Title: A PROCESS FOR PREPARING PURE CITICOLINE (CDP-CHOLINE)

Abstract: Disclosed herein is a process for preparing highly pure Citicoline (CDP-Choline) or sodium salt of Citicoline with the aid of dicarboxylic acid or its salts. The process of the present invention results in Citicoline with a purity of more than 99% measured by HPLC.

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A PROCESS FOR PREPARING PURE CITICOLINE (CDP-CHOLINE)

Field of the Invention

The present invention relates to industrially feasible and economical process for preparing highly pure Citicoline (CDP-Choline) or sodium salt of Citicoline with the aid of dicarboxylic acid or its salts.

Background of the Invention

Citicoline (CDP-Choline), naturally occurring nucleotide, is a neuroprotective indicated for the treatment of ischemic stroke and head trauma in patients. Citicoline (CDP-Choline) is represented by formula (I).

US patent no. 3,666,748 discloses a process for preparing Citicoline sodium by reaction of 4-morpholino-N,N'-dicyclohexylcarboxamidine chloride salt of choline phosphormorpholidate (I) with cytidine-5-monophosphate in free form or its salts with base in a solvent such as o-chlorophenol, m-cresol, acetonitrile, pyridine and the like. The Citicoline thus obtained is purified through a column chromatograph packed with activated carbon followed by elution to get ammonium salt of citicoline, which is further converted to citicoline followed by citicoline sodium.

US patent no. 3,787,392 discloses a process for preparing Citicoline by adding the acidic calcium phosphoryl choline chloride tetra hydrate to the solution of phosphormorpholidate cytidine 5-monophosphate and DCC in methanol followed by isolation and purification by means of chromatography column containing anion exchanger (Dowex 1x2 type formate form; 50-100 mesh) which is further converted to its sodium salt by neutralizing with sodium hydroxide.

Further, US patent no. 3,803,125 discloses a process for preparing citicoline by reacting morpholidiate cytidine 5'-monophosphate with calcium phosphoryl choline chloride tetra hydrate in solvent system of an aliphatic alcohol or dialkyl ketone or dimethyl formamide at pH from 1 to 6.5. The product thus obtained is further isolated; purified by means of
chromatography column containing anion exchanger; concentrated; and neutralized with aqueous solution of sodium hydroxide to get citicoline sodium.

All the above prior art processes have several disadvantages from industrial and economical point of view, in particular, these methods employ costly and hazardous solvents in higher volume during reaction as well as for isolation. Not only that, quantity and quality of the desired product is hampered due to various by-products engendered during these processes as these by-products are highly soluble along with the desired product. In addition, column chromatography is employed for removal of all these by-products, which is costly, prolonged and non-feasible process on large scale.

To address the above-mentioned problems, the present invention provides a process for preparation of Citicoline sodium, which does not suffer from the above-mentioned disadvantages and provides desired compound with high yield and quality, in a short period of time, without formation of substantial amounts of disconcerting by-products.

**Summary of the Invention**

It is an object of the present invention to provide an efficient, commercially viable and highly productive process for preparing sodium salt of Citicoline (CDP-Choline) and thereby isolation of the same without means of column chromatography.

One more object of the invention is to provide industrially feasible process to attain highly pure sodium salt of Citicoline (CDP-Choline) with minimum impurities where the purity attained is ≥ 99%, measured by HPLC.

In another object, the present invention provides a process of removing the soluble impurities by means of dicarboxylic acid or its salt, during the preparation of sodium salt of Citicoline (CDP-Choline).

In one aspect, the invention provides a process for preparing Citicoline (CDP-Choline), wherein the process comprises reacting a cytidine S’-monophospahte or its amide salts with calcium phosphoryl choline chloride tetra hydrate or its amide salts in presence of dicyclohexyl carbodiimide (DCC) and a solvent, wherein a dicarboxylic acid or its salt is employed in the process to obtain citicoline with a purity of more than 99% measured by HPLC.

In another aspect, the process of the present invention, further provides a process for preparing highly pure sodium salt of citicoline by reacting the pure citicoline obtained by the process of this invention with aqueous solution of sodium hydroxide.
The dicarboxylic acid used in the present invention is either in the form of free acid or its base salts. In one aspect, the dicarboxylic acid is selected from the group consisting of oxalic acid, malonic acid, succinic acid, glutaric acid. In another aspect, the base of dicarboxylic acid is selected from the group consisting of organic bases such as amidates, amines or inorganic base such as alkali or alkaline earth metal.

In another aspect of the present invention, the solvent for carrying out the process is selected from the group consisting of aliphatic alcohols from C₁-₄ atoms, ketones such as acetone, methyl isobutyl ketone and the like or mixture thereof. In a preferred aspect, the solvent employed in the process is methanol.

In the present invention, the dicarboxylic acid or its salts lessen the solubility of inorganic impurities such as calcium chloride, calcium hydroxide, unreacted choline phosphate, 5-CMP. This results in obtaining highly pure Citicoline or sodium salt of Citicoline without employing column chromatography.

The above and other objects of the present invention are attained according to following preferred embodiments of the present invention. However the scope of the invention is not restricted to the particular embodiments discussed herein after.

**Detailed Description of the Invention**

While the invention is susceptible to various modifications and alternative forms, specific embodiment thereof will be described in detail below. It should be understood, however that it is not intended to limit the invention to the particular forms disclosed, but on the contrary, the invention is to cover all modifications, equivalents, and alternative falling within the scope of the invention as defined by the appended claims.

In accordance to one embodiment of the present invention, there is provided a process for preparing Citicoline (CDP-Choline) or sodium salt of Citicoline having HPLC purity ≥ 99%, without employing column chromatography.

In accordance to another embodiment of the present invention, there is provided a process that employs dicarboxylic acid or its salts for lessening soluble inorganic impurities like calcium chloride, calcium hydroxide as well as unreacted choline phosphate, 5-CMP etc., during the process for preparing Citicoline or sodium salt of Citicoline, having HPLC purity ≥ 99%.

According to one of the embodiments of the present invention, there is provided a process for preparing Citicoline (CDP-Choline) where the process comprises condensing cytidine 5'-
monophosphosphate or its amidate salts with calcium phosphoryl choline chloride tetra hydrate or its amidate salts in presence of dicyclohexyl carbodiimide (DCC).

The reaction of cytidine 5'-monophosphosphate or its amidate salt with calcium phosphoryl choline chloride or its amidate salt is generally carried out in a solvent selected from the group consisting of but not limited to aliphatic alcohols from C\textsubscript{1}-C\textsubscript{4} atoms; ketone such as acetone, methyl isobutyl ketone and the like or mixture thereof; preferably methanol. The reaction temperature generally ranges from 60°C to the reflux of the solvent employed.

According to the process of the present invention, dicarboxylic acid is exercised either in the form of free acid or its base salts, provided that dicarboxylic acid or its salts are employed at different juncture during the Citicoline (CDP-Choline) synthesis. Dicarboxylic acid may include oxalic acid, malonic acid, succinic acid, glutaric acid and the like. The base used herein for preparing the dicarboxylic acid salt is selected from the group consisting of but not limited to organic bases such as amidates, amines; or inorganic base such as alkali or alkaline earth metal.

Amidates used herein may be used as mono and dialkyl-substituted amidates, the alkyl radicals of C\textsubscript{1}-C\textsubscript{4} atoms, such as dimethylamidate, mono and diethyl-amidate, mono and dipropyl amidate, isopropyl-amidates and butyl-amidates, as well as the mixed amidates. Further the alkyl radicals, which are joined together directly or via a heteroatom to form a ring, for example, the morpholidates, piperidates, cyclohexylimidates and anilidates may also be used. Amines, including triethylamine, diisopropyl amine, etc. may also be used herein.

The choice for the use of dicarboxylic acid or its base salt is dependent on the starting material taken for the Citicoline preparation. Dicarboxylic acid is used herein at the start of the reaction, when cytidine 5'-monophosphosphate is reacted with phosphoryl choline chloride amidates. At this time dicarboxylic acid or its base salt is exercised with calcium phosphoryl choline chloride first to get the phosphoryl chlorine chloride, which is then converted to its amidates salt by reacting it with amidanes.

Further, on the other hand when Cytidine 5'-monophosphosphate amidates is condensed with phosphoryl choline chloride, dicarboxylic acid or its base salt is exercised on completion of condensation reaction.

The employment of dicarboxylic acid or its amidates contribute an important role in preparation of highly pure Citicoline having purity \( \geq 99\% \) without the use of column chromatography. The dicarboxylic acid or its salts employed in the process, convert the soluble impurities, generated during the reaction, into insoluble impurities, and the desired
product with diminished amounts of impurities remains soluble in the solvent of reaction. Thereby insoluble impurities can be removed by a simple method of filtration, instead of non-feasible, prolonged column chromatography.

Thus, the citicoline produced by the process of this invention is easily recovered by known methods. For example, the resulting reaction mixture is extracted with a solvent, and the solvent extract is distilled out to obtain crude citicoline. Further, the solution of citicoline in water is purified by means of charcoal treatment and then concentrated to dryness to recover the objective compound with the purity of \( \geq 99\% \). Moreover, citicoline is treated with sodium hydroxide to get citicoline sodium with purity of \( \geq 99\% \) measured by HPLC.

The following examples are illustrative of the instant invention. These examples are not intended to limit the scope of the invention as defined herein above.

**Example 1**

To a solution of calcium phosphoryl choline chloride tetra hydrate (50.0 gm) in water, a solution of oxalic acid in RO water (19.5 gm oxalic acid in 90 ml RO water) was added at 45-50°C. The reaction mass was filtered and distilled out to get residue followed by addition of methanol. To the above solution, solution of morpholine and DCC in methanol was added. The temperature of the reaction was raised to 50-55°C and to this, solution of cytidine 5'-monophosphate in methanol (12.2 gm in 40 ml methanolic HCl and 20 ml methanol) was added and reaction was maintained. The pH 3.5 of reaction mixture was maintained by methanolic HCl. Reaction mass was cooled and IPA was added after completion of the reaction. The precipitated product, citicoline, was filtered and dried. The crude Citicoline (16.0 gm) was dissolved in water and treated with charcoal to get the purified Citicoline acid which on reaction with aqueous sodium hydroxide gave Citicoline Sodium with purity \( \geq 99\% \).

**Example 2**

To the solution of cytidine 5'-monophosphate (5'-CMP) (100 gm) in methanol (750 ml), solution of morpholine (75 gm) and DCC (100 gm) in methanol was added at room temperature. The temperature of the reaction was raised to 50-55°C for a time period of 3-7 hrs followed by cooling the reaction mass and filtered to get morpholidiate cytidine 5'-monophosphate in mother liquor. To this, solution of calcium phosphoryl choline chloride (200 gm) in methanol was added and the temperature of reaction mass was raised to 50-55°C and maintained at pH of 3.5 by methanolic HCl. The reaction mass was cooled and filtered to get crude Citicoline by adding IPA. Further, morpholidiate salt of oxalic acid (138.3 gm) was
added to the solution of crude citicoline in methanol at 30-35°C followed by the addition of IPA to get the precipitated Citicoline, which is further treated with activated charcoal in water followed by filtration. To filtrate containing purified Citicoline, aqueous solution of sodium hydroxide was added at room temperature followed by addition of ethanol and the temperature of reaction mass was raised to 50-55°C. The precipitated product was filtered and dried where the purity of citicoline sodium is ≥ 99% measured by HPLC. (265 gm).
We Claim:

1. A process for preparing pure Citicoline (CDP-Choline), the process comprising:
   reacting a cytidine 5'-monophosphate or its amide salts with calcium phosphoryl choline chloride tetra hydrate or its amide salts in presence of dicyclohexyl carbodiimide (DCC) and a solvent,
   wherein a dicarboxylic acid or its salt is employed in the process to obtain citicoline with a purity of more than 99% measured by HPLC.

2. The process as claimed in claim 1, further comprising preparing highly pure sodium salt of citicoline by reacting the pure citicoline with sodium hydroxide.

3. The process as claimed in claim 1, wherein the dicarboxylic acid is used either in the form of free acid or its base salts.

4. The process as claimed in any one of the preceding claims, wherein dicarboxylic acid is selected from the group consisting of oxalic acid, malonic acid, succininc acid and glutaric acid.

5. The process as claimed in any one of the preceding claims, wherein the base of dicarboxylic acid is selected from the group consisting of organic bases such as amidates, amines or inorganic base such as alkali or alkaline earth metal.

6. The process as claimed in claim 1, wherein the solvent is selected from the group consisting of aliphatic alcohols from C1-4 atoms, ketones such as acetone, methyl isobutyl ketone and the like or mixture thereof.

7. The process as claimed in claim 1, wherein the solvent is methanol.

8. The process as claimed in any of the preceding claims, wherein the dicarboxylic acid or its salts lessen the solubility of inorganic impurities such as calcium chloride, calcium hydroxide, unreacted choline phosphate, 5-CMP.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC: C07H 19/10 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REGISTRY, CAPLUS, EPDOC, WPI

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>US 3666748 A (MISKIO HONJO, YOSHIYASU FURUKAWA) 30 May 1972 (30.05.1972) Examples, cited in the application</td>
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search: 19 July 2013 (19.07.2013)

Date of mailing of the international search report: 05 August 2013 (05.08.2013)

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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