTS: Below is a summary table showing the appearance of the CAP 7.1 solution in 5% glucose solution.

Day	**Day 1**						**Day 2**			
Time	**17:00**	**18:00**	**19:00**	**20:00**	**21:00**	**22:00**	**7:00**	**8:00**	**15:00**	**17:00**
Preparation time	0	1h	2h	3h	4h	5h	14h	15h	22h	24h

Appearance (*)	C	C	C	C	C	C	C	C	C	C

(*) Appearance clear solution with no particles. It will be Compliant (C) if this criterion is met, or Non-Compliant (NC) if it is not met.

It can be concluded that the CAP 7.1 solution is stable in 5% glucose solution for a period of 24 hours.

Test 6

The objective was to confirm stability by testing a new batch of formulation at two different fill volumes, and also at 3 different pH levels.

The conditions for this new pre-stability study were:

- Storage in a refrigerator (2-8°C)
- Atmosphere O_2
- Vertical position of the vial (Fluorotec® cap)
- pHs: 5-6, 6-7 and 7.3-7.8 (3 different formulations)
- Sampling time points: time 0, 1 week, 3 weeks, 6 weeks, 3 months and 5 months.

The formulation was prepared to provide 2000ml of solution as follows:

	Amounts weighed
CAP 7.1	20 g
PEG 300	1.3 g
Polysorbate 80	160 g
Ethanol	484 g
Benzyl alcohol	162 g

The order of addition of the materials was as follows: CAP 7.1, ethanol, benzyl alcohol, PEG 300 and polysorbate 80. Initially stirred at 700 rpm until the addition of PEG 300 when the speed was increased to 1000 rpm for 1 hour.

From this solution the following was taken:

- 760 ml and adjusted to pH 5-6 by adding 941 mg citric acid. The final pH of the solution was 5.8 and the appearance of the solution was cloudy.
- 450 ml and adjusted to pH 6-7 by adding 294 mg citric acid. The final pH of the solution was 6.5 and the appearance of the solution was transparent.

The vials for the 3 solutions have been filled at 10 ml for the pH's 5-6 and 6-7 and 20 ml for the pH's 5-6 and 7.3-7.8.

RESULTS: The results of this study are shown in Tables 4B-6B below. In summary:

1) In the 3 formulations, a new impurity occurred (rrt: 0.28) that remained constant over time. After further investigation, it was found that this impurity came from the benzyl alcohol solvent used and was unrelated to the stability of the formulation.

2) The filling volume did not affect the analytical level. The results obtained were of the same order regardless of the filling volume.

3) The formulation with a more acidic pH tended to have a lower increase in levels of the impurity cis-CAP7.1. At the 5M time point, 0.42% was obtained with the 7.3-7.8 formulation, whereas at pH 5-6 and 6-7, it was 0.22-0.24%

4) Purity remained stable over time for the 3 formulations.

5) In the stability in use study, it can be concluded that the solution was stable for 24 h at room temperature (20-25°C/60% RH) for the two concentrations of 0.7 and 2.9 mg/ml.

The results obtained from the osmolality test performed with an infusion of 0.9% saline solution was 1040 mOsm/kg. It is preferable for infusion solutions to be closer to blood osmolality.

Table 4B:

Time	Concentration (mg/mL)		
	pH 7.3 – 7.8	pH 6 – 7	pH 5 – 6
bulk (zero)	10.55	10.98	11.73
1 week, dosage 10 mL	-	11.02	11.78
1 week, dosage 20 mL	10.63	-	11.77
3 weeks, dosage 10 mL	-	11.34	11.94
3 weeks, dosage 20 mL	10.82	-	11.87
6 weeks, dosage 10 mL	-	11.21	11.9
6 weeks, dosage 20 mL	10.83	-	11.92
3 months, dosage 10 mL	-	11.04	11.77
3 months, dosage 20 mL	10.53	-	11.71
5 months, dosage 10 mL	-	10.88	11.26
5 months, dosage 20 mL	10.47	-	11.72

Table 5B (SSRR):

Bulk (t = 0)	pH 7.3 – 7.8			pH 6-7		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.23**	Impurity Cis-CAP7.1	0.98	**0.18**
	Unknown impurity	0.28	**0.53**	Unknown impurity	0.28	**0.59**
	Unknown impurity	0.95	**0.11**	Unknown impurity	0.95	**0.09**
	Totals	-	**0.87**	Totals	-	**0.86**
	pH 5 - 6 (dosage 10 mL)			pH 5 - 6 (dosage 20 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.2**	Impurity Cis-CAP7.1	-	-
	Unknown impurity	0.28	**0.55**	Unknown impurity	-	-
	Unknown impurity	0.95	**0.10**	Unknown impurity	-	-
	Totals	-	**0.85**	Totals	-	-

1 week	pH 7.3 – 7.8			pH 6-7		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.21**	Impurity Cis-CAP7.1	0.98	**0.18**
	Unknown impurity	0.28	**0.60**	Unknown impurity	0.28	**0.56**
	Unknown impurity	0.95	**0.10**	Unknown impurity	0.95	**0.11**
	Totals	-	**0.91**	Totals	-	**0.85**
	pH 5 - 6 (dosage 10 mL)			pH 5 - 6 (dosage 20 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.22**	Impurity Cis-CAP7.1	0.98	**0.22**
	Unknown impurity	0.28	**0.60**	Unknown impurity	0.28	**0.57**
	Unknown impurity	0.95	**0.10**	Unknown impurity	0.95	**0.10**
	Totals	-	**0.92**	Totals	-	**0.89**

Table 5B (cont):

3 weeks	pH 7.3 – 7.8			pH 6-7		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.24**	Impurity Cis-CAP7.1	0.98	**0.25**
	Unknown impurity	0.28	**0.55**	Unknown impurity	0.28	**0.56**
	Unknown impurity	0.95	**0.10**	Unknown impurity	0.95	**0.09**
	Totals	-	**0.89**	Totals	-	**0.9**
	pH 5 - 6 (dosage 10 mL)			pH 5 - 6 (dosage 20 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.21**	Impurity Cis-CAP7.1	0.98	**0.22**
	Unknown impurity	0.28	**0.52**	Unknown impurity	0.28	**0.53**
	Unknown impurity	0.95	**0.10**	Unknown impurity	0.95	**0.09**
	Totals	-	**0.83**	Totals	-	**0.85**

6 weeks	pH 7.3 – 7.8			pH 6-7		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.25**	Impurity Cis-CAP7.1	0.98	**0.17**
	Unknown impurity	0.28	**0.58**	Unknown impurity	0.28	**0.57**
	Unknown impurity	0.95	**0.09**	Unknown impurity	0.95	**0.11**
	Totals	-	**0.92**	Totals	-	**0.85**
	pH 5 - 6 (dosage 10 mL)			pH 5 - 6 (dosage 20 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.17**	Impurity Cis-CAP7.1	0.98	**0.2**
	Unknown impurity	0.28	**0.56**	Unknown impurity	0.28	**0.53**
	Unknown impurity	0.95	**0.10**	Unknown impurity	0.95	**0.10**
	Totals	-	**0.83**	Totals	-	**0.83**

Table 5B (cont):

3 months	pH 7.3 - 7.8 (dosage 20 mL)			pH 6-7 (dosage10 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.34**	Impurity Cis-CAP7.1	0.98	**0.23**
	Unknown impurity	0.28	**0.55**	Unknown impurity	0.28	**0.52**
	Unknown impurity	0.95	**0.08**	Unknown impurity	0.95	**0.09**
	Totals	-	**0.97**	Totals	-	**0.84**
	pH 5 - 6 (dosage 10 mL)			pH 5 - 6 (dosage 20 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.27**	Impurity Cis-CAP7.1	0.98	**0.25**
	Unknown impurity	0.28	**0.54**	Unknown impurity	0.28	**0.51**
	Unknown impurity	0.95	**0.10**	Unknown impurity	0.95	**0.10**
	Totals	-	**0.91**	Totals	-	**0.86**

5 months	pH 7.3 - 7.8 (dosage 20 mL)			pH 6-7 (dosage10 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.42**	Impurity Cis-CAP7.1	0.98	**0.24**
	Unknown impurity	0.28	**0.58**	Unknown impurity	0.28	**0.57**
	Unknown impurity	0.95	**0.08**	Unknown impurity	0.95	**0.08**
	Totals	-	**1.08**	Totals	-	**0.89**
	pH 5 - 6 (dosage 10 mL)			pH 5 - 6 (dosage 20 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.24**	Impurity Cis-CAP7.1	0.98	**0.22**
	Unknown impurity	0.28	**0.59**	Unknown impurity	0.28	**0.59**
	Unknown impurity	0.95	**0.09**	Unknown impurity	0.95	**0.07**
	Totals	-	**0.91**	Totals	-	**0.88**

Note: Concentration LOQ = 0.1%

Table 6B (pH):

	Temperature 2-8 °C		
Time	**pH 7.3 – 7.8**	**pH 5 – 6**	**pH 6 – 7**
Bulk (t = 0)	7.27	5.2	6.38
1 week	NP	NP	NP
3 weeks	7.89	6.22 (10 ml) / 6.06 (20 ml)	7.03
6 weeks	7.73	6.22 (10 ml) / 6.06 (20 ml)	6.82
3 months	7.94	6.21 (10 ml) / 5.94 (20 ml)	6.72
5 months	7.80	6.11 (10 ml) / 6.27 (20 ml)	6.94

Test 7

To confirm the results of Test 6, a new batch of formulation was prepared at a concentration of 10 mg/ml. The testing conditions were:

- Storage in a refrigerator (2-8°C).
- Atmosphere O_2
- Vertical position of the vial (Fluorotec® cap)
- pHs: 5-6, 6-7 and 7.3-7.8.
- Sampling frequency: time 0, 2 months, 3 months and 5 months.

Osmolality at the concentration of 0.8 mg/ml with saline solution and water was also studied.

A total batch of 1,300 ml of solution was prepared according to the following formulation:

	Amounts weighed
CAP 7.1	13.0 g
PEG 300	845 g
Polysorbate 80	104 g
Ethanol	315 g
Benzyl alcohol	40.6 g

The order of addition of the materials was as follows: CAP 7.1, ethanol, benzyl alcohol, PEG 300 and polysorbate 80. Initially stirred at 700 rpm until the addition of PEG 300 when the speed was increased to 1000 rpm for 1 hour.

The initial pH of the solution was 8.08. The following were taken from the total solution:

- 443.8 g and adjusted to a pH of 7-8 by adding 90 mg of citric acid. The final pH obtained was 7.45.
- 443.0 g and adjusted to a pH of 6-7 by adding 191 mg of citric acid. The final pH obtained was 6.45.
- 443.0 g and adjusted to a pH of 5-6 by adding 409 mg of citric acid. The final pH obtained was 5.74.

The 3 solutions at different pH were filled to 10 ml.

RESULTS: The results of the test are set out in Tables 7B-9B below. In summary:

1) Regarding impurity cis-CAP 7.1, it was observed that its increase for the 3 formulations was similar and did not exceed 0.3% after the 5M stability test.
2) The unknown impurity of rrt: 0.95 was stable over time and remained at <0.1%.

The results of the osmolality test were:

- Water infusion solution and a concentration of 0.8 mg/ml: 565 mOsm/Kg.
- Infusion solution 0.9% NaCl and a concentration of 0.8 mg/ml: 924 mOsm/Kg

It can be seen that the osmolality that most closely resembles blood is a water infusion solution.

Table 7B:

Time	Concentration (mg/mL)		
	pH 7-8	pH 6 – 7	pH 5 – 6
bulk (zero)	**10.87**	**11.13**	**11.77**
2 months	**10.72**	**10.57**	**11.37**
3 months	**10.48**	**11.02**	**11.52**
5 months	**10.85**	**10.93**	**11.56**

Table 8B (SSRR):

	pH 7-8 (dosage10 mL)			pH 6-7 (dosage10 mL)			pH 5 - 6 (dosage 10 mL)		
Bulk (t = 0)	**Impurity**	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.97	**0.19**	Impurity Cis-CAP7.1	0.97	**0.16**	Impurity Cis-CAP7.1	0.97	**0.22**
	Totals	-	**0.29**	Totals	-	**0.25**	Totals	-	**0.31**
2 months	pH 7-8 (dosage10 mL)			pH 6-7 (dosage10 mL)			pH 5 - 6 (dosage 10 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.30**	Impurity Cis-CAP7.1	0.97	**0.26**	Impurity Cis-CAP7.1	0.97	**0.22**
	Unknown impurity	0.95	**0.09**	Unknown impurity	0.95	**0.10**	Unknown impurity	0.95	**0.10**
	Totals	-	**0.39**	Totals	-	**0.36**	Totals	-	**0.32**
3 months	pH 7-8 (dosage10 mL)			pH 6-7 (dosage10 mL)			pH 5 - 6 (dosage 10 mL)		
	Impurity	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.30**	Impurity Cis-CAP7.1	0.98	**0.25**	Impurity Cis-CAP7.1	0.97	**0.26**
	Unknown impurity	0.95	**0.09**	Unknown impurity	0.95	**0.09**	Unknown impurity	0.95	**0.09**
	Totals	-	**0.39**	Totals	-	**0.34**	Totals	-	**0.35**

	pH 7-8 (dosage10 mL)			pH 6-7 (dosage10 mL)			pH 5 - 6 (dosage 10 mL)		
5 months	**Impurity**	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**	**Impurity**	**RRT**	**% Impurity**
	Impurity Cis-CAP7.1	0.98	**0.27**	Impurity Cis-CAP7.1	0.98	**0.24**	Impurity Cis-CAP7.1	0.97	**0.23**
	Unknown impurity	0.95	**0.08**	Unknown impurity	0.95	**0.08**	Unknown impurity	0.94	**0.09**
	Totals	-	**0.35**	Totals	-	**0.32**	Totals	-	**0.32**

Note: Concentration LOQ = 0.1%

Table 9B (pH):

	Temperature 2-8 °C		
Time	pH 7.3 – 7.8	pH 5 – 6	pH 6 – 7
Bulk (t = 0)	7.45	5.74	6.45
2 months	7.46	6.35	7.12
3 months	7.71	6.47	7.32
5 months	7.74	6.51	7.39

Test 8

The objective was to assess the osmolality and stability at RT for 24 hr of a WFI infusion solution at 0.5mg/ml final concentration of CAP7.1.

A 100ml formulation was prepared according to the following:

	Amounts weighed
CAP 7.1	1.0 g
PEG 300	65.0 g
Polysorbate 80	8.0 g
Ethanol	24.2 g
Benzyl alcohol	3.12 g

The formulation was diluted in water for injection to provide 800ml of an infusion solution at 0.5 mg/ml. A pH of 7.91 was obtained.

RESULTS: The osmolality test was performed at a water infusion volume of 800 ml. The result was 392 mOsm/Kg, meaning the infusion solution exhibited an osmolality close to blood osmolality.

In the stability study in use, it is concluded that the solution was stable for 24 hours at room temperature (20-25°C/60% RH) for the concentration of 0.5 mg/ml.

Conclusions

From the tests carried out and described above, it can be concluded that:

A solution formula of CAP 7.1 with a concentration of 10 mg/ml was established. The formula is detailed as follows:

	Composition/ml
CAP 7.1	10.00 mg
PEG 300	650.0 mg
Polysorbate 80	80.00 mg
Ethanol	241.60 mg
Benzyl alcohol	31.20 mg

The optimum pHs for the solution are neutral pH (7.3-7.8) and pH 6-7. To achieve pH 6-7, citric acid must be added.

At pH 5-6 it was observed that the batch solution became cloudy; however, when the solution was filled into a vial, this appearance could pass unnoticed.

Optimal storage conditions for the solution were at 2-8°C (refrigerator conditions) and in an O_2 atmosphere.

Fluorotec® cap did not affect the solution, so it will be used to close the vials.

The benzyl alcohol used in the formulation should be kept under refrigerator conditions (2-8°C) and under a N_2 atmosphere.

The optimum conditions for perfusion according to the osmolality results obtained are a dose of 400 mg of API and a concentration in water of 0.5 mg/ml.

a polysorbate;

Ethanol;

Benzyl alcohol.

5

CLAIMS

1. **A liquid pharmaceutical formulation** comprising:
etoposide toniribate;
a polysorbate; and
ethanol.

2. The liquid pharmaceutical formulation of claim 1, comprising etoposide toniribate at a concentration in the range of from 50 mg/ml to 100 mg/ml.

3. The liquid pharmaceutical formulation of claim 1 or claim 2, wherein the polysorbate is polysorbate 80.

4. The liquid pharmaceutical formulation of any preceding claim wherein the polysorbate concentration is in the range of from 600 mg/ml to 800 mg/ml.

5. The liquid pharmaceutical formulation of any preceding claim wherein the polysorbate concentration is 750 mg/ml.

6. The liquid pharmaceutical formulation of any preceding claim wherein the ethanol concentration is in the range of from 200 mg/ml to 300 mg/ml.

7. The liquid pharmaceutical formulation of any preceding claim wherein the ethanol concentration is 250 mg/ml.

8. The liquid pharmaceutical formulation of any preceding claim, wherein the liquid pharmaceutical formulation comprises:
50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml Ethanol.

9. The liquid pharmaceutical formulation of any one of claims 1-7, wherein the liquid pharmaceutical formulation comprises:
91 mg/ml Etoposide toniribate;
631 mg/ml Polysorbate 80; and
252 mg/ml Ethanol.

10. The liquid pharmaceutical formulation of any one of claims 1-7, wherein the liquid pharmaceutical formulation consists, or consists essentially of:
50 mg/ml Etoposide toniribate;
750 mg/ml Polysorbate 80; and
250 mg/ml Ethanol.

11. The liquid pharmaceutical formulation of any one of claims 1-7, wherein the liquid pharmaceutical formulation consists, or consists essentially of:
91 mg/ml Etoposide toniribate;
631 mg/ml Polysorbate 80; and
252 mg/ml Ethanol.

12. The liquid pharmaceutical formulation of any preceding claim, wherein the liquid pharmaceutical formulation has a pH in the range of from pH 3 to 4.

13. The liquid pharmaceutical formulation of any preceding claim, wherein the liquid pharmaceutical formulation has a pH of 3.7.

14. **A method of preparing an infusion solution** comprising diluting the liquid pharmaceutical formulation according to any one of claims 1-13 in a diluent.

15. **An infusion solution** prepared by diluting a pharmaceutical formulation according to any of claims 1-13 in a diluent.

16. **An infusion solution** comprising the liquid pharmaceutical formulation according to any one of claims 1-13 and a diluent.

17. The method according to claim 14 or the infusion solution according to claim 15 or 16, wherein the diluent is selected from: water for injection; 5% glucose solution; 0.45% NaCl saline, and 0.9% NaCl saline.

18. The method or an infusion solution according to claim 17, wherein the diluent is water for injection.

19. The method or the infusion solution according to any one of claims 14-18, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 1 mg/ml to 10 mg/ml.

20. The method or the infusion solution according to any one of claims 14-19, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 1 mg/ml to 5 mg/ml.

21. The method or the infusion solution according to any one of claims 14-20, wherein the etoposide toniribate concentration in the infusion solution is 3.1 mg/ml.

22. **A kit** comprising: the liquid pharmaceutical formulation of any one of claims 1-13; and a diluent.

23. The kit of claim 22, wherein the diluent is selected from: water for injection; 5% glucose solution; 0.45% NaCl saline, and 0.9% NaCl saline.

24. The liquid pharmaceutical formulation according to any one of claims 1-13 or the infusion solution according to any one of claims 15-21, for use in therapy.

25. The liquid pharmaceutical formulation according to any one of claims 1-13 or the infusion solution according to any one of claims 15-21, for use in a method of treating cancer.

26. The liquid pharmaceutical formulation according to any one of claims 1-13 or the infusion solution according to any one of claims 15-21, for use in a method of treating biliary tract cancer

27. **A method of treating cancer in a patient in need thereof**, the method comprising administering to the patient an effective amount of the liquid pharmaceutical formulation according to any one of claims 1-13, or the infusion solution according to any one of claims 15-21.

28. **A liquid pharmaceutical formulation** comprising:
Etoposide toniribate;
PEG;

a polysorbate;
Ethanol;
Benzyl alcohol.

29. The liquid pharmaceutical formulation of claim 28, comprising etoposide toniribate at a concentration in the range of from 10 mg/ml to 20 mg/ml, optionally at a concentration of 10 mg/ml or 20mg/ml.

30. The liquid pharmaceutical formulation of claim 28 or claim 29, wherein the polysorbate is polysorbate 80.

31. The liquid pharmaceutical formulation of any one of claims 28 to 30, wherein the PEG is PEG 200-600.

32. The liquid pharmaceutical formulation of any one of claims 28 to 31, wherein the PEG is PEG 200-400.

33. The liquid pharmaceutical formulation of any one of claims 28 to 32, wherein the PEG is PEG 300.

34. The liquid pharmaceutical formulation of any one of claims 28 to 33, wherein the liquid pharmaceutical formulation has a pH in the range of from pH 5.0 to 7.8.

35. The liquid pharmaceutical formulation of any one of claims 28-34, wherein the liquid pharmaceutical formulation has a pH in the range of from 7.3 to 7.8.

36. The liquid pharmaceutical formulation of any one of claims 28-24, wherein the liquid pharmaceutical formulation has a pH in the range of from 6.0 to 7.0.

37. The liquid pharmaceutical formulation of any one of claims 28-34, wherein the liquid pharmaceutical formulation has a pH in the range of from 5.0 to 6.0.

38. The liquid pharmaceutical formulation of any one of claims 28 to 37, wherein the liquid pharmaceutical formulation further comprises citric acid.

39. The liquid pharmaceutical formulation of any one of claims 28 to 38, wherein the liquid pharmaceutical formulation comprises:
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

40. The liquid pharmaceutical formulation of any one of claims 28 to 38, wherein the liquid pharmaceutical formulation comprises:
20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml dehydrated ethanol; and
31 mg/ml Benzyl alcohol.

41. The liquid pharmaceutical formulation of any one of claims 28 to 37, wherein the liquid pharmaceutical formulation consists of, or consists essentially of:
10 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml dehydrated ethanol; and
31 mg/ml Benzyl alcohol.

42. The liquid pharmaceutical formulation of any one of claims 28 to 37, wherein the liquid pharmaceutical formulation consists of, or consists essentially of:
20 mg/ml Etoposide toniribate;
650 mg/ml PEG 300;
80 mg/ml Polysorbate 80;
242 mg/ml Ethanol; and
31 mg/ml Benzyl alcohol.

43. **A method of preparing an infusion solution** comprising diluting the liquid pharmaceutical formulation according to any one of claims 28-42 in a diluent.

44. **An infusion solution** prepared by diluting the liquid pharmaceutical formulation according to any of claims 28-42 in a diluent.

45. **An infusion solution** comprising the liquid pharmaceutical formulation according to any one of claims 28-42 and a diluent.

46. The method according to claim 43 or the infusion solution according to claim 44 or 45, wherein the diluent is selected from: water for injection; 5% glucose solution; and 0.9% NaCl saline.

47. The method or the infusion solution according to claim 46, wherein the diluent is water for injection.

48. The method or the infusion solution according to any one of claims 43-47, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 0.1 mg/ml to 1 mg/ml.

49. The method or the infusion solution according to any one of claims 43-48, wherein the etoposide toniribate concentration in the infusion solution is in the range of from 0.5 mg/ml to 0.8 mg/ml.

50. The method or the infusion solution according to any one of claims 43-49, wherein the etoposide toniribate concentration in the infusion solution is 0.5 mg/ml or 0.8 mg/ml.

51. **A kit** comprising: the liquid pharmaceutical formulation of any one of claims 28-42; and a diluent.

52. The kit of claim 51, wherein the diluent is selected from: water for injection; 5% glucose solution; and 0.9% NaCl saline.

53. The liquid pharmaceutical formulation according to any one of claims 28-42, or an infusion solution according to any one of claims 43-45, for use in therapy.

54. The liquid pharmaceutical formulation according to any one of claims 28-42 or the infusion solution according to any one of claims 43-50, for use in a method of treating cancer.

55. The liquid pharmaceutical formulation according to any one of claims 28-42 or the infusion solution according to any one of claims 43-50, for use in a method of treating biliary tract cancer

56. **A method of treating cancer in a patient in need thereof**, the method comprising administering to the patient an effective amount of the liquid pharmaceutical formulation according to any one of claims 28-42, or the infusion solution according to any one of claims 43-50.