RESEARCH ARTICLE

Modification of Choline Derivatives and the Study of their Pharmacological Activity

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ABSTRACT

Organicmoleculeshavebiologicalactivityforavarietyofstructuralfeatures,someactivitiesareassociatedwiththestructural basis of a known molecule, and others are associated with the type and orientation of additive modifications. However, acetylcholine (ACh) is the main neurotransmitter of the parasympathetic nervous system, the part of the autonomic nervous system that contracts smooth muscles, dilates blood vessels, increases body secretion, and slows the heartrate.

Inthecentralnervoussystem,AChhasseveralrulesanditplaysanimportantroleinmemoryandlearning,aswellas,inthe abnormal deficiency of ACh in the brain in people with Alzheimer's disease. In the past, it has been attempted to use ACh chlorideascholinergicstimulants,but,unfortunately,ithasbeenfoundthatitdoesnothavealastingeffectbecauseofitstoo short action duration due to its rapid hydrolysis by acetylcholinesterase (AChE) enzymes and the lack ofspecificity.

Keywords: Choline, Modification, Choline Esterase, Choline Acetyltransferase, Alzheimer disease, EDTA chelating agent.
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INTRODUCTION

Brain chemical ACh plays a very necessary role in methods of regulating human body structure, which is related to the study of body functions. This does not serve the development of agents that imitate the effect of ACh, as well as, those which block the effect of ACh as medically supporting agents. Scientists are getting better information from these developments about treating special diseases, like brain disease, Parkinson's disease, and urine storage sac (bladder).

For professional pharmacists, it is important to know how the autonomic nervous system influences usual body structure related to function and how they can be targeted in controlling sicknesses. Dale[1] explained the actions of esters and ethers of choline on organs and their relationship to muscarine. Since then, the medical drug experts, physiologists, chemists, and scientists who study the chemicals in living things, have used the knowledge gained to know the actions of the cholinergic nerve and its brain chemical.

Loewi first found ACh in frog heart in the year 1921. It was found as a substance released by vagus nerve stimulation. At that time, the complex difficulty of action of ACh on cholinergic nerve cells and receptors was unknown. Subsequently, the advancement in the application of biotechnology and chemistry developed probes that uncovered this difficulty.[2]

Cholinergic nerves are found in the peripheral nervous system and central nervous system (CNS) of humans. Investigators are releasing the mystery that surrounds thinking-related damage, especially, Alzheimer's disease. Synaptic terminals in the brain, corpus striatum, hippocampus, and a few other areas in the CNS are rich in ACh and in the enzymes that create and break down this brain chemical. Multiple experiments have shown that agonists and antagonists of cholinergic receptors change output of brain chemicals, including ACh,

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from brain preparations. In spite of the unobvious function of ACh in the brain, it is known that it is involved in memory and behavioral activity in humans.[3] ACh receptors are classified into two types, *viz*., nicotinic and muscarinic. They have a different composition, location, and pharmacological action. Nicotine and muscarine, respectively, are the naturally occurring alkaloids that bind the nicotinic and muscarinic ACh receptors that has led to the said classification. There is a sub-classification for the receptors, on the basis of the differences in the location and the specification for the identified agonists and antagonists of nicotinic and muscarinic ACh receptors.

When ACh binds to the nicotinic receptor, it will lead to the change in permeability of the membrane allowing the cations Ca^{2-}, Na^{-}, and K^{-} to pass through it. This will help the depolarization of the endplate, which will lead to the contraction of the muscle at the neuromuscular junction or, as occurs in autonomic ganglia, a continuation of the nerve impulse. It is important to know that the neuromuscular nicotinic ACh receptors can be used in surgical operations because of their autoimmune antibodies in myasthenia gravis and muscle relaxants. Some drugs block the nicotinic receptors which can help to control hypertension.

In 1980, it had appeared that the action of ACh could not be settled by one muscarinic receptor, that is why more studies were taken in 1980 to improve the usefulness of these receptors to be used as a target to treat many diseases that became predominant especially in elderly people. The functions of the cholinergic neurons are to synthesize, store, and release ACh. The other function of these neurons is the formation of two other enzymes, which are called choline acetyltransferase (ChAT) and AChE. They are produced in the soma of the neuron and distributed throughout the neuron by the axoplasmic flow. The location of AChE is outside the neuron and it is connected with the neuroglial cells in the synaptic cleft. In the nerve ending occurs the preparation of Ach, which takes a place by transporting an acetyl group from acetyl-coenzyme A (CoA) to choline with the help of the enzyme choline acetyltransferase. The latter enzyme plays an important role in the synthesis of ACh. The enzyme and the neurotransmitter are present in the cholinergic neurons,[1-31] as well as, in some non-nervous tissues.[1,2,4,5] Their function is unknown in the non-nervous tissues. On the other hand, the choline esterase (ChAc) is considered as a special marker for cholinergic neurons.

In order to study the characteristic features of this enzyme at the molecular level and to localize it in cholinergic cells by immune histochemical methods, with a precondition that this enzyme should have high purification. From the enzyme properties and the localization, it will help to clarify the cholinergic transmission mechanism, as well as, the allocation of the cholinergic neurons in the nervous system.[32] Most of the choline which is needed for ACh synthesis comes from the breakdown of ACh in the synapses and then the choline will be regained again with the help of the sodium ions in the presynaptic terminal to synthesize ACh.[25]

AChE is the enzyme that is responsible for the breakdown of ACh. AChE is a type-B carboxylesterase enzyme which is primarily located in the synaptic cleft with a smaller concentration in the extra junctional area. It is secreted by the muscle and stays attached by collagen. 50% of released ACh because of this enzyme is broke down into choline and acetate in a very short time (in less than 1 ms). Then, again the liberated choline will be used for the production of ACh in the nerve terminal. AChE has a high specific activity, which functions at a rate, which is near to that of a diffusion-controlled reaction.[33] The powerful acute toxicity of organophosphorus (OP) poisons is the generation of drugs for the treatment of Alzheimer's disease.[34] AChE contains two subsites, *viz*., "esteratic" and "anionic."[35-37]

It is important that the neuronal activity in the peripheral and central nervous systems is potently regulated by ACh.[38,39] However, the clearly stated/particular (things that are given/ work that is done) of cholinergic neuromodulation to circuit function in the healthy brain and in psychiatric illness have been very hard to cut apart, due to its pleiotropic actions on nerve-related excitability, synaptic transmission, and network patterns (of relationships, movement, or sound). Recently, clever inventions in the field of molecular (the study of tiny chemical assembly instructions inside of living things), (body structure/ related to the study of body functions), and human imaging has opened up new dimensions to understand the circuits and behavior of neuromodulation shapes. Here, we discuss the progress in the field of cholinergic signaling that is added/given to the circuits which are involved in major depressive sickness/problem (MDD), the two groups of the psychiatric sicknesses/problems, and very serious mental disorder. Technical innovation will be given further stimulus to support the treatment of psychiatric diseases.

It is important to know that the abnormalities in the cholinergic system may lead to multiple diseases, for example, myasthenia gravis.[40,41] This may also lead to other diseases, like Alzheimer's and Parkinson's disease.[42,43] Recent researches are related to the dysfunction in the cholinergic system and the important role it plays in diseases, like schizophrenia and depression.

AIM OF THE STUDY

Modification of choline ester derivatives and the study of the pharmacological activity to counteract the problems of rapid hydrolysis and short duration of action of acetylcholine.

PHARMACOLOGICAL ACTIVITY OF ACH IN SCHIZOPHRENIA AND ATTENTION

The symptoms of schizophrenia can be divided into two types, *viz*., positive and negative. Positive symptoms are disordered thoughts, delusions, and hallucinations. While, the negative symptoms are blunted affect and social withdrawal.[44] It has been known that the brain chemicals, like serotonin and dopamine, in addition to Ach, lead to the contribution of this disease. Stimulation of the cholinergic nerve cells in the basal forebrain ontogenetically improved the visual performance in the mouse cortex.[45] In spite of many years of continuous

working to understand the role of Ach, which is considered as evasive, in new studies which are related to human imaging gave a spot on the contribution of the cholinergic system in behavior. So, it is important to know that in order to treat neuropsychiatric diseases drugs should be directed to a targeted receptor subtypes and cell classes.

Researches must be directed forward to assure the link between the clinical and preclinical researches. To have a proper health and a good well being it is important to take choline which can be taken from the diet.[46] Many diets contain this substance which is quite enough for health maintenance. The US Department of Agriculture website showed which types of food contain choline, i.e., free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin, and betaine—a metabolite of choline.[47] Adequate intake (AI) recommendations, issued by the Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences, which they said that 7.5 mg per kg of body weight is enough for a normal person,[46] but the quantities increase in special cases like pregnancy and lactation to meet the requirements of the fetus and babies.[48-50] Lower choline intake may lead to a lot of disorders, for example, liver dysfunction and other disorders,[51-53] and *vice versa* high intakes help to reduce the risk of some cancers.[54-58] Whether it is choline or its related metabolites which are considered as quaternary amine and they are very important in the structure of cell membranes, metabolism of methyl, neurotransmission, etc., this amine is widely distributed in tissues as phosphatidylcholine and sphingomyelin. There are a lot of studies that investigate the choline function and metabolism.[59-61]

On the contrary, there are a lot of studies that make sure that there is a link between the cholinergic activity and the normal behaviors ruptured in patients with schizophrenia. When ACh is applied locally it gives a great push in the improvement of neuronal activity in the primary visual cortex leading to increase attention. While other drugs that reverse the action which is called antagonists, like scopolamine, reduce attention.[44] Because of the short duration of action of ACh due to its hydrolysis by a certain enzyme which is called AChE, and due to the importance of this neurotransmitter for the treatment of many neuronal diseases, this point gave an orientation to look forward to improving ways to get all the benefits of this neurotransmitter in treating many diseases depending on it.

PHARMACOLOGICAL CHARACTERISTICS OF CHOLINE ESTERS

Choline esters may be defined as a modified compound of choline which is used nowadays clinically. For example, according to a study done by Williams,[62] this study was considered as a patent this study summarized that all people when they become elderly they will suffer from presbyopia and cataract, which are age-related diseases, they are normally treated by using corrective lenses if treatment is not found.

A new formulation was done to reduce the risk of toxicity of the surrounding healthy tissues this formulation, which is formulated as an eye drop that consists of 0.25 to about 10% of a reducing agent that is choline ester it has been found that this eye drop ensures ocular delivery. Another study showed the use of choline esters to enhance the absorption of the drug by many other routes (nasal, buccal, vaginal, and sublingual) and to avoid the first-pass metabolism when given orally and this help to ensure drug stability in spite the oral route is one of the proffered routs but not all the drugs have good absorption so from here the idea started of choline esters to promote absorption and also it is proved that they may not necessarily segregate divalent cations (Mg^{----} or Ca^{++}) which are important for cell function. This gives an advantage over the chelating agent Ethylenediaminetetraacetic acid (EDTA). . It means with choline esters no damage of the tissue occurred according to many studies as compared with a surfactant activity (sodium lauryl sulfate).

Another patent was done by Jose A.[63]; this study was done for drugs with low bioavailability because of poor absorption so they were administrated with choline esters in a special form suitable for oral or rectal delivery. Another patent[64] was done to make a low choline odor of an organic compound, like choline ellagate to help with the delivery. Choline deficiency occurs in men and post-menopausal women with a low-calorie diet. Sometimes choline is used to support the delivery and uptake of the nutrients to reach the daily recommended dose for example in fortified food.

STANDARDS OF CHOLINE AND CHOLINE ESTERS IN USP, EP, AND RUSSIAN PHARMACOPEIA[92-94]

USP Reference Standards

Choline Bitartrate

$C_9H_{19}NO_7$ 253.25

2-hydroxyethanaminium,-*N*,*N*,*N*-trimethyl, [*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1)

(2-hydroxyethyl) trimethylammonium-L-(+)-tartrate salt (1:1) [*87-67-2*]

- Choline bitartrate contains not less than 99 percent and not more than 100.5 percent of $C_9H_{19}NO_7$, calculated on the anhydrous basis.

Identification:

A: Infrared absorption <197K>.

B: Dissolve 1-gram of choline bitartrate with 20 mL of water, and add 2 mL of potassium chloride solution (1 in 4). A white precipitate of potassium bitartrate is formed.

Specific rotation <781S>: between + 17.5 and + 18.5º.

Test solution: 400 mg per mL, in water.

pH <791>: between 3 and 4, in a solution (1 in 10).

Water, method I <921>: Not more than 0.5%.

Residue on ignition <281>: Not more than 0.1%.

Table 1: Choline ester preparations which are registered in the State Register of Medicines[92]

No. p / p	*Trade name*	*International nonproprietary name or grouping (chemical) name*	*Release form*	*Name of holder or holder of drug registration certificate*	*Country of holder or holder of drug registration certificate*	*Registration number*	*State registration date*	*Expiration date reg. beats*	*Re-registration date of RU*	*Condition*	*Decision date*
1	Holy Alpha	Choline alfoscerate	solution for infusion and intramuscular injection;	Limited Liability Company "Velfarm" (LLC "Velfarm")	Russia	LP-005108	10/15/2018	10/15/2023	12/10/2018	D	12/10/2018
2	Alfokholin-Lekfarm	Choline alfoscerate	solution for intravenous and intramuscular administration;	Joint limited liability company "Lekpharm" (JLLC "Lekpharm")	Republic of Belarus	LP-004869	05/29/2018	05/29/2023	-	D	09/06/2019
3	Cereton®	Choline alfoscerate	oral solution;	Closed Joint-Stock Company FarmFirma Soteks (CJSC PharmFirma Soteks)	Russia	LP-004829	04/26/2018	04/26/2023	-	D	04/26/2018
4	Choline alfoscerate	Choline alfoscerate	oral solution;	Closed Joint-Stock Company Berezovsky Pharmaceutical Plant (CJSC BFZ)	Russia	LP-004777	04/03/2018	04/03/2023	-	D	04/03/2018
5	Choline alfoscerate	Choline alfoscerate	solution for infusion and intramuscular injection;	PJSC "Biosynthesis"	Russia	LP-004753	03/26/2018	03/26/2023	-	D	03/26/2018
6	Choline alfoscerate	Choline alfoscerate	solution for intravenous and intramuscular administration;	Atoll Limited Liability Company (Atoll LLC)	Russia	LP-003816	08/31/2016	08/31/2021	09/26/2019	D	09/26/2019
7	Choline alfoscerate	Choline alfoscerate	solution for intravenous and intramuscular administration, 250 mg / mL (ampoule) 4 mL x 3/5 (pack of cardboard)	FarmIntellect Limited Liability Company (PharmIntellect LLC)	Russia	LP-003771	08/10/2016	08/10/2021	07/25/2019	D	-
8	Choline alfoscerate	Choline alfoscerate	solution for intravenous and intramuscular administration;	Open Joint-Stock Company "Borisov Plant of Medical Preparations" (OJSC "BZMP")	Republic of Belarus	LP-003768	08/08/2016	08/08/2021	08/10/2018	D	08/10/2018

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9	Choline alfoscerate	Choline alfoscerate	solution for intravenous and intramuscular administration;	Ellara Limited Liability Company (Ellara LLC)	Russia	LP-003241	10/08/2015	10/08/2020	10/10/2017	D	07/30/2019
10	Nooprin®	Choline alfoscerate	solution for intravenous and intramuscular administration;	Joint-stock company "Novosibkhimpharm" (JSC "Novosibkhimpharm")	Russia	LP-003243	10/08/2015	10/08/2020	08/09/2018	D	08/14/2019
11	Gliatilin	Choline alfoscerate	solution for infusion and intramuscular injection;	Italfarmako S.p.A.	Italy	LP-003090	07/14/2015	07/14/2020	-	D	12/10/2018
12	Choline alfoscerate	Choline alfoscerate	solution for intravenous and intramuscular administration;	Open Joint-Stock Company Kurgan Joint-Stock Company of Medical Preparations and Products "Synthesis" (OJSC "Synthesis")	Russia	LP-002522	07/04/2014	-	07/05/2019	D	07/05/2019
13	Delecitis	Choline alfoscerate	solution for infusion and intramuscular injection;	LLC "ITF"	Russia	LP-001577	03/07/2012	-	08/10/2017	D	08/10/2017
14	Gliatilin	Choline alfoscerate	oral solution;	Italfarmako S.p.A.	Italy	LP-001540	02/27/2012	-	06/28/2017	D	12/11/2018
15	Delecitis	Choline alfoscerate	capsules;	LLC "ITF"	Russia	LP-001536	02/27/2012	-	09/06/2017	D	09/06/2017
16-	Choline alfoscerate	Choline alfoscerate	solution for intravenous and intramuscular administration;	Limited liability company "COMPANY" DECO "(LLC" COMPANY "DECO")	Russia	LP-001431	01/12/2012	-	10/07/2019	D	10/07/2019
17	Otinum	Choline Salicylate	ear drops;	Meda Pharma gmbH and Co.KG	Germany	P N011858 / 01	11/22/2011	-	07/02/2014	D	10/02/2019
18	Noocholin Rompharm	Choline alfoscerate	solution for intravenous and intramuscular administration;	K.O. Rompharm Company S.R.L.	Romania	LP-000715	09/29/2011	-	10/06/2016	D	09/06/2019
19	Cereton®	Choline alfoscerate	solution for intravenous and intramuscular administration;	Closed Joint-Stock Company FarmFirma Soteks (CJSC PharmFirma Soteks)	Russia	LS-002652	09/21/2011	-	-	D	07/29/2019
20	Holisal®	Choline Salicylate + Cetalkonium Chloride	dental gel;	LLC "VALANTE"	Russia	P N012118 / 01	08/24/2010	-	04/14/2014	D	09/27/2019

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21	Cholitilin®	Choline alfoscerate	solution for intravenous and intramuscular administration;	Closed Joint-Stock Company Canonfarm Production (CJSC Canonfarm Production)	Russia	LSR-005773/10	06/23/2010	--	07/25/2017	D	08/29/2019
22	Cerepro®	Choline alfoscerate	solution for intravenous and intramuscular administration;	Joint-stock company "VEROPHARM" (JSC "VEROPHARM")	Russia	LS-000476	06/02/2010	-	07/15/2019	D	07/15/2019
23	Cerepro®	Choline alfoscerate	capsules;	Joint-stock company "VEROPHARM" (JSC "VEROPHARM")	Russia	LS-000475	05/31/2010	-	04/04/2018	D	03/21/2019
24	Cholitilin®	Choline alfoscerate	capsules;	Closed Joint-Stock Company Canonfarm Production (CJSC Canonfarm Production)	Russia	LSR-004850/10	05/28/2010	-	03/07/2019	D	03/07/2019
25	Gleazer	Choline alfoscerate	solution for intravenous and intramuscular administration;	EcoFarmPlus JSC	Russia	LSR-007815/09	10/05/2009	-	06/20/2019	D	06/20/2019
26	Cereton®	Choline alfoscerate	capsules;	Closed Joint-Stock Company FarmFirma Soteks (CJSC PharmFirma Soteks)	Russia	LSR-005608/09	07/13/2009	-	10.21.2015	D	08/12/2019
27	Mundizal	Choline Salicylate	gel for topical application;	Mundifarma gmbH	Germany	P N011976 / 01	02/15/2008	-	08/15/2014	D	08/25/2017
28	Gliatilin	Choline alfoscerate	solution for intravenous and intramuscular administration;	Italfarmako S.p.A.	Italy	P N011966 / 02	12/17/2007	-	02/20/2018	D	07/03/2019
29	Gliatilin	Choline alfoscerate	capsules;	Italfarmako S.p.A.	Italy	P N011966 / 01	12/17/2007	-	09/17/2019	D	09/17/2019

Arsenic, method I <211>: Proceed as directed in the test for arsenic under choline chloride; the limit is 2 µg per gram.
Lead <251>: Proceed as directed in the test for lead under choline chloride; not more than 0.3 µg per gram is found.
Heavy metals, method II <231>: 10 µg per gram.
Limit of total amines: Proceed as directed in the test for the limit of total amines under choline chloride; not more than 10 µg per gram.

Test solution: Transfer 10 grams of choline bitartrate to a beaker containing a plastic-coated stirring bar, add 70 mL of sodium hydroxide TS and 130 mL of water and stir until dissolved.

Chromatographic purity: Buffer solution, mobile phase, standard solution, and chromatographic system—Proceed as directed in the test for chromatographic purity under choline chloride.

Test solution: Transfer about 500 mg of choline bitartrate, accurately weighed, to a centrifuge tube; add 2 mL of water, and swirl to dissolve. Add 0.5 mL of potassium chloride solution (7.5 in 25), centrifuge, and transfer 1 mL of the supernatant to a 24 mL screw-capped vial. Dry at 120ºC for 2 hours. Add 400 mg of 3,5-dinitrobenzoyl chloride and 10 mL of acetonitrile, and mix. Cap the vial, and heat at 55ºC for 2 hours. Cool to room temperature, add 5 mL of water and allow to stand for 5 minutes. Quantitatively transfer this solution to a 50 mL volumetric flask, dilute with mobile phase to volume, and mix. Pipet 2 mL of the solution to a 25 mL volumetric flask, dilute with mobile phase to volume and mix.

Procedure: Separately inject equal volumes (about 20 µL) of the standard solution and the test solution into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of each impurity in the portion of choline bitartrate taken by the formula:

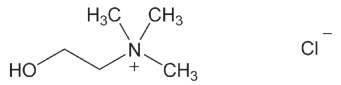
$$(253.25/139.62)62{,}500(C/W)(r_i/r_S)$$

Where, 253.25 and 139.62 are the molecular weights of choline bitartrate and choline chloride, respectively; *C* is the concentration of USP choline chloride, RS, in mg per mL, in the standard solution; W is the weight, in mg, of choline bitartrate taken to prepare the test solution; r_i is the peak response for each impurity, other than that of the choline bitartrate derivative and 3,5-dinitrobenzoic acid; and r_S is the peak response for the choline chloride derivative in the standard solution; not more than 0.3% of any individual impurity is found, and not more than 2% of total impurities is found.

Residual solvents <467>: Meets the requirements, except that the limit for 1,4-dioxane is 10 µg per gram.

Assay: Transfer about 200 mg of choline bitartrate, accurately weighed, to a conical flask, and dissolve with 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically (see Titrimetry <541>). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 25.32 mg of $C_9H_{19}NO_7$ (Table 1).

USP Choline Chloride Reference Standard



$C_5H_{14}ClNO$ 139.62
(2-Hydroxyethyl)trimethylammonium chloride.
2-Hydroxy-*N*,*N*,*N*,-trimethylethanaminium chloride [*67-48-1*].

- Choline chloride contains not less than 99 percent and not more than 100.5 percent of $C_5H_{14}ClNO$, calculated on the anhydrous basis.

Identification

A: Infrared absorption <196 K>.

B: A solution (1 in 20) met Chloride test requirements <190>.

pH <790>: Between 4 and 7 (1 in 10).

Water, method I <920>: Less than 0.5%.

Residue on ignition <280>: Less than 0.05%.

Arsenic, method I <210>: Add 30 mL of water to 5 mL of hydrochloric acid (HCl) to dissolve the sample: the limit is 2 µg per gram.

Lead <250>: Replace chloroform with methylene chloride to prepare solution of the extract of dithizone extraction and standard dithizone.

Ammonium hydroxide-sodium hydroxide solution: Transfer 8.5 grams of sodium hydroxide (NaOH) solution (1 in 2) in a plastic bottle, and add 110 mL of ammonium hydroxide and mix contents.

Standard solution: Transfer 1.1 mL of the diluted standard lead solution to a separatory funnel containing 30 mL of water.

Test solution: Dissolve 4 grams in a separatory funnel that contains 30 mL of water.

Procedure: Add 5 mL of ammonium citrate solution and 3 mL of potassium cyanide solution to standard and test solution. Extract each of the resulting solutions thrice with 6 mL portions of the dithizone extraction solution, shake for 60 seconds, and drain-off each extract into another separator. Add 20 mL of nitric acid (1 in 100) to the combined dithizone solutions and shake for 30 seconds. Discard methylene chloride layer. Add 5 mL of ammonia cyanide solution, 3 mL of ammonium hydroxide-sodium hydroxide solution, and 12 mL of standard dithizone solution. Shake for 50 seconds. Allow the phases to separate. Now, measure the absorbance of the lower layer at 520 nm with a suitable spectrophotometer, found less than 0.4 µg per gram. The absorbance of the test solution is not more than the absorbance of the standard solution.

Heavy metals, method II <231>: 0.001%

Limit of total amines

Standard solution: Weigh an accurate quantity of trimethylamine hydrochloride and dissolve the same in water. Dilute quantitatively and stepwise if necessary, in order to obtain a solution with known concentration of 400 µg per mL.

Test solution: Transfer 15 grams of choline chloride to a beaker with a plastic-coated stirring bar. To this, add 150 mL of water and 40 mL of sodium hydroxide TS. Stir the contents until dissolved.

System suitability solution: Weigh an accurate quantity of trimethylamine hydrochloride and dissolve the same in water. Dilute quantitatively and stepwise if necessary, in order to obtain a solution that contains 15 µg of trimethylamine hydrochloride per mL. Transfer 15 mL of this solution to a beaker containing a plastic-coated stirring bar, add 150 mL of water and 25 mL of sodium hydroxide TS. Stir the contents until they get dissolved.

Electrode system: Use an ammonia-specific gas-sensing electrode with internal reference connected to a pH meter which is capable of measuring potentials with a minimum reproducibility of ±0.11 mV (see pH <790>).

Standard response line: Transfer 25 mL of sodium hydroxide TS to an appropriate beaker, and add water in the quantity to total a volume of 210 mL. Add a plastic-coated stirring bar, thereafter insert electrode into the solution and record the potential in mV. Continue stirring, and at every interval of 5 minutes, add 0.25, 0.65, 1.5, and 2.5 mL of standard solution. Record the potential after each addition of the standard solution. Plot the logarithms of the cumulative trimethylamine concentrations (0.55, 1.55, 2.55, and 5.5 µg per mL) *vs.* potential in mV. Now, determine the slope (S) of the standard response line for the electrode.

System suitability: Proceed with the system suitability solution as directed for test solution in the procedure, and measure the potentials. The trimethylamine equivalent is found to be between 8.65 and 11.55 mg per liter.

Procedure: Rinse the electrode, insert it into the test solution, stir, and record the potential in mV. Add 0.2 mL of the standard solution, and record the potential. Add another 0.2 mL of the standard solution, and record the potential. It may be noted that a third aliquot of 0.3 mL needs to be added if the total change after the second addition of the standard solution is not more than 10 mV. Calculate the quantity, in µg per gram of total amines in the portion of choline chloride taken by the formula:

$$500V_A / (F - 1)W$$

Where, V_A is the total volume of the standard solution added to the test solution; W is the weight in grams of choline chloride taken to prepare the test solution; and the correction factor, F, is calculated by the formula:

$$\text{antilog } [(mV_F - mV_0)/S]$$

Where, mV_F is the final reading in mV, after addition of the standard solution; mV_0 is the initial reading in mV of the test solution; S is the slope of the standard response line for the electrode. Less than 0.0011% was found.

Chromatographic purity

Buffer solution: Dissolve 7.2 grams of anhydrous dibasic sodium phosphate in 1 liter of water. Adjust with phosphoric acid in order to obtain pH 2.5.

Mobile phase: Prepare a filtered and degassed mixture of buffer solution and acetonitrile (70:30).

Standard solution: Transfer an accurately weighed amount, less than 150 mg of USP choline chloride RS to a 25 mL screw-capped vial. Add 380 mg of 3,5-dinitrobenzoyl chloride and 12 mL of acetonitrile. Cap the vial, heat to 55ºC, and continue heating for 2 hours. Cool to room temperature (RT), and add 6 mL of water. Allow the mixture to stand for 5 minutes. Now, quantitatively transfer the solution to a 25 mL volumetric flask, dilute with acetonitrile to volume, and mix. Dilute a volume of this solution with the mobile phase in order to obtain a solution that has a known concentration of 3 µg of USP choline chloride RS per mL.

Test solution: Transfer about 120 mg of choline chloride, accurately weighed, to a 25 mL screw-capped vial. Dry at 125ºC for 2.2 hours. Add 410 mg of 3,5-dinitrobenzoyl chloride and 12 mL of acetonitrile. Cap the vial, heat it to 55ºC, and then, continue heating it for 2.2 hours. Cool to RT, and add 5.5 mL of water. Allow the mixture to stand for 5 minutes. Now, quantitatively transfer the solution to a 50 mL volumetric flask, dilute with mobile phase to volume, and mix. Pipet 3 mL of the solution to a 25 mL volumetric flask, dilute with mobile phase to volume and mix.

Chromatographic system (refer to chromatography <621>): The liquid chromatograph is equipped with a 208 nm detector and a 4.6 mm × 25 cm column, which contains L7 packing. The column temperature is maintained at 30ºC. The flow rate is about 1 mL per minute. The standard solution was chromatographed, and the peak responses were recorded as was directed for the procedure: the capacity factor, k', is not less than 2, and the relative standard deviation (RSD) determined from the choline chloride derivative peak is not more than 6%.

Procedure: Separately inject equal volumes, about 25 µL, of standard and test solution into the chromatograph. Record the chromatograms, and measure all the peak responses. Calculate the percentage of each impurity in the portion of choline chloride taken by the formula:

$$62{,}500(C/W)(r_i/r_S)$$

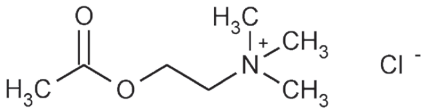
Where, C is the concentration in mg per mL of USP choline chloride RS in the standard solution; W is the weight in mg of choline chloride that was taken to prepare the test solution; r_i is the peak response for each impurity, other than that for the choline chloride derivative and 3,5-dinitrobenzoic acid obtained from the test solution; and r_S is the peak response for the choline chloride derivative obtained from the standard solution. The individual impurity found was less than 0.35% and the total impurity found was less than 2%.

Residual solvents <466>: Meets the requirements, except that the limit for 1,4-dioxane is 10 µg per gram.

Assay: Transfer an accurately weighed quantity of choline chloride, about 120 mg, to a conical flask, dissolve in 35 mL of water, and add 3 drops of acetic acid. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically (see Titrimetry <541>). Perform a blank determination, and make

any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 13.96 mg of $C_5H_{14}ClNO$.

USP Acetylcholine Chloride Reference Standard



$C_7H_{16}ClNO_2$ 181.66

Ethanaminium, 2-(acetyloxy)-*N*,*N*,*N*-trimethyl-, chloride. Choline acetate (ester) chloride [*60-31-1*].

- Acetylcholine chloride contains not less than 98 percent and not more than 102 percent of $C_7H_{16}ClNO_2$, calculated on the dried basis.

Identification

A: Infrared absorption <196 K>.

B: Add 5.5 mL of silver nitrate TS to 5.5 mL of solution (1 in 10). A white, curdy precipitate is formed, which is soluble in ammonium hydroxide and insoluble in nitric acid.

Melting range, class I <740>: Between 149 and 152ºC.

Acidity: Dissolve 110 mg in 12 mL of recently boiled water, and add one drop of bromothymol blue TS immediately. It is observed that less than 0.55 mL of 0.01 N sodium hydroxide produces a color change.

Loss on drying <730>: Dry it at 105ºC for 3.5 hours. It lost less than 1.1% of its weight.

Residue on ignition <280>: This was less than 0.21%.

Chloride content: Transfer accurately weighed 280 mg to a porcelain casserole, and add 140 mL of water and 1 mL of dichlorofluorescein TS. Mix the solution. Now, titrate with 0.1 N silver nitrate VS until silver chloride flocculates and the mixture turns faint pink in color. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). The quantity of Cl calculated on dried basis was found to be more than 19.355% but less than 19.785%.

Assay: Accurately weight 400 mg of acetylcholine chloride, and dissolve in 15 mL of water in conical flask with a glass stopper. Add 41 mL of 0.1 N sodium hydroxide VS, and heat on steam bath for 30 minutes. Insert the glass stopper and allow the mixture to cool. Now, add phenolphthalein TS, and then titrate excess alkali with 0.1 N sulfuric acid VS. A blank determination is performed (refer residual titrations under titrimetry <541>). Each mL of 0.1 N sodium hydroxide is equivalent to 18.17 mg of $C_7H_{16}ClNO_2$.

EUROPEAN PHARMACOPEIA

Acetylcholine chloride reference standard acetylcholini chloridum C7H16ClNO2 Mr 181.7 [60-31-1], definition 2-(scetyloxy)-N,N,N-trimethylethanaminium chloride. Content: 98.55 to 101.55% (dried substance).

Identification

First identification: B, E.

Second identification: A, C, D, E.

A. Melting point (2.2.13): This is from 149.1 to 152.2°C. The substance to be examined in put into a capillary tube. It is dried in an oven at about 100 to 106°C for 3.1 hours. Then, the tube is sealed and melting point is determined.

B. Infrared absorption spectrophotometry (2.2.23). Comparison: Acetylcholine chloride CRS.

C. Examine the chromatograms obtained in the test for related substances. The result is that the principal band in the chromatogram obtained with test solution (b) is similar in position, color, and size to the one obtained with reference solution (b).

D. Add 12 mL of dilute sodium hydroxide solution R to 16 mg, and then 2.5 mL of 0.025 M potassium permanganate. Then, heat the contents. By the vapors formed, the color of the litmus paper R gets changes from red to blue.

E. 0.55 mL of solution S (refer tests) gives reaction (a) of chlorides (2.3.2). Test solution S. Dissolve 5.5 grams in carbon dioxide (CO_2)-free water R and then, dilute to 55 mL using same solvent.

Appearance of Solution

Solution S is clear (2.2.2) and less intensely colored than the reference solution Y6 or BY6 (2.2.1, method II).

Acidity

Dilute 1.2 mL of solution S to make it 12 mL using CO_2-free water R. Add 0.055 mL of phenolphthalein solution R. It was found that color of the indicator got changed to pink with less than 0.45 mL of 0.01 M sodium hydroxide.

Related Substances—Thin-Layer Chromatography (2.2.26)

The solutions are to be prepared immediately before their use.

Test solution (a): 0.35-gram of the substance which is to be examined is dissolved in methanol R. Then the mixture if diluted to 3.5 mL using the same solvent.

Test solution (b): Using methanol R, 1.5 mL of the test solution (a) is then diluted to 10.5 mL.

Reference solution (a): Using methanol R, 1.5 mL of the test solution (a) is then diluted to 100 mL.

Reference solution (b): Using the same solvent, 25 mg of acetylcholine chloride CRS dissolved in methanol R is diluted.

Reference solution (c): 0.5 mL of test solution (a) is added to 25 mg of choline chloride R dissolved in methanol R. This solution is further diluted to 2.5 mL using methanol R.

Plate: TLC silica gel plate R.

Mobile phase: Mix 25 volumes of a 45 g/liter solution of ammonium nitrate R, 25 volumes of methanol R, and 65 volumes of acetonitrile R.

Application: 6 µL in the form of bands of 10.5 × 2.5 mm.

Development: Over 2/3rd of the plate.

Detection: Spray with potassium iodobismuthate solution R3.

System suitability: The chromatogram obtained with reference solution (c) shows two clearly separated bands.

Limits: Impurity, if any. Other than the principal band, all other bands obtained with the test solution (a) in the chromatogram,

are less intense than the principal band obtained with 1% of the reference solution (a) chromatogram.

Trimethylamine: Take 15 mL of sodium carbonate solution R and then dissolve 0.12-gram in it. Now, heat the solution to boiling. Litmus paper R did not change its color from blue and no vapors appeared.

Heavy metals (2.4.7): The maximum limit is 12 ppm. It is observed that 10 mL of solution S is in compliance with limit test A. The standard solution of lead (Pb) is used to prepare the standard of 1.5 ppm Pb R.

Loss on drying (2.2.30): The maximum loss observed is 1%. In order to determine the loss on drying, 1.5 grams was dried in an oven for 3.5 hours at 110°C.

Sulfated ash (2.4.13): The maximum sulfated ash that was observed is 0.15%. During the test for loss on drying, the sulfated ash was determined in the residue.

Assay: For this, 0.25-gram was dissolved in 25 mL of carbon dioxide-free water R. The solution was then neutralized using 0.01 M sodium hydroxide. 0.16 mL of phenolphthalein solution R was used as an indicator. Then, 25 mL of 0.1 M sodium hydroxide was added. The mixture was allowed to stand for 40 minutes. Thereafter, the mixture was titrated with 0.1 M HCl. It is observed that 1.5 mL of 0.1 M NaOH is equivalent to 18.29 mg of $C_7H_{16}ClNO_2$.

RUSSIAN PHARMACOPIEA

Acetylcholine chloride [60-31-1]. $C_7H_{16}ClNO_2$. (M.M. 181.66). N-[2-(acetyloxy) ethyl] -N, N, N-trimethylammonium chloride. It is in the form of crystalline powder. It can be easily dissolved in cold water, as well as, 96% alcohol. It is practically insoluble in ether. It decomposes in hot water and alkali solutions. It is to be stored at a temperature of -20ºC.

Pharmaceutical Analysis for Choline Ester Derivatives

A high-performance liquid chromatography (HPLC)/ electrospray ionization mass spectrometry (EIMS) is used to detect choline and ACh in a pharmaceutical preparation. In pharmaceutical preparations that contain choline esters, it is important to use certain detective methods for choline or acetylcholine in these preparations.

ACh and choline detection using ultra-violet (UV) is extremely difficult since strongly UV chromophore, which strongly absorb, are absent in the molecule(s).[65-67] That is why there are other methods are suggested which include electrochemical,[68-72] or spectrophotometric detection,[73] since in both these methods, prior enzymatic reaction is required, and various other methods that involve materials, which are labelled radioisotopically.[74-76] The first method for ACh detection was done by the use of mass spectroscopy with gas chromatography. This method is specially used for quaternary ammonium species. It is observed that there is significant improvement over the methods which were earlier available, e.g., bioassay.[77] However, this approach is still not direct. The reason being that it requires the conversion of quaternary ammonium species to volatile tertiary amine through demethylation reaction, and this reaction could be achieved chemically[78] or through pyrolysis.[79] Ionization methods, like, thermo spray ionization[80] and fast-atom bombardment[81] were immediately put to use for the analysis of ACh and other related compounds,[82,83] since they enabled the combination of online HPLC separations using highly sensitive mode of mass spectrometric detection. Further, no pre-treatment of non-volatile or thermally fragile analytes was required in these ionization methods. A lot of renovations were done to introduce a new method which is called electro spray ionization (ESI).[84] This method is better than other methods when it used with mass spectroscopy to detect ACh and with a separation method like HPLC.[64]

Another study used refractive index due to the absence of UV chromophore, but the disadvantage of this method that it is not suitable for the detection of low levels ACh that come up against when it is needed to make analysis to ensure the presence of this neurotransmitter in certain matrices, like cerebrospinal fluids. Some new methods are used to change ACh to hydrogen peroxide following electrochemical detection, but this method offers a low detection limit.

It has been confirmed that ACh can be detected by the use of certain reagents, like alkyl sulfonate, which is considered as an ion-pairing agent for HPLC,[85-89] other methods, like mass spectroscopy, are also used for ACh detection,[82,84,90-94] but it is important to know that this method requires a volatile phase.

CONCLUSION

According to the previous studies which had been done for the modification of choline esters and their uses nowadays as drugs for the treatment of many diseases, this gave us the inspiration to look forward to further modifications of choline esters, which will have the same effect as ACh with higher activity and efficacy. So our study is relevant at the present time and will be expected as a significant breakthrough in science.

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