FORM 2

THE PATENTS ACT, 1970
(39 of 1970)

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THE PATENT RULES, 2003

COMPLETE SPECIFICATION
(See Section 10; rule 13)

LYOPHILIZED CENTHAQUINE CITRATE INJECTION FORMULATION AND A METHOD FOR THE SAME

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The following specification particularly describes the invention and the manner in which it is to be performed:

FIELD OF INVENTION

The present invention relates to a stable lyophilized injectable formulation comprising Centhaquine or its water–soluble salts which can be administered via intravenous route and is suitable for therapeutic use in managing hypovolemic shock.

BACKGROUND OF INVENTION

Centhaquine citrate is a white crystalline powder with empirical formula $C_{28}H_{33}N_3O_7$ and a molecular weight of 523.58. The chemical name of Centhaquine citrate is 2–[2–[4–(3–methylphenyl)–1–piperazinyl] ethyl] quinoline citrate ((Figure 1).Centhaquine as a free base is insoluble in water and may not be suitable for developing a formulation for intravenous use, while citrate salt is water soluble.

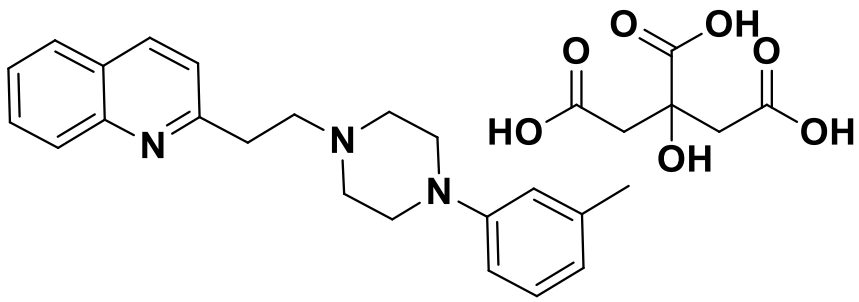

Figure 1: Chemical structure of Centhaquine citrate

The particle size of the “Centhaquine citrate” can considerably affect its solubility during manufacturing of its lyophilized injectable formulations. The particle size of Centhaquine citrate ranges from 5 to 500 µm. Specifically, 10% of the particles are within the range of 5-8 µm, 50% of the particles fall within the range of 100 to 250 µm, and 90% of the particles range from 200 to 500 µm.

The Centhaquine free base is a crystalline anhydrate that is non-hygroscopic. Centhaquine salt is developed and has different polymorphic forms wherein type A is mono-MeOH (Methanol) solvate and can deliquesce when exposed to humidity higher than 75%RH, whereas type B is a non-hygroscopic anhydrate. A stable crystal form was identified in aqueous (with low, medium, and high-water activity), and in an organic system, thermodynamic solid form screening was performed under different solvent systems including acetone: water; DMSO (Dimethylsulfoxide): Water; MeOH; IPAc (Isopropyl Acetate); Acetone. Based on the thermodynamic polymorph screening and results type A is MeOH solvate, and it was only obtained from the MeOH system; whereas type B is an anhydrate and obtained from mono-MeOH systems. The critical solvent activity was conducted in different systems including either from the following system MeOH/H_2O; MeOH/IPAc; MeOH/IPAc/H_2O and MeOH/Acetone at RT and 60°C. The anhydrate type B is more stable when MeOH activity is equal to or smaller than 0.2 at RT and 0.4 at 60°C. To improve the solubility, several temperature points were selected and 50°C was chosen for the final first-run crystallization temperature; to improve the yield, several scale batches were set, and the anhydrate phase was obtained with approximate solubility greater than 100 mg/mL.

The research studies showed that lower doses of Centhaquine increases the blood pressure in animals experiencing blood loss. This prompted a further investigation which demonstrated that Centhaquine is indeed a highly effective resuscitative agent for hypovolemic shock. A formulation of Centhaquine that is freely water soluble is highly desirable for its use as a resuscitative agent in hypovolemic shock (Reniguntala et al., 2015).

Centhaquine possesses a distinctive mode of action in comparison to other agents used for resuscitation. It exerts its effects through the stimulation of 2B adrenergic receptors, thereby inducing venous constriction, which enhances the return of blood from the venous circulation to the heart. This increase in blood in the heart causes more blood to be pumped out, due to an increase in ventricular contraction.

Furthermore, Centhaquine acts on 2A adrenergic receptors, leading to a reduction in sympathetic drive, and reduces the heart rate, allowing the heart to fill up with more blood. Besides, Centhaquine does not exhibit beta-adrenergic agonist activity, which mitigates the risk of cardiac arrhythmias (Gulati et al., 2019 and 2021). Centhaquine is a promising therapeutic agent for patient resuscitation, as it can convert the venous unstressed blood volume to stressed blood volume, thereby enhancing cardiac output, and improving blood circulation in hypovolemic shock. This mechanism of action renders Centhaquine a suitable candidate for improving hemodynamic status in critically ill patients.

The lyophilized Centhaquine citrate injection 1.0 mg should be administered at a dose of 0.01 mg/kg body weight as an intravenous infusion over 1 hour in 100 mL normal saline in patients of hypovolemic shock. The next dose of Centhaquine injection should be administered if systolic blood pressure falls below or remains below 90 mmHg, but not before 4 hours of the previous dose and the total number of doses per day should not exceed 3 doses. Centhaquine injection administration, if needed, may continue for two days subject to a maximum of 6 doses within the first 48 hrs of treatment.

The regulatory authorities enforce strict criteria for ensuring the quality of pharmaceutical products. Manufacturers are required to provide evidence that their product is almost free of impurities, that any impurities are within admissible limits, and that these standards can be replicated for every batch of an injectable pharmaceutical product. To determine the safety and efficacy of active pharmaceutical ingredients (API) or pharmaceutical compositions, several tests are required, including assays for purity, testing for related substances, testing for content uniformity, osmolarity testing, and moisture content testing. The assay test is used to determine the purity of the test product in comparison to a standard of known purity. The related substances test is used to quantify all the impurities present in the product. Lastly, the content uniformity test is conducted to ensure that batches of injectable products contain a uniform amount of drug or API. The

preferred approach for assessing the API or pharmaceutical formulation or composition is often by High Performance Liquid Chromatography (HPLC).

Reniguntala et al. synthesized and characterized the citrate salt of Centhaquine, determined its purity by HPLC and evaluated its effects on various cardiovascular parameters in anesthetized male Sprague–Dawley rats. The results showed that centhaquine citrate had greater cardiovascular activity than centhaquine free base, as evidenced by a greater decrease in mean arterial pressure, pulse pressure, heart rate, cardiac output, stroke volume, and stroke work. The study concludes that centhaquine citrate may be a more effective cardiovascular agent than Centhaquine (Reniguntala et al., 2015).

The safety and tolerability of Centhaquine was evaluated in a phase I clinical study (CTRI/2014/06/004647; NCT02408731) using a double-blind, randomized, and placebo-controlled approach. The study involved single and multiple ascending doses and demonstrated that healthy male volunteers tolerated the drug well without any serious adverse events. Some non–serious adverse events occurred at 10 to 15 folds higher doses than the therapeutic dose (0.01 mg/kg), including hypotension, high lactic acid, fall in respiratory rate, dryness of mouth, and drowsiness, but they were temporary and resolved without any intervention (Gulati et al., 2016; Gulati et al., 2020).

Gulati et al. (2021) conducted a clinical trial (CTRI/2019/01/017196, NCT04045327) to evaluate the safety and efficacy of centhaquine in patients with hypovolemic shock. The study enrolled patients with hypovolemic shock. The demographics of patients and baseline vitals were comparable between the control and Centhaquine groups. The study found that resuscitation with Centhaquine resulted in a significantly greater number of patients with improved blood lactate and base deficit compared to the control group. The use of vasopressors was lower in the Centhaquine group during the first 48 hours of resuscitation, and the stroke volume improved, as indicated by a significant increase in pulse pressure.

Additionally, the shock index was significantly lower in the Centhaquine group from 1 hour to 4 hours of resuscitation. The study showed that Centhaquine improved acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS), and an 8.8% absolute reduction in 28 day all-cause mortality was observed in the Centhaquine group. The study suggests that Centhaquine is an efficacious resuscitative agent for treating hypovolemic shock (Gulati et al., 2021).

OBJECTIVES OF THE INVENTION

The primary objective of the invention is to provide a method for producing an injectable composition comprising lyophilized Centhaquine citrate injection that can be reconstituted using diluent like normal saline (sodium chloride injection 0.9% w/v) or water for injection before administration.

Another objective is to formulate Centhaquine citrate in such a manner that it manifests high solubility characteristics in aqueous solutions, thereby rendering it suitable for parenteral administration.

An another objective of the present invention is to introduce an innovative approach to examine Centhaquine citrate, its impurities, and associated compounds without encountering the usual difficulties.

SUMMARY OF INVENTION

The present invention is directed to a stable and sterile pharmaceutical formulation that includes lyophilized Centhaquine citrate injection 1.0 mg. This formulation is composed of several ingredients, including Edetate Sodium (EDTA), Polysorbate 80 (Tween 80), Mannitol, Sodium Phosphate, Dibasic anhydrous, Sodium citrate dihydrate, Citric acid, and Water for injection (WFI). To develop a solution for medical use, the pharmaceutical formulation is dissolved in an aqueous medium.

The inclusion of various components in the formulation, such as EDTA and tween 80, acts as a solubilizer. Mannitol, a sugar alcohol, serves as a bulking agent, while anhydrous Sodium Phosphate Dibasic and Sodium citrate act as buffering agents and regulate the pH of the formulation. Citric acid is used to adjust the pH and also act as a preservative. The pharmaceutical formulation is thus carefully designed to provide maximum stability, efficacy and safety.

The lyophilized preparation may contain an appropriate quantity of Centhaquine, although it is desirable for it to contain a therapeutically effective dose of Centhaquine. Specifically, it is preferred that the lyophilized form of Centhaquine citrate be included in the preparation at a concentration of 1.0 mg. The Centhaquine in the lyophilized formulation is mainly in the form of Centhaquine citrate.

The invention provides methods for producing stable and sterile pharmaceutical products containing Centhaquine, specifically as a free base or in the salt form of citrate, acetate, hydrochloride as “Centhaquine citrate” or as “Centhaquine acetate” or as “Centhaquine hydrochloride” but not limited to these and their different polymorphic forms (Type A- mono-MeOH solvate, Type B anhydrate but not limited to these). The method for producing a stable, sterile pharmaceutical product containing lyophilized Centhaquine involves preparing a composition containing “Centhaquine citrate or Centhaquine acetate or Centhaquine hydrochloride” and lyophilizing it. The method for producing a stable, sterile pharmaceutical formulation containing lyophilized Centhaquine citrate involves preparing a liquid composition containing Centhaquine citrate and excipients in an aqueous solvent. This composition is then cooled and frozen at a temperature of about –40°C for at least 120 to 600 minutes. Afterward, the frozen mixture undergoes a primary drying stage, where a vacuum is applied to remove the aqueous solvent while changing the temperature to a primary drying temperature of –30°C to 25°C for at least 30 to 1320 minutes. Following the primary drying stage, the first intermediate undergoes a secondary drying stage where a vacuum is applied to remove the aqueous solvent

from the intermediate. The temperature is changed to a first secondary drying temperature of about 40°C for at least 30 minutes, followed by maintaining the intermediate at the same temperature for at least 180 minutes. The secondary drying process is then continued again at the same temperature for another 120 minutes. The lyophilization cycle took a total of about 93.5 hours to freeze–dry the composition, with primary drying taking about 76 hours and secondary drying taking about 5.5 hours to produce the lyophilized pharmaceutical formulation. Overall, this method provide a reliable and effective means for producing a stable and sterile pharmaceutical product containing Centhaquine citrate.

Additionally, the inventions also provide the method of preparation of lyophilized formulation, which enhances the stability of formulation and the method that will improve the solubility of Centhaquine citrate injection.

In addition, the invention encompasses a technique for treating a medical condition in a patient in need thereof. This approach involves administering the suitable therapeutic quantity of the aforesaid drug formulation to the patient after reconstitution in 10 mL of sodium chloride injection (0.9% w/v) or water for injection and then intravenous infusion over 1 hour in 100 ml of normal saline or any other fluids used for resuscitation like crystalloids, colloids, blood products but not limited to these.

Through research and experimentation, a novel and reproducible HPLC analytical method has been developed and validated for the analysis of Centhaquine citrate drug substance in its lyophilized formulation. The method is particularly useful for the identification and quantification of the drug substance (API) in drug product and related substances that emerge during the manufacturing process and during storage period. The innovative methodology contributes to the improvement of analytical capabilities for Centhaquine citrate analysis, which can lead to a better understanding of the drug’s behaviour, properties, and potential applications. This

method employs a mobile phase consisting of one or more liquids, and the proportions of these liquids are modified to a predetermined gradient.

An additional aspect of the invention involves a technique for evaluating the concentration of impurities in Centhaquine citrate samples or pharmaceutical dosage forms containing Centhaquine citrate. This technique involves analyzing the sample to detect the presence of any of the compounds (impurity) A and B described in the invention. Another aspect of the current invention offers a method to identify compounds (related substance or impurity) A and B by utilizing an HPLC technique to detect and analyze the impurities in Centhaquine citrate.

Additional apects of the invention involve the provision of two mobile phases, designated as Mobile Phase A and Mobile Phase B. Mobile Phase A is composed of 25 millimolar (mM) Potassium Dihydrogen Orthophosphate (KH_2PO_4) and includes a pH adjusting agent, either a 1 M solution of potassium hydroxide (KOH) or a diluted solution of orthophosphoric acid (OPA, 85%). The pH of Mobile Phase A is kept at a value of 3.0. The mobile phase B is chosen from a set of solvents including methanol, acetonitrile, isopropanol, propanol, or their mixtures. Among these, acetonitrile is the preferred option, as a substitute, one may opt for either methanol or a blend thereof.

The invention described involves an HPLC technique that uses a mobile phase with a gradient programming strategy for related substances. The program starts with a mobile phase consisting of mobile phase A and mobile phase B. Comprehensive details of the gradient program is described in the description.

In alternative aspects , the HPLC technique as per the present invention adeptly identifies and measures all impurities, including but not limited to compound A, namely 1–(3–methylphenyl) Piperazine, and compound B, specifically 2–Vinylquinoline, within a single analytical procedure.

The present invention entails a method of testing the purity of Centhaquine citrate, wherein the sample to be tested may consist of (a) Centhaquine API, (b) Centhaquine formulation, or (c) a salt form of Centhaquine or (d) a formulation of Centhaquine salt. Ideally, the sample tested using this method is of high grade to be incorporated into a pharmaceutical composition.

DETAILED DESCRIPTION OF THE INVENTION

A first embodiment of the present invention is a lyophilized sterile composition of Centhaquine citrate which after reconstitution with water for injection or 0.9% aqueous sodium chloride solution releases Centhaquine citrate to exert its therapeutic effects.

The terms “Pharmaceutical Dosage Forms” or “Dosage Form” refer to specific formulations or forms in which drugs are prepared and administered to patients for any therapeutic benefit. These forms can be classified based on the route of administration, such as intravenous, oral, topical, or inhalation. They can vary based on their physical state into liquid, solid, or semisolid.

The “stable pharmaceutical composition” as used here refers to a drug or lyophilized formulation that, when stored properly, preserves its physical, chemical, and therapeutic attributes for prolonged.

The present invention relates to a lyophilized Centhaquine citrate-based formulation for intravenous administration and method for preparation thereof that is advantageous for the treatment of hypovolemic shock.

According to an embodiment of the present invention, a method of preparation of a stable, sterile lyophilized Centhaquine citrate-based formulation for intravenous administration, comprising: (i) an active pharmaceutical ingredient in the range of 0.0004% to 1.0% w/w; (ii) at least five water-soluble excipients in the range of 0.0004 to 90% w/w; and water for injection.

In an embodiment, the invention comprises Centhaquine citrate as an active ingredient, and at least one water soluble excipient, but not limited to ethylene diamine tetra acetic acid – disodium (EDTA), polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, mannitol, citric acid monohydrate, where the soluble excipient serves the function to modify the pH, solubility and as a bulking agent.

The lyophilized pharmaceutical composition, wherein the Centhaquine citrate is present in the range of about 0.0004 to 1.0% w/w.

The lyophilized pharmaceutical composition, wherein the Ethylene diamine tetra acetic acid – disodium salt (EDTA) is present in the range of about 0.0004% to about 5.0% w/w.

The lyophilized pharmaceutical composition, wherein the polysorbate 80 is present in the range of about 1.0% to about 20.0% w/w.

The lyophilized pharmaceutical composition, wherein the Sodium Phosphate, Dibasic, anhydrous is present in the range of about 5.0% to about 50.0% w/w.

The lyophilized pharmaceutical composition, wherein the sodium citrate dihydrate is present in the range of about 1.0% to about 30.0% w/w.

The lyophilized pharmaceutical composition, wherein the mannitol is present in the range of about 20.0% to about 90.0% w/w.

The lyophilized pharmaceutical composition, wherein the Citric acid monohydrate is added in sufficient quantity to adjust (if required) the pH of the bulk solution in the rage between 7.5 to 8.5.

The lyophilized pharmaceutical composition, wherein Centhaquine citrate is present at about 1.0 mg; Ethylene diamine tetra acetic acid – disodium salt is present at about 1.0 mg; polysorbate 80 is present at about 21.4 mg (0.02 ml); sodium phosphate dibasic anhydrous is present at about 32.40 mg; sodium citrate dihydrate is present at about 6.5 mg and mannitol is present at about 150 mg.

The lyophilized pharmaceutical composition, wherein the mannitol is a freeze-drying filler and citric acid monohydrate is a pH adjusting agent.

The lyophilized pharmaceutical composition, wherein the composition further comprises sodium chloride.

The lyophilized pharmaceutical composition, wherein the composition further comprises water.

The lyophilized pharmaceutical composition comprising about 1.0 mg of Centhaquine citrate; about 1.0 mg of EDTA; about 21.4 mg of polysorbate 80; about 32.40 mg of sodium phosphate dibasic anhydrous; about 6.5 mg of sodium citrate dihydrate; about 150 mg of mannitol.

The lyophilized pharmaceutical composition, wherein the said composition comprises, total impurities not more than 3.0%; or any unspecified impurities not more than 0.5%; or 2–Vinyl quinoline impurity not more than 1.0%, and 1– (3–methylphenyl) Piperazine impurity not more than 1.5%.

The lyophilized pharmaceutical composition, wherein the said composition comprises: total impurities not more than 0.69%; or any unspecified impurities not more than 0.15%; or 2–Vinyl quinoline impurity not more than 0.03% and 1– (3–methylphenyl) Piperazine impurity not more than 0.46%.

In another embodiment, the invention provides a stable, sterile pharmaceutical formulation comprising lyophilized Centhaquine citrate injection, which after its reconstitution with sodium chloride (0.9%) solution or water for injection releases Centhaquine citrate in a therapeutically effective concentration.

The lyophilized pharmaceutical composition is reconstituted by 0.9% sodium chloride solution or water for injection.

A method of treating hypovolemic shock comprising administering the lyophilized pharmaceutical composition of Centhaquine citrate according to a subject in need thereof.

The method wherein the hypovolemic shock condition is selected from any patient with fall in blood pressure, or increased blood/plasma lactate levels due to loss of blood or body fluids.

The method wherein the lyophilized pharmaceutical composition is administered intravenously as a bolus dose or infusion.

The pharmaceutical formulation wherein the lyophilized formulation is stable at temperature 25 ºC ± 3 ºC and 60 % RH ± 5 % RH for not less than 12 months. The pharmaceutical formulation wherein the lyophilized formulation of Centhaquine citrate is stable at temperature 2–8°C, for not less than 24 months.

A reconstituted liquid composition comprising Centhaquine citrate, Ethylene diamine tetra acetic acid – disodium (EDTA), Polysorbate 80, Sodium Phosphate, Dibasic, anhydrous, Sodium citrate dihydrate, Mannitol, Water for injection or 0.9% aqueous sodium chloride solution.

The reconstituted liquid composition, wherein the Centhaquine citrate, EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol are provided as a lyophilized powder.

The reconstituted liquid composition, wherein the composition is prepared by reconstituting the lyophilized powder of Centhaquine citrate, EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol in water for injection or 0.9% aqueous sodium chloride solution.

The reconstituted liquid composition, wherein the Centhaquine citrate is present in the range from about 0.0004% to about 1.0% w/w; the other water soluble ingredients including EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol are present from about 0.0004% to about 90% w/w.

The reconstituted liquid composition, wherein Centhaquine citrate is present at about 1.0 mg; EDTA about 1.0 mg, polysorbate 80 about 21.4 mg; sodium phosphate dibasic anhydrous about 32.40 mg; sodium citrate dihydrate about 6.5 mg; mannitol is present at about 150 mg.

The reconstituted liquid composition, wherein Centhaquine citrate is present at 0.1mg /ml strength.

The reconstituted liquid composition, wherein the said composition comprises, total impurities not more than 3.0%; or any unspecified impurities not more than 0.5%; or 2–Vinyl quinoline impurity not more than 1.0% and 1– (3– methylphenyl) Piperazine impurity not more than 1.5%.

The reconstituted liquid composition, wherein the said composition comprises, total impurities not more than 0.69%; or any unspecified impurities not more than

0.15%; or 2–Vinyl quinoline impurity not more than 0.03% and 1– (3–methylphenyl) Piperazine impurity not more than 0.46%.

The reconstituted liquid composition, wherein the said reconstituted liquid composition has an osmolality between 200 to 350 mOsmol/L. The composition, wherein the said reconstituted liquid composition has an osmolality of about 307 mOsmol/L.

The composition, wherein the said reconstituted liquid composition has a pH of about 6.0 to 9.0.

A method of treating hypovolemic shock comprising administering the liquid pharmaceutical composition to a subject in need thereof.

The method wherein the hypovolemic shock condition is selected from any patient with fall in blood pressure, or increased blood/plasma lactate levels due to loss of blood or body fluids.

The lyophilized pharmaceutical formulation further optimally comprises ingredients listed in **Table** 1.

Table 1: Ingredients along with the composition used in the preparation of lyophilized Centhaquine citrate injection

Name of Ingredients	**Strength (mg/2.5 mL)**	**Qty/batch [gm/25.0 L]**
Centhaquine citrate	1.00	10.00
Ethylene diamine tetra acetic acid – disodium (EDTA)	1.00	10.00
Polysorbate 80	0.020 mL	214.00

Sodium Phosphate Dibasic Anhydrous	32.40	324.00
Sodium citrate dihydrate	6.50	65.00
Mannitol	150.0	1500.00
Citric acid monohydrate	Q.S.to adjust pH	Q.S.to adjust pH
Nitrogen	Q.S.	Q.S.
Water for Injection	Q.S to 2.5 mL	Q.S to 25 L

Table 2: Composition of In-process bulk solution and Lyophilized finished formulation of Centhaquine citrate injection 1.0 mg

S.No.	Name of Ingredients	Composition of in-process bulk solution of Centhaquine citrate injection (Per 2.5 mL)	Composition of Lyophilized Centhaquine citrate injection (Per Vial)
1.	Centhaquine citrate	1.00 mg	1.00 mg
2.	Ethylene diamine tetra acetic acid – disodium salt	1.00 mg	1.00 mg
3.	Polysorbate 80	0.020 mL	21.4 mg
4.	Sodium Phosphate, Dibasic, anhydrous	32.40 mg	32.40 mg
5.	Sodium citrate dihydrate	6.50 mg	6.50 mg
6.	Mannitol	150.0 mg	150.0 mg
7.	Citric acid monohydrate	Q.S.to adjust pH if required	Q.S.to adjust pH if required
8.	Water for Injection	Q.S to 2.5 mL	---

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Manufacturing Procedure

The manufacturing procedure for formulating a lyophilized injection of Centhaquine citrate includes the following steps:

Dispense API-Centhaquine citrate, excipients given in Table 1, and primary packing materials. Clean and sterilize filling components with water for injection and autoclave, and wash and sterilize vials in a tunnel sterilizer for depyrogenation.

- Compounding:

a) Collect 120% of the batch size i.e., 30 kg WFI (Considering 1.00 wt/mL) in a 30L manufacturing vessel. Cool the WFI and purge with sterile nitrogen (purging pressure 0.5 kg/cm²–1.0 kg/cm²) to achieve dissolved oxygen content of not more than (NMT) 2 PPM and temperature 15°C–20°C.

b) Keep 80% of batch size WFI in 30 L manufacturing tank and transfer the remaining WFI to the holding tank. This WFI will be used for rinsing, buffer preparation, and final volume makeup purposes.

c) After receiving the WFI release report, the temperature of WFI in the manufacturing vessel should be 15°C–20°C and the dissolved oxygen content of NMT 2 PPM.

d) Add the required quantity of Centhaquine citrate under continuous stirring in the vessel at 350–400 RPM and mix for 120–180 minutes or till the clear solution is obtained. Rinse the container with 100 mL of WFI and add it to the manufacturing tank. Check for clarity of the solution. Record the temperature (Target: 15°C–20°C), stirring speed, stirring time and dissolved oxygen (Target: NMT 2PPM).

e) Add the dispensed quantity of EDTA under continuous stirring in a 30 L manufacturing vessel at 350–400 RPM and mix for 10 to 15 minutes or till the clear solution is obtained. Rinse the polybag with 100 mL of WFI and add it to the manufacturing tank. Check for clarity of the solution. Record the temperature (Target: 15°C–20°C), stirring speed, stirring time, and dissolved

oxygen (Target: NMT 2PPM).

f) Add the dispensed quantity of Polysorbate 80 under continuous stirring in a 30 L manufacturing vessel at 350–400 RPM and mix for 15 to 20 minutes or till a clear solution is obtained. Rinse the container with 100 mL of WFI (3 times X100 mL) and add it to the manufacturing tank. Check for clarity of the solution. Record the temperature (Target: 15°C–20°C), stirring speed, stirring time, and dissolved oxygen (Target: NMT 2 PPM).

g) Add the dispensed quantity of Sodium Phosphate Dibasic Anhydrous under continuous stirring in a 30 L manufacturing vessel at 350–400 RPM and mix for 10 to 15 minutes or till a clear solution is obtained. Rinse the polybag with 100 mL of WFI and add it to the manufacturing tank. Check for clarity of the solution. Record the temperature (Target: 15°C–35°C), stirring speed, stirring time, and dissolved oxygen (Target: NMT 2 PPM).

h) Add the dispensed quantity of Sodium citrate dihydrate under continuous stirring in a 30 L manufacturing vessel at 350–400 RPM and mix for 15–20 min or till a clear solution is obtained. Rinse the polybag with 100 mL of WFI and add it to the manufacturing tank. Check for clarity of the solution. Record the temperature (Target: 15°C–35°C), stirring speed, stirring time, and dissolved oxygen (Target: NMT 2 PPM).

i) Add the dispensed quantity of Mannitol under continuous stirring in a 30 L. manufacturing vessel at 350–400 RPM and mix for 15 to 20 min or till a clear solution is obtained. Rinse the polybag with 100 mL of WFI and add it to the manufacturing tank. Check the appearance and clarity of the solution.

j) Measure the pH, and if required adjust the pH in the range of 7.5 –8.5 using 1M citric acid solution and stir the solution with a stirring speed of 350–400 RPM for 5–10 min, after each buffer addition.

k) Final volume Make–up 100 % i.e., 25.01/25.87 Kg (25.0 L X 1.035 wt/ml.) with the WFI at 20–35°C. Finally, stir the bulk solution at 350–400 RPM for 15 to 20 minutes and submit the sample for analysis. Control the temperature of the bulk solution at 20°C–35°C, Dissolved oxygen (NMT 2.0 PPM), check pH (7.5 to 8.5) and check the appearance (clear, light to pale yellow).

l) Cover the bulk solution under a nitrogen blanket with NMT 0.5kg/cm² and close the vessel until initiation for further processing and then proceed with the filtration process.

- **Filling and partial stoppering:** Perform the filling and flush the filled vials with pre and and post-purging with nitrogen. The minimum fill volume is 2.55 mL, the target fill volume is 2.60 mL, and the maximum fill volume is 2.65 mL. Once filled, the vials are partially stoppered and transferred to the lyophilizer through a mobile LAF and loaded into it.

- **Lyophilization:** Lyophilization after completion of loading the vials into the lyophilizer chamber, starts the lyophilization cycle.

Lyophilization

The invention also presents a technique for manufacturing a sterile and stable product, which includes lyophilized Centhaquine citrate injection. This process involves formulating a mixture of Centhaquine citrate-API and excipients in water for injection and then subjecting it to lyophilization, The approach encompasses a set of procedures:

a) Formulating a liquid mixture that consists of Centhaquine citrate and an aqueous solvent.
b) Precooling the composition or mixture to a temperature of approximately 5°C,
c) Freezing the mixture to a temperature of around -40°C to generate a frozen mixture and maintaining the freezing temperature for at least 120 minutes to at least 600 minutes.
d) To develop the first intermediate product, the frozen mixture undergoes a primary drying process, which involves reducing the pressure by applying a vacuum to eliminate the aqueous solvent while adjusting the temperature of the frozen mixture to a primary drying temperature between -30°C to

25°C. This primary drying temperature is maintained for a minimum of 30 minutes and up to 1320 minutes. The entire primary drying process carried out in multiple stages, continued for approximately 76 hours.

e) By implementing a secondary drying stage, the initial intermediate undergoes a process wherein a vacuum is applied to decrease the pressure to a level sufficient for eliminating any residual aqueous solvent from the intermediate.

   i. Altering the first intermediate temperature to a first secondary drying temperature of approximately 40°C, wherein keeping this temperature constant for no less than 30 minutes; and

   ii. The intermediate was maintained at the same temperature for at least 180 minutes.

   iii. The secondary drying process was resumed for another 120 minutes at the same temperature. The lyophilized pharmaceutical formulation was obtained after a total of 5.5 hours of secondary drying.

f) The composition was freeze–dried in a lyophilization cycle that persisted for about 93.5 hours. After the cycle was finished, the vacuum was released with Nitrogen gas filtered through a 0.2µ filter, and the vials were partially stoppered under a vacuum of less than 150 mbar. Then the vacuum was fully broken with sterile Nitrogen gas and the vials were unloaded from the lyophilizer.

Full stoppering Sealing Visual Inspection

After completion of the lyophilization, fully stopper the vials under a partial vacuum below 150 mBar. Then break the vacuum completely with sterile nitrogen gas and unload the vials from the lyophilizer. After completion of stoppering, unload the lyophilized vials and perform the vial sealing and carry out the inprocess checks like clarity and leak test of scaled vials. Perform the visual inspection and send the samples for finished product analysis.

A lyophilized Centhaquine-based injectable formulation, comprising, an active pharmaceutical ingredient-Centhaquine citrate in the range 0.0004% to about 1.0% w/w; the other water soluble ingredients including EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol in the rage of about 0.0004% to 90% w/w; and water for injection.

Labelling & Packing: Label each vial with a printed label and pack the vial as per packing specification.

Finished Product release specification.

Table 3 outlines the specifications that are required for the final product to meet the desired standards. It provides a detailed description of the necessary characteristics that must be present in the finished product. These specifications serve as guidelines for the manufacturing process and ensure that the final product meets the required quality standards.

Table 3. Finished Product release specification.

S. No:	Tests	Specifications
1.	Description	White to off–white lyophilized cake
2.	**Identification:**	
3.	A. By HPLC (UV)	The retention time of the Centhaquine citrate peak in the chromatogram of the test sample should correspond to that in the chromatogram of the reference standard preparation as obtained in the assay method.

	B. By HPLC (PDA)	The PDA spectrum of Centhaquine citrate in the test sample should correspond to that in the reference standard as obtained in the assay method.
4.	Reconstitution Time	Content should be dissolved within 3 minutes
5.	Clarity of reconstituted solution	The solid should dissolve completely and the solution should be visually clear.
6.	**Particulate matter: Average number of particles per container**	
	Particle ≥ 10 µm	Not more than 6000 particles/container
	Particle ≥ 25 µm	Not more than 600 particles/container
7.	pH	Between 6.0 –9.0
8.	Moisture Content (%)	Not more than 5.0%
9.	Osmolarity	Between 200 to 350 mOsmol/L
10.	Uniformity of dosage unit (By content uniformity)	Maximum acceptance value should be less than or equal to 15.0
11.	Assay By HPLC	Not less than 90.00 % and not more than 110.0% of the labeled amount of Centhaquine citrate.
12.	**Related substances by HPLC**	
	2–Vinyl quinoline	Not more than 1.0 %
	1– (3– methylphenyl) Piperazine	Not more than 1.5 %
	Unspecified Impurity	Not more than 0.5 %
	Total impurity	Not more than 3.0 %
13.	Bacterial Endotoxin test	Not more than 500 EU/mg
14.	Sterility testing	Should be sterile
15.	EDTA Content (%)	Not less than 80.0 % and not more than 120.0 % of a stated amount of EDTA

Primary packaging and secondary packaging

The primary packaging of lyophilized injectable products typically consists of sterile vials made of glass, which are sealed with a rubber stopper and an aluminum flip-off seal. This packaging is designed to maintain the sterility and stability of the product, protect it from external factors such as light and moisture, and allow for the convenient and safe administration of the drug. Lyophilized Centhaquine citrate injection vials can be packaged either separately or in combination with a diluent like normal saline (0.9% aqueous Sodium chloride injection), or water for injection.

A kit or a co-pack comprising a vial containing lyophilized composition of Centhaquine citrate and an ampoule containing 0.9% sodium chloride aqueous solution or water for injection. The vial of lyophilized composition of Centhaquine citrate injection with or without an ampoule of diluent solution is packed in a plastic tray and this plastic tray containing the vial of a pharmaceutical composition of lyophilized Centhaquine citrate injection with or without diluent ampoule is labeled and packaged in a carton. The lyophilized pharmaceutical composition of Centhaquine citrate injection comprises 1.0 mg of Centhaquine citrate; about 1.0 mg of EDTA; about 0.02 mL of polysorbate 80; about 32.40 mg of sodium phosphate dibasic anhydrous; about 6.5 mg of sodium citrate dihydrate and about 150 mg of mannitol. The pack or co-pack comprises single or multiple vials containing lyophilized pharmaceutical composition of Centhaquine citrate and single or multiple ampoules of 0.9% sodium chloride aqueous solution, or water for injection.

A pack comprising a vial containing a lyophilized pharmaceutical composition of Centhaquine citrate.

A kit or a co-pack comprising, a vial containing a lyophilized pharmaceutical composition of Centhaquine citrate; and an ampoule containing 0.9% sodium chloride aqueous solution or water for injection.

A pack, wherein the vial containing a lyophilized pharmaceutical composition of Centhaquine citrate comprises, about 1.0 mg of Centhaquine citrate, about 1.0 mg of Ethylene diamine tetra acetic acid – disodium (EDTA), about 21.4 mg of

polysorbate 80, about 32.40 mg of sodium phosphate dibasic anhydrous, about 6.5 mg of sodium citrate dihydrate, about 150 mg of mannitol.

The kit or co-pack, wherein the kit or co-pack comprises a single vial containing lyophilized pharmaceutical composition of Centhaquine citrate and a single ampoule/vial of 0.9% sodium chloride aqueous solution or water for injection.

The kit or co-pack, wherein the vial containing a lyophilized pharmaceutical composition of Centhaquine citrate comprises, about 1.0 mg of Centhaquine citrate, about 1.0 mg of Ethylene diamine tetra acetic acid – disodium (EDTA), about 21.4 mg of polysorbate 80, about 32.40 mg of sodium phosphate dibasic anhydrous, about 6.5 mg of sodium citrate dihydrate and about 150 mg of mannitol.

In the present invention, various forms of lyophilized Centhaquine citrate formulation, as well as various methods of administration, including intraperitoneal, intra-arterial and intravenous routes are disclosed. The invention covers lyophilized powdered form of Centhaquine citrate, liquid injections, infusion systems, and prefilled syringes (prefilled standard primary syringe and/or cartridge container with dual chamber), for the proper delivery of Centhaquine citrate.

The solution of sterile lyophilized Centhaquine citrate injection as an infusion in 500 mL and 1000 mL packing wherein the solution system in sterile infusion bag comprises but is not limited to normal saline (0.9% Sodium chloride injection), or water for injection, or dextrose solution, or Lactated Ringer's solution.

The packaging configurations as a convenient administration option considered include prefilled standard primary syringes of injection of Centhaquine citrate in a solution, including but not limited to normal saline, water for injection, dextrose solution, or Lactated Ringer's solution or lyophilized Centhaquine citrate in a cartridge container within a dual-chamber reconstitution system with integrated needle retraction mechanism, wherein the reconstitution system comprises but not

limited to normal saline (0.9% Sodium chloride injection), or water for injection, or dextrose solution, or Lactated Ringer's solution. Table 4 outlines the recommended packaging material for Centhaquine citrate.

Table 4. Primary Packaging material

S. No	Material Description
1.	10 mL Amber USP Type–I Glass Tubular vials
2.	20 mm slotted Grey Bromo butyl sterile rubber closure
3.	20 mm C/L Flip off Aluminum seal with plain white plastic

Storage stability of the lyophilized Centhaquine citrate injection

The lyophilized formulation of Centhaquine citrate injection was found to be stable for not less than 24 months when stored at 2°C – 8°C. The lyophilized formulation of Centhaquine citrate was found stable for not less than 12 months when stored at 25 ºC ± 2 ºC and 60 % RH ± 5 % RH.

Assay Method for Centhaquine citrate injection

Preparation of Mobile Phase A:

Accurately weigh and transfer about 0.770 gm of Ammonium acetate (10 mM) into 1000 mL of HPLC water and mix. Sonicate to dissolve and degas. Adjust the weight and dilution of ammonium acetate according to the requirement for analysis, keeping the final concentration the same.

Preparation of Mobile Phase B:

Prepare a suitable quantity of a mixture of acetonitrile and methanol in the ratio of 85:15 (% v/v) and mix. Sonicate and degas.

Chromatographic conditions (Assay)

The chromatographic conditions for assay were carefully set to ensure reliable and accurate results. An analytical column, specifically the Shim–pack was used. The flow rate was set to 1.0 mL/min, and a pump ratio of mobile phase A to mobile phase B was set at a 32:68 gradient. Overall, these specific parameters were chosen to ensure the separation and detection of the target analyte with high sensitivity and accuracy (**Table 5**).

Table 5. Chromatographic Conditions

Parameters	Condition
Analytical column	Shim–pack GIST C18, 5µm, (4.6 X 250) mm
Flow rate	1.0 mL/min
Pump ratio	Mobile Phase A: Mobile Phase B (32:68)
Injection volume	20 µL
Detection wavelength	UV at 239 nm
Autosampler Temperature	5°C
Column Oven Temperature	25°C
Run time	20 min
Retention time	9.0±1.0 min

Assay Method Validation Summary:

This analytical method validation contains the method, acceptance criteria, and results generated during the analytical method validation of Identification, Assay (In Process and finish Product), and Content Uniformity for Centhaquine citrate in

Lyophilized Centhaquine citrate injection 1.0 mg/vial. A summary of validation, consisting of a variety of parameters along with their corresponding acceptance criteria and outcomes, has been briefly presented in **Table 6**.

Table 6. Assay Method Validation Summary

Parameter	Acceptance criteria	Results Obtained
Specificity		
Interference	There should not be any interference from blank, Placebo, and impurity peaks at the Retention Time of the Centhaquine citrate peak.	There is no interference observed in blank placebo and impurity peaks at the retention time of Centhaquine citrate.
% Difference	The absolute difference between the mean assay results of the Unspiked (control) sample and spiked sample solution should be NMT 2.0	0.4
Peak Purity	Peak purity of the main peak shall pass in both spiked and unspiked samples. **Note**: In open lab CDS 2.4 software peak purity shall be not less than 990.	Complies.

Parameter	Acceptance criteria	Results Obtained	
Identification by HPLC: (UV/PDA detector)	The retention time of the Centhaquine citrate peak in the chromatogram of the test sample should correspond to that in the chromatogram of the reference standard preparation as obtained in the assay.	The retention times of Centhaquine citrate are comparable in standard and sample.	
Identification by HPLC: (PDA detector)	The PDA spectrum of Centhaquine citrate in the test sample solution should correspond to that in the reference standard as obtained in the assay method.	The spectrum of Centhaquine citrate in the test sample solution matched the spectrum of the standard solution.	
Assay (%)	Assay results of unspiked (control) and spiked samples should be Not less than 90.0 and not more than 110.0 of the labelled amounts of Centhaquine citrate.	**Unspiked sample**	**Spiked Sample**
		98.4	98.0
Forced degradation	Diluent peaks shall not interfere with the main peak.	There is no interference observed in blank and placebo peaks at the retention time of Centhaquine citrate.	
	The peak purity of the main peak shall pass in both the control and stressed sample solution.	Complies.	

<table>
<tr><th>Parameter</th><th>Acceptance criteria</th><th colspan="4">Results Obtained</th></tr>
<tr><td rowspan="2"></td><td>All known and unknown peaks should be resolved from the main analyte peak.</td><td colspan="4">Known and Unknown peaks do not interfere with the peak of interest and all peaks are well resolved from the main Analyte Peak.</td></tr>
<tr><td>Degradation should be between 5% and 20 % in at least one stress condition.</td><td colspan="4">Complies.</td></tr>
<tr><td rowspan="5">Linearity and range</td><td rowspan="2">The correlation coefficient and regression coefficient shall be NLT 0.999 and NLT 0.998 respectively.</td><td colspan="2">Correlation coefficient (r)</td><td colspan="2">Regression coefficient (r^2)</td></tr>
<tr><td colspan="2">1.000</td><td colspan="2">0.9999</td></tr>
<tr><td>The % Y intercept should be ± 2% of the Area Response at 100% concentration.</td><td colspan="4">0.9</td></tr>
<tr><td rowspan="2">% RSD for precision at lower and higher levels should be NMT 2.0.</td><td colspan="2">Linearity Lower Level</td><td colspan="2">Linearity Higher Level</td></tr>
<tr><td colspan="2">0.2</td><td colspan="2">0.1</td></tr>
<tr><td rowspan="11">Accuracy</td><td rowspan="11">The individual % recovery and mean % recovery should be NLT 98.0 and NMT 102.0.</td><td>SPL</td><td>50 %</td><td>100 %</td><td>150 %</td></tr>
<tr><td>SPL</td><td>99.3</td><td>99.2</td><td>98.9</td></tr>
<tr><td>SPL</td><td>99.3</td><td>98.9</td><td>98.4</td></tr>
<tr><td>SPL</td><td>98.8</td><td>99.2</td><td>98.4</td></tr>
<tr><td>SPL</td><td>98.8</td><td rowspan="3">NAP</td><td>98.2</td></tr>
<tr><td>SPL</td><td>98.8</td><td>98.2</td></tr>
<tr><td>SPL</td><td>98.5</td><td>98.2</td></tr>
<tr><td>AV</td><td>98.9</td><td>99.1</td><td>98.4</td></tr>
<tr><td>%</td><td>0.3</td><td>0.2</td><td>0.3</td></tr>
</table>

Parameter	Acceptance criteria	Results Obtained
Precision		
System Precision	The relative standard deviation of peak areas of Centhaquine citrate from six replicate injections of standard solution should not be more than 2.0%.	0.2
	The relative standard deviation of the peak Retention time of Centhaquine citrate from six replicate injections of standard solution should not be more than 1.0%.	0.7
Method Precision		
For Assay	The % RSD of Assay results for 6 replicate sample preparations should be NMT 2.0.	0.4
	Standard and bracketing standard solutions should meet the acceptance criteria of System Suitability	Complies
For Content Uniformity	The maximum acceptance value should be less than or equal to 15.0.	4.2
Intermediate precision		
For Assay	The % RSD of Assay results for 6 replicate sample preparations should be NMT 2.0.	0.3

Parameter	Acceptance criteria	Results Obtained		
	Overall %RSD for 12 determinations between method precision and intermediate precision should be NMT 3.0.	0.4		
	Standard and bracketing standard solutions should meet the acceptance criteria of System Suitability.	Complies		
For Content Uniformity	Maximum acceptance value should be less than or equal to 15.0.	2.0		
Solution stability:	The similarity factor between the initial standard and standard solution injected at different time intervals should be between 0.98 to 1.02.	Standard solution.		
		Complies		
	The % difference for Assay results of sample preparation between initial and different time intervals should be within ± 2.0.	Sample Solution.		
		Complies.		
	Standard and bracketing standard solutions should meet the acceptance criteria of System Suitability.	Complies.		
Mobile phase Stability	The column efficiency (USP plate count) determined from the Centhaquine peak should not be	**USP Plate Count**		
		Initial	Day 1	Day 2

Parameter	Acceptance criteria	Results Obtained		
	less than 2000 Theoretical plates from the first injection of the	4595	6856	6631
	Tailing Factor determined from the Centhaquine peak should not be more than 2.0 from the first injection of the standard solution.	**Tailing Factor**		
		Initial	Day 1	Day 2
		1.0	1.1	1.1
	The relative standard deviation of area counts of Centhaquine peak from five replicate of standard solution injections should not be more than 2.0%.	**% RSD**		
		Initial	Day 1	Day 2
		0.2	0.1	0.1
Robustness	The column efficiency (USP plate count) determined from the Centhaquine peak should not be less than 2000 theoretical plates from the first injection of the standard solution. The Tailing Factor determined from the Centhaquine peak should not be more than 2.0 from the first injection of the standard solution. The relative standard deviation of area counts of Centhaquine peak from five replicate of standard solution injections should not be	Complies.		

Method Validation of Related Substance in Centhaquine citrate injection

Preparation of Mobile Phase– A (25 mM KH_2PO_4, pH 3.0)

Accurately weigh and transfer about 3.4 gm of Potassium Dihydrogen Orthophosphate (KH_2PO_4) into a beaker of 1000 mL. Transfer 900 ml of HPLC water, sonicate and dissolve. Adjust the pH of this solution to 3.0 ± 0.05 with 1 M KOH solution or diluted orthophosphoric acid (OPA) solution. Transfer into a 1000 mL volumetric flask and make up the volume with HPLC water. Mix and sonicate for 5 minutes and filter through a 0.45 µm membrane filter. Store the solution at room temperature (25±3ºC). Adjust the weight and dilutions according to the requirement for analysis, keeping the final concentration and pH same.

Preparation of Mobile Phase– B

Transfer Acetonitrile (100 %) into a reagent bottle or HPLC mobile phase bottle and label the bottle indicating the date of transfer.

Chromatographic Conditions (Related Substances)

The present embodiment provides an improved means for the identification and quantification of one or more substances, such as 1–(3–methylphenyl) Piperazine and 2–Vinylquinoline, within a given sample. It should be noted that for the above–mentioned compounds, the terms "impurity" and "compound" are utilized synonymously unless specifically indicated otherwise. The conditions for chromatographic analysis of related substances in a sample were established using a specific set of parameters to achieve effective and efficient separation and analysis of related substances in the given sample (**Table 7**).

Table 7. Chromatographic conditions (RS)

Parameters	Condition
Analytical column	Xtimate C18, 5 µm, (4.6 X 250mm)
Ghost–Buster Column	4.6 x 50 mm
Flow rate	0.5 mL/min

Injection volume	50 µL
Detection wavelength	239 nm
Autosampler Temperature	5 °C
Column Oven Temperature	25 °C
Run time	120 min
Retention time (Centhaquine)	60.0 ± 6 min
Relative Retention Time (1–(3–methylphenyl) Piperazine)	About 0.238
Relative Retention Time (2–Vinylquinoline)	About 0.349

Gradient program

The present invention relates to a gradient program utilized in chromatography, which is a technique employed for separating and analyzing mixtures. The program provides specific instructions for time, mobile phase A percentage, and mobile phase B percentage to be used during the chromatography process. The mobile phase acts as the solvent that passes through the chromatography column and carries the mixture under analysis. Mobile phase A and mobile phase B are two distinct solvents mixed in different proportions at specific times to achieve separation of the mixture. The detailed description of the gradient program for method validation of related substances is described in **Table 8**.

Table 8. Gradient Program for method validation of related substance

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.01	80	20
20.00	80	20
30.00	70	30
45.00	70	30
55.00	60	40
70.00	60	40

80.00	40	60
90.00	40	60
100.00	80	20
120.00	80	20
120.01	Stop	Stop

Method Validation Summary (RS):

The analytical method validation summary contains the method, acceptance criteria, and results generated during the analytical method validation of related substance for Centhaquine citrate in the drug product- Lyophilized Centhaquine citrate injection 1.0 mg per vial, which is used for the estimation of known, unknown and total impurities in the drug product for Lyophilized Centhaquine citrate injection 1.0 mg.

The current innovation is beneficial as it utilizes a selective, sensitive, linear, precise, accurate, and robust method for analyzing related substances in Centhaquine and/or its salts, particularly Centhaquine citrate. Moreover, this invention is incredibly sensitive, making it possible to detect and quantify related substances in Centhaquine and/or its salts at levels well below the established acceptance limits set by WHO and in the ICH Guidelines. Additionally, the proposed method makes it easy to identify and quantify all degradation impurities that arise during the storage of Centhaquine samples. This was determined by conducting forced degradation studies following the ICH QlA Guidelines and validated as per the ICH Q2A Guidelines, which cover various parameters like, system suitability, force degradation, the limit of detection (LOD), the limit of quantitation (LOQ), linearity and range, accuracy, precision (system precision, method precision, and intermediate precision), solution stability, mobile phase stability, and robustness (**Table 9**).

Table 9. Related Substance Method Validation Summary

Parameter	Acceptance criteria	Results obtained
Specificity		
Blank and Placebo interference	There should not be any interference of blank and placebo peaks at the retention time of the main analyte and its impurities. All impurities should be separated from each other.	There was no interference from blank, placebo peak at the retention time of Centhaquine citrate, and Known Impurities were observed. Centhaquine citrate and its impurities are well separated from co–eluents and the main peak.
Peak Purity	Peak purity of the main analyte peak shall pass in both spiked and unspiked samples.	The Peak Purity for Centhaquine citrate and its known impurities in standard, unspiked sample and spiked samples met the acceptance criteria.
System suitability	The standard solution should meet the acceptance criteria of System Suitability.	Complies
Forced degradation	Diluent and placebo shall not interfere with the main analyte peak and its impurities.	There was no interference from blank, placebo peak at the retention time of Centhaquine citrate and Known Impurities were observed.
	All known and unknown peaks should be well resolved from the main analyte peak.	All known impurities were adequately separated from each other

<table>
<tr><th>Parameter</th><th>Acceptance criteria</th><th colspan="3">Results obtained</th></tr>
<tr><td></td><td>The peak purity of the main peak shall pass in both the control and stressed sample solution.
The mass balance should be 95.0% to 105%</td><td colspan="3">The peak purity and mass balance met the acceptance criteria.</td></tr>
<tr><td></td><td>The standard solution should meet the acceptance criteria of System Suitability.</td><td colspan="3">Complies.</td></tr>
<tr><td rowspan="8">Limit of detection (LOD) and Limit of Quantification (LOQ)</td><td rowspan="4">The % RSD for peak area due to Centhaquine citrate and Known Impurities at LOQ Level should be NMT 15.0.</td><td colspan="3">Precision (% RSD)</td></tr>
<tr><td>Centhaquine citrate</td><td colspan="2">4.1</td></tr>
<tr><td>1–(3–methylphenyl) Piperazine)</td><td colspan="2">1.4</td></tr>
<tr><td>2–Vinylquinoline</td><td colspan="2">1.8</td></tr>
<tr><td rowspan="4">The S/N ratio of LOD should be NLT 3 and for LOQ it should be NLT 10.</td><td colspan="3">S/N ratio</td></tr>
<tr><td>Parameter</td><td>LOD</td><td>LOQ</td></tr>
<tr><td>Centhaquine citrate</td><td>6</td><td>18</td></tr>
<tr><td>1–(3–Methylphenyl) Piperazine)</td><td>16</td><td>28</td></tr>
<tr><td></td><td>2–Vinylquinoline</td><td>18</td><td>41</td></tr>
<tr><td>The standard solution should meet the acceptance criteria of System Suitability.</td><td colspan="3">Complies.</td></tr>
<tr><td></td><td></td><td colspan="3">Correlation–coefficient (r)</td></tr>
</table>

Parameter	Acceptance criteria	Results obtained				
Linearity and Range	The Correlation –coefficient (r) value should be ≥ 0.997.	Centhaquine citrate	0.9993			
		1–(3–Methylphenyl)	1.0000			
		2–	1.0000			
	The Y–intercept should be ± 5.0% of the active response at a 100% concentration.	**%Y intercept**				
		Centhaquine citrate	–3.8			
		1–(3–Methylphenyl)	–0.1			
		2–	–0.4			
	The % RSD for peak area due to Centhaquine citrate and Known Impurities at Linearity Higher precision should be NMT 5.0.	**Linearity Higher precision (% RSD)**				
		Centhaquine citrate	1.8			
		1–(3–Methylphenyl)	0.1			
		2–	0.5			
	The standard solution should meet the acceptance criteria of System Suitability.	Complies.				
Accuracy	The individual % recovery and mean % recovery at LOQ to 200% level should be NLT 80.0 and NMT 120.0 for Centhaquine citrate and Known impurities.	**Centhaquine citrate**				
		SPL	LOQ	50%	100%	200 %
		SPL 1	109.1	115.1	117.1	104.4
		SPL 2	110.6	115.8	117.3	102.2

Parameter	Acceptance criteria	Results obtained				
	The % RSD of each level recovery for Centhaquine citrate and Known impurities should be NMT 15.0.	SPL 3	110.0	115.8	117.0	102.4
		SPL 4	108.0	NAP		102.1
		SPL 5	106.5			102.2
		SPL 6	113.6			102.0
		Average	**109.6**	**115.6**	**117.1**	**102.6**
		% RSD	**2.2**	**0.3**	**0.1**	**0.9**
		1–(3–Methylphenyl) Piperazine				
		SPL	LOQ	50%	100%	200 %
		SPL 1	105.0	113.1	110.8	108.2
		SPL 2	105.5	107.1	109.8	106.1
		SPL 3	99.4	110.8	109.6	107.3
		SPL 4	105.7	NAP		101.1
		SPL 5	105.5			104.0
		SPL 6	103.9			102.4
		Average	**104.2**	**110.3**	**110.1**	**105.7**

Parameter	Acceptance criteria	Results obtained				
		% RSD	**2.3**	**2.7**	**0.6**	**2.0**
		2–Vinylquinoline				
		SPL	LOQ	50%	100%	200 %
		SPL 1	98.8	108.7	105.2	106.2
		SPL 2	97.2	115.1	106.2	102.4
		SPL 3	97.6	117.9	109.0	103.4
		SPL 4	98.0	NAP		104.8
		SPL 5	100.3			103.6
		SPL 6	101.8			100.1
		Average	**99.0**	**113.9**	**106.8**	**103.4**
		% RSD	**1.8**	**4.1**	**1.8**	**2.0**
	The standard solution should meet the acceptance criteria of System Suitability.	Complies.				
Precision						

Parameter	Acceptance criteria	Results obtained
System Precision (Injector repeatability)	The % RSD for areas of Centhaquine citrate peaks obtained from six replicate injections of the standard preparation should not be more than 10.0.	0.3
	The % RSD for the Retention time of Centhaquine citrate peaks obtained from six replicate injections of the standard preparation should not be more than 2.0.	0.2
	The column efficiency (USP plate count) determined from the Centhaquine peak should not be less than 2000 theoretical plates from the first injection of the standard solution.	578977
	The tailing factor determined from the Centhaquine peak should not be more than 2.0 from the first injection of the standard solution.	1.2

Parameter	Acceptance criteria	Results obtained		
Method Precision	The % of unknown impurities in unspiked sample results should be within the specification limit.	Complies.		
	The % RSD of unknown impurity (≥ LOQ) results for six spiked samples should be NMT 15.0.	Below Quantification Limit (BQL).		
	%RSD of individual known impurity results for six spiked samples should be NMT 10.0%.	**1–(3–methylphenyl) Piperazine**	**2–Vinylquinoline**	**Total Impurities**
		3.2	4.8	2.1
	The standard solution should meet the acceptance criteria of System Suitability.	Complies.		
Intermediate Precision	The % of unknown impurities in unspiked sample results should be within the specification limit.	Complies.		
	The % RSD of unknown maximum impurity (≥ LOQ) results for six spiked samples should be NMT 15.0.	BQL.		

<table>
<tr><th>Parameter</th><th>Acceptance criteria</th><th colspan="3">Results obtained</th></tr>
<tr><td rowspan="5"></td><td rowspan="2">The %RSD of individual known impurity results for six spiked samples should be NMT 10.0%.</td><td>1–(3–methylphenyl) Piperazine</td><td>2–Vinylquinoline</td><td>Total Impurities</td></tr>
<tr><td>3.5</td><td>0.0</td><td>3.1</td></tr>
<tr><td>The overall percentage RSD of unknown maximum impurity (≥ LOQ) and total impurities between method precision and Intermediate precision (12 spiked samples) should be NMT 15.0.</td><td colspan="3">BQL.</td></tr>
<tr><td rowspan="2">The overall percentage RSD of individual known impurity between method precision and Intermediate precision (12 spiked samples) should be NMT 10.0.</td><td>1–(3–methylphenyl) Piperazine</td><td>2–Vinylquinoline</td><td>Total Impurities</td></tr>
<tr><td>4.0</td><td>8.3</td><td>5.4</td></tr>
<tr><td></td><td>The standard solution should meet the acceptance criteria of system suitability.</td><td colspan="3">Complies.</td></tr>
<tr><td rowspan="2">Solution stability</td><td rowspan="2">The similarity factor between the initial standard</td><td rowspan="2">Time in hours</td><td colspan="2">Similarity factor</td></tr>
<tr><td>25°C</td><td>2–8°C</td></tr>
</table>

<table>
<tr><th>Parameter</th><th>Acceptance criteria</th><th colspan="4">Results obtained</th></tr>
<tr><td rowspan="11"></td><td rowspan="3">and standard solution injected at different time intervals should be between 0.90 to 1.10.</td><td>Initial</td><td colspan="2">–</td><td>–</td></tr>
<tr><td>90 hours</td><td colspan="2">0.90</td><td>0.98</td></tr>
<tr><td colspan="4">The standard solution is stable up to 90 hours at room temperature (25°C) and 2–8°C.</td></tr>
<tr><td rowspan="8">The % difference in % known, unknown (above LOQ) and total impurities of sample solution between the initial and after the specified period should be NMT ± 15.0.</td><td>Name of Impurities</td><td>1–(3–methylphenyl) Piperazine) (%w/w)</td><td>2–Vinylquinoline (% w/w)</td><td>Total Impurities</td></tr>
<tr><td>Initial</td><td>–</td><td>–</td><td>–</td></tr>
<tr><td>3rd hour</td><td>4.762</td><td>–10.000</td><td>3.125</td></tr>
<tr><td>5th hour</td><td>0.000</td><td>–10.000</td><td>0.000</td></tr>
<tr><td>7th hour</td><td>0.000</td><td>–10.000</td><td>0.000</td></tr>
<tr><td>9th hour</td><td>0.000</td><td>–10.000</td><td>0.000</td></tr>
<tr><td>11th hour</td><td>0.000</td><td>–10.000</td><td>–6.250</td></tr>
<tr><td colspan="4"></td></tr>
</table>

<table>
<tr><th>Parameter</th><th>Acceptance criteria</th><th colspan="4">Results obtained</th></tr>
<tr><td></td><td>The standard solution should meet the acceptance criteria of System Suitability.</td><td colspan="4">Complies.</td></tr>
<tr><td rowspan="5">Mobile Phase stability</td><td rowspan="4">The column efficiency (USP Plate count) determined from Centhaquine peak should not be less than 2000 theoretical plates from the first injection of standard solution.
The relative standard deviation of area counts of Centhaquine peak from three replicates of standard solution injections, should not be more than 10.0%.
The tailing factor determined from the Centhaquine peak should not be more than 2.0 from the first injection of the Standard solution.</td><td colspan="4">Centhaquine Citrate (% RSD)</td></tr>
<tr><td></td><td>Initial</td><td>Day 2</td><td>Day 5</td></tr>
<tr><td>Tailing Factor</td><td>1.1</td><td>1.1</td><td>1.1</td></tr>
<tr><td>Theoretical Plates</td><td>387991</td><td>388861</td><td>369086</td></tr>
<tr><td>% RSD</td><td>0.5</td><td>0.9</td><td>0.2</td></tr>
<tr><td colspan="4">NAP</td></tr>
<tr><td>The standard solution should meet the acceptance criteria of System Suitability.</td><td colspan="4">Complies.</td></tr>
</table>

Parameter	Acceptance criteria	Results obtained
Robustness Change in wavelength variation ± 2nm. Change in flow variation ± 0.05mL Change column oven temperature ±5°C Change in pH variation in mobile phase A ± 0.2 unit. Change in Mobile Phase Gradient composition (± 2 absolute difference)	The column efficiency (USP plate count) determined from the Centhaquine peak should not be less than 2000 theoretical plates from the first injection of the standard solution. The relative standard deviation of area counts of Centhaquine peak from three replicates of standard solution injections should not be more than 10.0%. The tailing factor determined from the Centhaquine peak should not be more than 2.0 from the first injection of the standard solution. RRTs of known impurities shall be comparable with controlled conditions.	Complies.

We Claim:

1. A stable, sterile lyophilized Centhaquine injectable formulation, comprising:
    a) Centhaquine citrate
    b) Ethylene diamine tetra acetic acid – disodium (EDTA)
    c) Polysorbate 80
    d) Sodium phosphate dibasic anhydrous
    e) Sodium citrate dihydrate
    f) Mannitol
    g) Citric acid

2. The lyophilized pharmaceutical composition of claim 1, wherein the composition is a lyophilized powder.

3. The lyophilized pharmaceutical composition of claim 1, wherein the Centhaquine citrate is present in the range of about 0.0004 to 1.0% w/w.

4. The lyophilized pharmaceutical composition of claim 1, wherein the Ethylene diamine tetra acetic acid – disodium salt (EDTA) is present in the range of about 0.0004% to about 5.0% w/w.

5. The lyophilized pharmaceutical composition of claim 1, wherein the polysorbate 80 is present in the range of about 1.0% to about 20.0% w/w.

6. The lyophilized pharmaceutical composition of claim 1, wherein the Sodium Phosphate, Dibasic, anhydrous is present in the range of about 5.0% to about 50.0% w/w.

7. The lyophilized pharmaceutical composition of claim 1, wherein the sodium citrate dihydrate is present in the range of about 1.0% to about 30.0% w/w.

8. The lyophilized pharmaceutical composition of claim 1, wherein the mannitol

is present in the range of about 20.0% to about 90.0% w/w.

9. The lyophilized pharmaceutical composition of claim 1, wherein the Citric acid monohydrate is added in sufficient quantity to adjust (if required) the pH of the bulk solution in the rage between 7.5 to 8.5.

10. The lyophilized pharmaceutical composition comprising of claim 1; wherein Centhaquine citrate is present at about 1.0 mg; Ethylene diamine tetra acetic acid – disodium salt is present at about 1.0 mg; polysorbate 80 is present at about 21.4 mg (0.02 ml); sodium phosphate dibasic anhydrous is present at about 32.40 mg; sodium citrate dihydrate is present at about 6.5 mg and mannitol is present at about 150 mg.

11. The composition of claim 1, wherein the mannitol is a freeze-drying filler and citric acid monohydrate is a pH adjusting agent.

12. The lyophilized pharmaceutical composition of claim 1, wherein the composition further comprises sodium chloride.

13. The composition of claim 1, wherein the composition further comprises water.

14. The lyophilized pharmaceutical composition comprising:
    a) about 1.0 mg of Centhaquine citrate;
    b) about 1.0 mg of EDTA;
    c) about 21.4 mg of polysorbate 80;
    d) about 32.40 mg of sodium phosphate dibasic anhydrous;
    e) about 6.5 mg of sodium citrate dihydrate;
    f) about 150 mg of mannitol.

15. The lyophilized pharmaceutical composition of preceding claims, wherein the said composition comprises:

a) total impurities not more than 3.0%; or
b) any unspecified impurities not more than 0.5%; or
c) 2–Vinyl quinoline impurity not more than 1.0%
d) 1– (3– methylphenyl) Piperazine impurity not more than 1.5%.

16. The lyophilized pharmaceutical composition according to claim 10, wherein the said composition comprises:
a) total impurities not more than 0.69%; or
b) any unspecified impurities not more than 0.15%; or
c) 2–Vinyl quinoline impurity not more than 0.03%
d) 1– (3– methylphenyl) Piperazine impurity not more than 0.46%.

17. The lyophilized pharmaceutical composition according to any of the preceding claims, is reconstituted by 0.9% sodium chloride solution.

18. The lyophilized pharmaceutical composition according to any of the preceding claims, is reconstituted by water for injection.

19. A method of treating hypovolemic shock comprising administering the lyophilized pharmaceutical composition of Centhaquine citrate according to any of the preceding claims to a subject in need thereof.

20. The method of claim 19, wherein the hypovolemic shock condition is selected from any patient with fall in blood pressure, or increased blood/plasma lactate levels due to loss of blood or body fluids.

21. The method of claims 19-20, wherein the lyophilized pharmaceutical composition according to any of the preceding claims is administered intravenously as a bolus dose or infusion.

22. The pharmaceutical formulation of claims 1 to 14, wherein the lyophilized

formulation is stable at temperature 25 ºC ± 3 ºC and 60 % RH ± 5 % RH for not less than 12 months.

23. The pharmaceutical formulation of claims 1 –14, wherein the lyophilized formulation of Centhaquine citrate is stable at temperature 2–8°C, for not less than 24 months.

24. A reconstituted liquid composition comprising:
    a) Centhaquine citrate
    b) Ethylene diamine tetra acetic acid – disodium (EDTA)
    c) Polysorbate 80
    d) Sodium Phosphate, Dibasic, anhydrous
    e) Sodium citrate dihydrate
    f) Mannitol
    g) Water for injection or 0.9% aqueous sodium chloride solution.

25. The liquid composition of claim 24, wherein the Centhaquine citrate, EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol are provided as a lyophilized powder.

26. The liquid composition of claim 24, wherein the composition is prepared by reconstituting the lyophilized powder of Centhaquine citrate, EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol in water for injection or 0.9% aqueous sodium chloride solution.

27. The liquid composition of claim 24, wherein the Centhaquine citrate is present in the range from about 0.0004% to about 1.0% w/w; the other water soluble ingredients including EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol are present from about 0.0004% to about 90% w/w.

28. The liquid composition of claims 24-27, wherein Centhaquine citrate is present at about 1.0 mg; EDTA about 1.0 mg, polysorbate 80 about 21.4 mg; sodium phosphate dibasic anhydrous about 32.40 mg; sodium citrate dihydrate about 6.5 mg; mannitol is present at about 150 mg.

29. The liquid composition of claim 24 – 28, wherein Centhaquine citrate is present at 0.1mg /ml strength.

30. The liquid composition according to the claims 24 - 29, wherein the said composition comprises:
    a) total impurities not more than 3.0%; or
    b) any unspecified impurities not more than 0.5%; or
    c) 2–Vinyl quinoline impurity not more than 1.0%
    d) 1– (3– methylphenyl) Piperazine impurity not more than 1.5%.

31. The liquid composition according to claim 24, wherein the said composition comprises:
    a) total impurities not more than 0.69%; or
    b) any unspecified impurities not more than 0.15%; or
    c) 2–Vinyl quinoline impurity not more than 0.03%
    d) 1– (3– methylphenyl) Piperazine impurity not more than 0.46%.

32. The liquid composition of claims 24 – 31, wherein the said reconstituted liquid composition has an osmolality between 200 to 350 mOsmol/L.

33. The composition of claim 24, wherein the said reconstituted liquid composition has an osmolality of about 307 mOsmol/L.

34. The composition of claims 24-33, wherein the said reconstituted liquid composition has a pH of about 6.0 to 9.0.

35. The composition of claims 24-35 is administered intravenously as an infusion or bolus dose.

36. A method of treating hypovolemic shock comprising administering the liquid pharmaceutical composition according to any of the claims 24 –36 to a subject in need thereof.

37. The method of claims 36, wherein the hypovolemic shock condition is selected from any patient with fall in blood pressure, or increased blood/plasma lactate levels due to loss of blood or body fluids.

38. A pack comprising a vial containing a lyophilized pharmaceutical composition of Centhaquine citrate;

39. A kit or a co-pack comprising:
    a) a vial containing a lyophilized pharmaceutical composition of Centhaquine citrate; and
    b) an ampoule containing 0.9% sodium chloride aqueous solution or water for injection.

40. A pack of claim 38, wherein the vial containing a lyophilized pharmaceutical composition of Centhaquine citrate comprises:
    a) about 1.0 mg of Centhaquine citrate
    b) about 1.0 mg of Ethylene diamine tetra acetic acid – disodium (EDTA)
    c) about 21.4 mg of polysorbate 80
    d) about 32.40 mg of sodium phosphate dibasic anhydrous
    e) about 6.5 mg of sodium citrate dihydrate
    f) about 150 mg of mannitol.

41. The kit or co-pack according to claim 39, wherein the kit or co-pack comprises a single vial containing lyophilized pharmaceutical composition of Centhaquine

citrate and a single ampoule/vial of 0.9% sodium chloride aqueous solution or water for injection.

42. The kit or co-pack of claims 39, wherein the vial containing a lyophilized pharmaceutical composition of Centhaquine citrate comprises:
    a) about 1.0 mg of Centhaquine citrate
    b) about 1.0 mg of Ethylene diamine tetra acetic acid – disodium (EDTA)
    c) about 21.4 mg of polysorbate 80
    d) about 32.40 mg of sodium phosphate dibasic anhydrous
    e) about 6.5 mg of sodium citrate dihydrate
    f) about 150 mg of mannitol.

43. A process of preparation of lyophilized composition of preceding claims comprising:
    a) dissolving Centhaquine citrate, EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol in water for injection
    b) filter the solution through a 0.2µ membrane filter
    c) fill the individual vials up to the target fill volume; and
    d) lyophilization of the filled vials.

44. The process according to claim 43, wherein the plugs on the vials are half stoppered, followed by loading the vials in freeze dryer for lyophilization to obtain the lyophilized Centhaquine-based injectable formulation.

45. The process according to claim 43, wherein the solution in step (a) is stirred at 350-400 rpm.

46. The manufacturing process according to claim 43, wherein the pH of the solution is adjusted in the range of 7.0 to 8.5 if required.

47. The process according to claim 43, wherein the process is carried at about 15°C to 40°C and the lyophilization process at a reduced temperature of about -40°C to about 5°C.

48. The method as claimed in claim 43-47, wherein said vials are unloaded from said freeze dryer and sealed to obtain said lyophilized Centhaquine-based injectable formulation.

49. A lyophilized Centhaquine-based injectable formulation, comprising:
an active pharmaceutical ingredient-Centhaquine citrate in the range 0.0004% to about 1.0% w/w;
the other water soluble ingredients including EDTA, polysorbate 80, sodium phosphate dibasic anhydrous, sodium citrate dihydrate, and mannitol in the rage of about 0.0004% to 90% w/w; and water for injection.

K. Setiya

Dated this: 30th March 2024

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INPA-2570
Agent for the Applicant(s)

ABSTRACT

LYOPHILIZED CENTHAQUINE CITRATE INJECTION FORMULATION AND A METHOD FOR THE SAME

A lyophilized Centhaquine citrate -based injectable formulation, comprising Centhaquine citrate in the range from about 0.0004 to about 1.0% w/w, Ethylene diamine tetra acetic acid – disodium salt is present in the range of from about 0.0004 to about 1.0% w/w, Polysorbate 80 is present in the range of from about 1.0 to about 20% w/w, Sodium Phosphate Dibasic, anhydrous is present in the range of from about 5.0 to about 50% w/w, Sodium citrate dihydrate is present in the range of from about 1.0 to about 30% w/w, and mannitol is present in the range of from about 20 to about 90% w/w. A reconstituted liquid composition comprising Centhaquine citrate; Ethylene diamine tetra acetic acid – disodium salt, Polysorbate 80, Sodium Phosphate, Dibasic, anhydrous, Sodium citrate dihydrate, and mannitol and water for injection or 0.9% aqueous sodium chloride solution and process for the preparation of a lyophilized pharmaceutical composition of Centhaquine citrate comprising: dissolving Centhaquine citrate, Ethylene diamine tetra acetic acid – disodium salt, Polysorbate 80, Sodium Phosphate, Dibasic, anhydrous, Sodium citrate dihydrate, mannitol in water for injection; and adjusting the pH with Citric acid if required; filter the solution through 0.2µ membrane filter; fill the individual vials up to the target fill volume; and lyophilization of the filled vials.