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THE STUDY OF DEGRADATION PRODUCTS OF CHLORPROMAZINE HYDROCHLORIDE BY THE METHOD OF LIQUID-MASS SPECTROSCOPY IN DRUGS FOR INJECTION

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Key words: chlorpromazine hydrochloride; chlorpromazine sulfoxide; chlorpromazine N-oxide; nor-chlorpromazine; chromatography mass-spectrometry; degradation products

The analysis of model samples of the drug Aminazin has been performed, its active substance is chlorpromazine hydrochloride. These model solutions were subjected to sterilization with previous exposure at room temperature. It has been found that depending on duration of the exposure during sterilization the degradation of the drug occurs in two possible ways – with formation of opalescence or without it. The schemes of degradation with formation of either one product – chlorpromazine sulfoxide, or some products – chlorpromazine sulfoxide, chlorpromazine N-oxide, nor-chlorpromazine and others have been proposed. It has been found that opalescence of solutions is caused by formation of degradation products – chlorpromazine N-oxide and nor-chlorpromazine that are slightly soluble in water. The analysis of model samples was performed using the liquid chromatography/mass spectrometry methods developed by the authors. In the course of analysis the molecular weights corresponding to the abovementioned products were obtained. Therefore, the experimental confirmation of the schemes of degradation proposed has been obtained. As the result of the research conducted the chromatographic method for detection of impurities using liquid chromatography/mass spectrometry has been developed, and the main ways of degradation of chlorpromazine hydrochloride in aqueous solutions have been determined. According to the result of the research the recommendations to the manufacturing process have been developed, and measures for optimizing the composition of the drug have been proposed.

ВИВЧЕННЯ ПРОДУКТІВ ДЕГРАДАЦІЇ ХЛОРОПРОМАЗИНУ ГІДРОХЛОРИДУ МЕТОДОМ РІДИННОЇ МАС-СПЕКТРОСКОПІЇ У ІН'ЄКЦІЙНИХ ПРЕПАРАТАХ

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Ключові слова: хлорпромазину гідрохлорид; хлорпромазину сульфоксид; хлорпромазину N-оксид; нор-хлорпромазин; хроматомас-спектрометрія; продукти деградації

Проведений аналіз модельних зразків препарату Аміназин, діючою речовиною якого є хлорпромазину гідрохлорид. Вказані модельні розчини пройшли стерилізацію з попередньою витримкою при кімнатній температурі. Встановлено, що залежно від тривалості експозиції при стерилізації деградація препарату проходить за двома можливими шляхами – з утворенням опалесценції або без неї. Були запропоновані схеми деградації з утворенням або одного продукту хлорпромазину сульфоксиду, або декількох – хлорпромазину сульфоксиду, хлорпромазину N-оксиду, нор-хлорпромазину та ін. Було встановлено, що опалесценція розчинів викликається утворенням водонерозчинних продуктів деградації – хлорпромазину N-оксиду, нор-хлорпромазину. Аналіз модельних зразків проводився методом рідинної хроматомас-спектрометрії за методикою, розробленою авторами статті. Під час аналізу були отримані молекулярні маси, що відповідають вказаним вище продуктам. Таким чином, було отримано експериментальне підтвердження запропонованих схем деградації. У результаті проведеної роботи нами була розроблена хроматографічна методика виявлення домішок методом рідинної хроматомас-спектрометрії та визначені головні шляхи деградації хлорпромазину гідрохлориду у водних розчинах. Також у результаті проведених досліджень були розроблені рекомендації до виробничого процесу та запропоновані заходи з оптимізації складу препарату.

ИЗУЧЕНИЕ ПРОДУКТОВ ДЕГРАДАЦИИ ХЛОПРОМАЗИНА ГИДРОХЛОРИДА МЕТОДОМ ЖИДКОСТНОЙ МАСС-СПЕКТРОСКОПИИ В ИНЪЕКЦИОННЫХ ПРЕПАРАТАХ

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Ключевые слова: хлорпромазина гидрохлорид; хлорпромазина сульфоксид; хлорпромазина N-оксид; нор-хлорпромазин; хроматомас-спектрометрия; продукты деградации

Проведен анализ модельных образцов препарата Аминазин, действующим веществом которого является хлорпромазина гидрохлорид. Данные модельные растворы были подвергнуты стерилизации с предварительной выдержкой при комнатной температуре. Установлено, что в зависимости от длительности экспозиции при стерилизации деградация препарата происходит по двум возможным путям – с образованием опалесценции либо без нее. Были предложены схемы деградации с образованием либо одного продукта хлорпромазина сульфоксида, либо нескольких – хлорпромазина сульфоксида, хлорпромазина N-оксида, нор-хлорпромазина и пр. Было установлено, что опалесценция растворов вызывается образованием мало растворимых в воде продуктов деградации – хлорпромазина N-оксида, нор-хлорпромазина. Анализ модельных образцов проводился методом жидкостной хроматомас-спектрометрии по методике, разработанной авторами статьи. В ходе анализа были получены молеку-

лярные массы, соответствующие указанным выше продуктам. Таким образом, было получено экспериментальное подтверждение предложенных схем деградации. В результате проведенной работы нами была разработана хроматографическая методика обнаружения примесей методом жидкостной хроматомасс-спектрометрии и определены основные пути деградации хлорпромазина гидрохлорида в водных растворах. Также в результате проведенных исследований были разработаны рекомендации к производственному процессу и предложены меры по оптимизации состава препарата.

Today phenothiazine derivatives are widely used in the pharmaceutical industry. The application of these substances is stipulated by their neuroleptic, antihistaminic and anti-arrhythmic effect on the human body. Chlorpromazine is the first synthesized neuroleptic (1950), a parent compound of phenothiazine drugs (including trifluoperazine, promazine, fluphenazine, etc.) and the basis for many of antipsychotics and antidepressants. In this regard, drugs with antipsychotic activity, in which chlorpromazine hydrochloride is the active pharmaceutical ingredient, are the most widespread at the pharmaceutical market. One of the representatives of such drugs is Aminazin, solution for injection.

The substance of chlorpromazine hydrochloride is described in the articles of such pharmacopoeias as the European Pharmacopoeia PhEur 8.0 "Chlorpromazine hydrochloride" (07/2012:0475) [1], United States Pharmacopoeia USP37-NF32 c.2318 and State Pharmacopoeia of Ukraine (SPhU) 1.0 [3]. The USP37-NF32 has also the article [2] containing requirements for the quality control of solutions for injection. However, even the use of these regulations to control the quality of the raw material and finished products is not a guarantee of quality. There are some reasons for it, in particular because of the quality of the substance and the technological process of preparation of the solution since this substance is highly reactive, and the slightest deviation from the validated technologies of the drug production may lead to the loss of quality of the finished product.

According to the IUPAC classification [5] chlorpromazine hydrochloride (2-chloro-10-[3-(dimethylaminopropyl)]phenothiazine hydrochloride) being a derivative of phenothiazine (10H-dibenzo-[b,e]-1,4-thiazine) belongs to heterocyclic compounds containing the atoms of sulphur and nitrogen in the cycle.

A high reactivity of phenothiazine and chlorpromazine [5] is a consequence of the presence of easily oxidizable sulphur atom in the structure of these substances (Fig. 1).

Under the effect of such strong oxidizers as potassium permanganate, hydrogen peroxide these compounds may form phenothiazine oxide-5 and phenothiazine dioxide-5,5 due to oxidation of the sulphur atom. It is confirmed by the requirements of the USP article on control of the content of chlorpromazine sulfoxide in solutions for injection using the thin-layer chromatography [2].

The tertiary nitrogen in the structure of chlorpromazine hydrochloride can also be oxidized in neutral or acidic media to N-oxide. In addition, for pheno-

thiazine and its derivatives the electrophilic substitution reactions, in which these compounds act as electron donors, are typical [8].

Thus, there is a need of inhibition of the oxidation process of chlorpromazine hydrochloride with atmospheric oxygen when preparing the solution of the drug. The solution to this problem was to introduce the substances – sodium metabisulfite, sodium sulfite absorbing oxygen dissolved in the product in the composition of the drug by interacting with it and to purge the solution with nitrogen to displace oxygen.

However, despite the measures taken to remove oxygen from the solution of the drug there are cases of noncompliance of the drug quality to requirements of the Pharmacopoeia by "Transparency" indicator – opalescence is observed in solutions [4].

Therefore, manufacture of a drug corresponding to the requirements of the Pharmacopoeia requires formation of additional requirements to the technological process and the quality of the substance; it can be done knowing the cause of impurities and their structure. To study the possible degradation products of chlorpromazine hydrochloride the LC-MS/MS method has been developed.

In the conditions given below the chromatograms of the full ion current were obtained for the samples of the solution of chlorpromazine sterilized immediately after preparation and after exposure for 48 h. The samples were prepared from the substance of one and the same manufacturer.

The chromatograms and mass spectra of the substances detected are given in Figures below.

Experimental Part

To study the possible degradation products of chlorpromazine hydrochloride the model solutions corresponding to the composition of the drug were prepared and placed in glass vials similar to those used in the manufacture of the drug Aminazin. All model

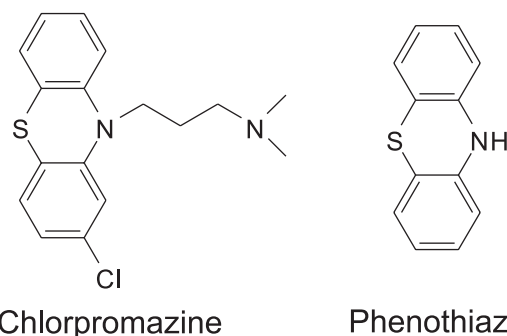


Fig. 1. The structural formulas of phenothiazine and chlorpromazine.

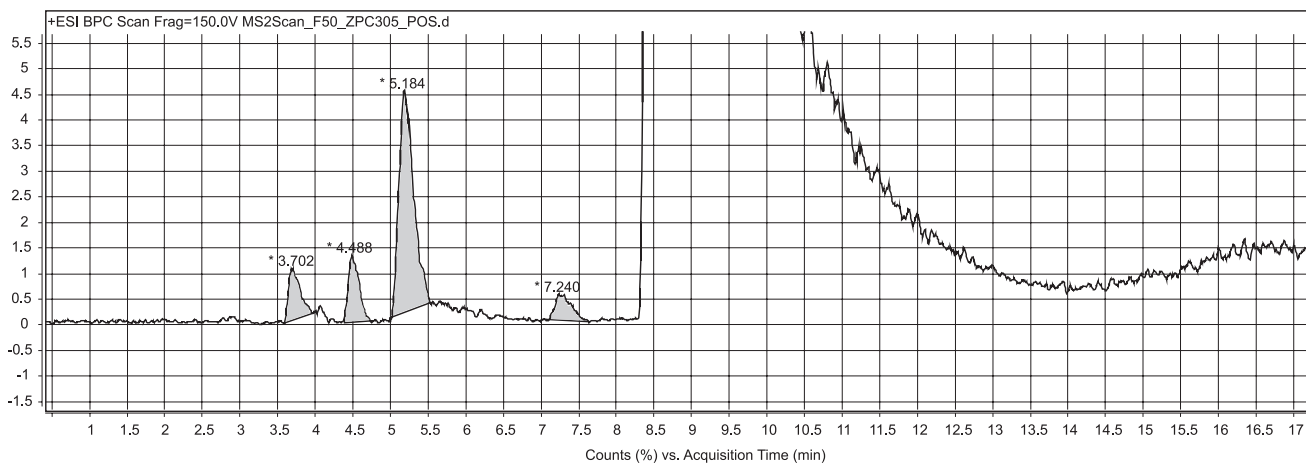


Fig. 2. The chromatogram of the full ion current obtained for the sample immediately after sterilization.

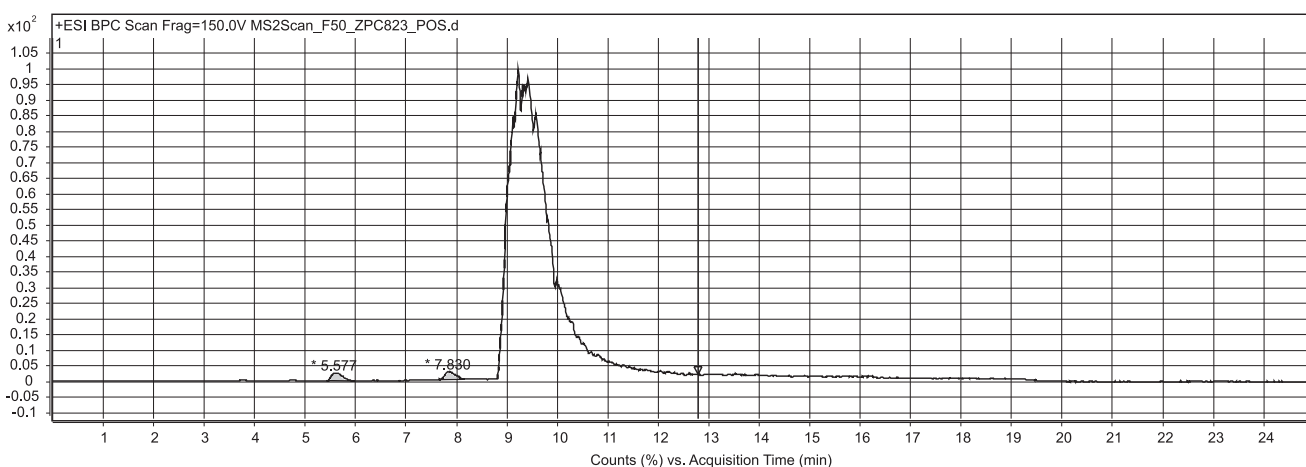


Fig. 3. The chromatogram of the full ion current obtained for the sample sterilized after exposure.

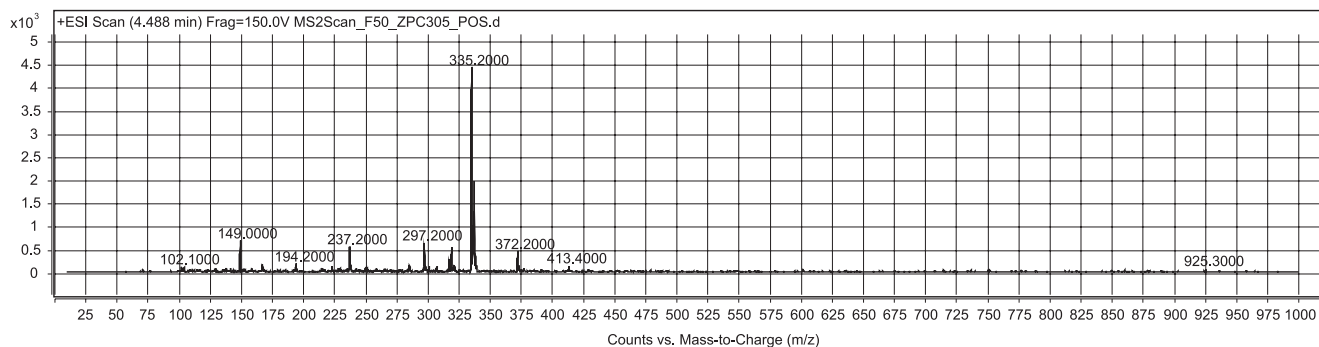


Fig. 4. The mass spectrum of chlorpromazine sulfoxide and N-oxide (the time of escape peak is 5.184 min).

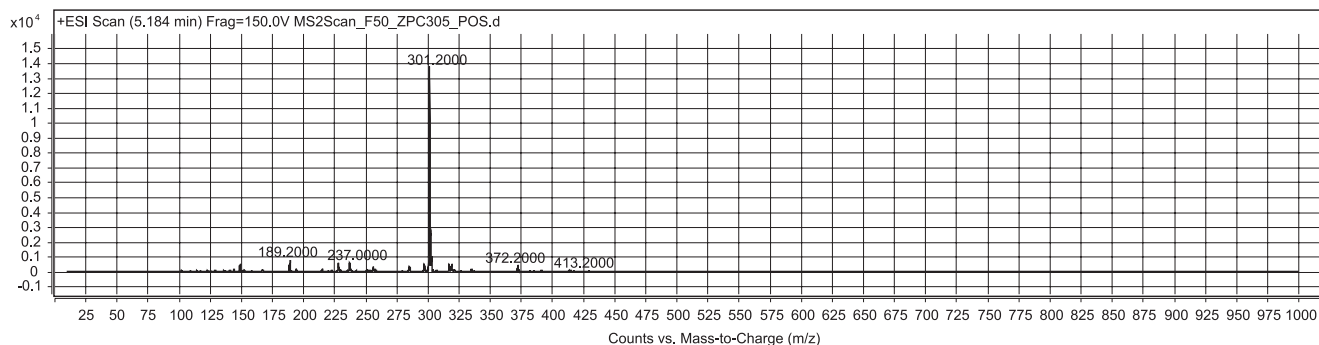


Fig. 5. The mass spectrum of nor-chlorpromazine (the time of escape peak is 4.488 min).

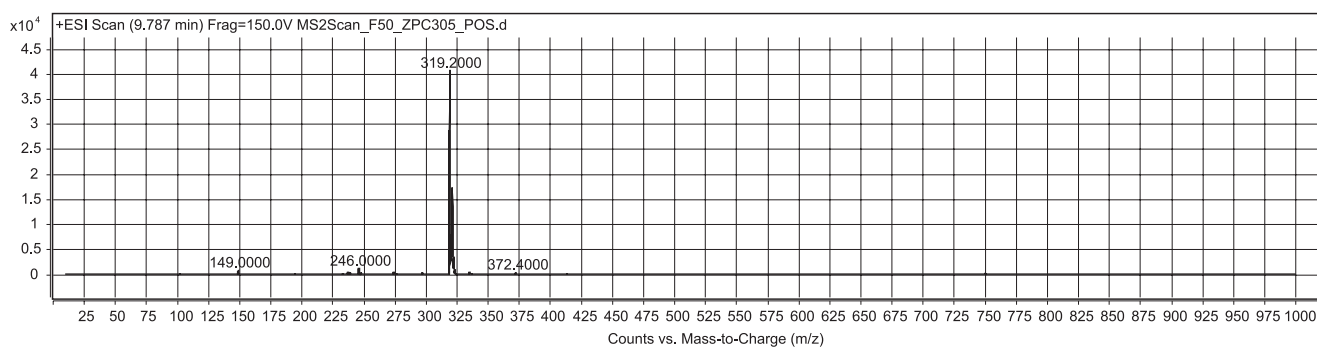


Fig. 6. The mass spectrum of chlorpromazine (the time of escape peak is 9.787 min).

solutions were prepared from the same batch of chlorpromazine hydrochloride substance of the Indian manufacturer. All indicators of the substance quality is fully consistent with the requirements of the article of the European Pharmacopoeia 8.0.

These solutions were sterilized in the conditions corresponding to the conditions of the drug production. It was observed that solutions sterilized immediately after preparation, did not meet the requirements of Pharmacopoeia [2] by "Transparency" indicator – opalescence appeared in these solutions. However, those solutions, in which 48 hours passed from their preparation and sterilization, were fully consistent with the requirements of the article by "Transparency" indicator.

Since for the study of the structure of possible degradation products a highly selective detector is required, an Agilent 6420 Triple Quad mass spectrometer was applied as a detection system. This detector was used together with an Agilent 1100 liquid chromatograph equipped with a diode array, four-channel pump for formation of a low pressure gradient, an autosampler and a column thermostat. To process the measurement results obtained the MassHunter software version B.05.00 was used.

The procedure of conducting chromatographic measurements is given below:

- the column with the size of 250×4.6 mm filled with a sorbent with the bonded phase of octyl silica gel (L1), the particle size is 5 μm , XTerra MS C18 (Waters) or similar, for which the requirements of the section "Chromatographic system suitability" are met;
- the flow rate – 1 ml/min;
- the temperature of the column thermostat – 30°C;
- the mobile phase A: 0.005 M solution of ammonium formate in water *R* degassed in any convenient way;
- the mobile phase B: 0.005 M solution of ammonium formate in the mixture of acetonitrile *R* – water *R* (90:10) degassed in any convenient way;
- the elution mode – gradient.

No.	Time, min	Mobile phase A	Mobile phase B	The elution mode
1	0-5	100	0	Isocratic
2	5-15	100 → 10	0 → 90	Linear gradient
3	15-16	10 → 100	90 → 0	Linear gradient
4	16-20	100	0	Isocratic

- the injection volume – 20 μl ;
- detector – mass spectrometer (Agilent 6420 Triple Quad);
- the detector settings:
- the ionization type: positive, electrospray (+ESI);
- the measurement mode: scanning in the mass range – 10-1000 amu;
- the voltage on the fragmentor – 100 V;
- the nitrogen temperature – 350°C;
- the nitrogen consumption – 10 ml/min;
- the nebulizer pressure – 35 PSI;
- the voltage on the capillary – 4 kV.

Results and Discussion

After analyzing the solutions on a liquid chromatography-mass spectrometer (Agilent 6420 Triple Quad) it was found in addition to the main mass of 319 amu the substances with the masses of 335, 305, 285 and 317 $[\text{M}+\text{H}]^+$ amu were present in solutions. It confirms the presence of chlorpromazine sulfoxide, chlorpromazine N-oxide, nor-chlorpromazine in the solution [6, 7].

Based on the data obtained it was determined that degradation of aqueous solutions of chlorpromazine during sterilization occurred according to the scheme given in Fig. 7. Thus, two possible ways of degradation were considered: with the excess and the lack of oxygen in the drug solution.

The above diagram explains well how in the case of the lack of oxygen (between preparation and sterilization enough time has passed and stabilizers are oxidized removing oxygen from the solution) chlorpromazine is oxidized to chlorpromazine sulfoxide. It is a crystalline substance that is readily soluble in water and does not cause opalescence of the solution. In the case of the oxygen excess (sterilization immediately after preparation, the reducing agents

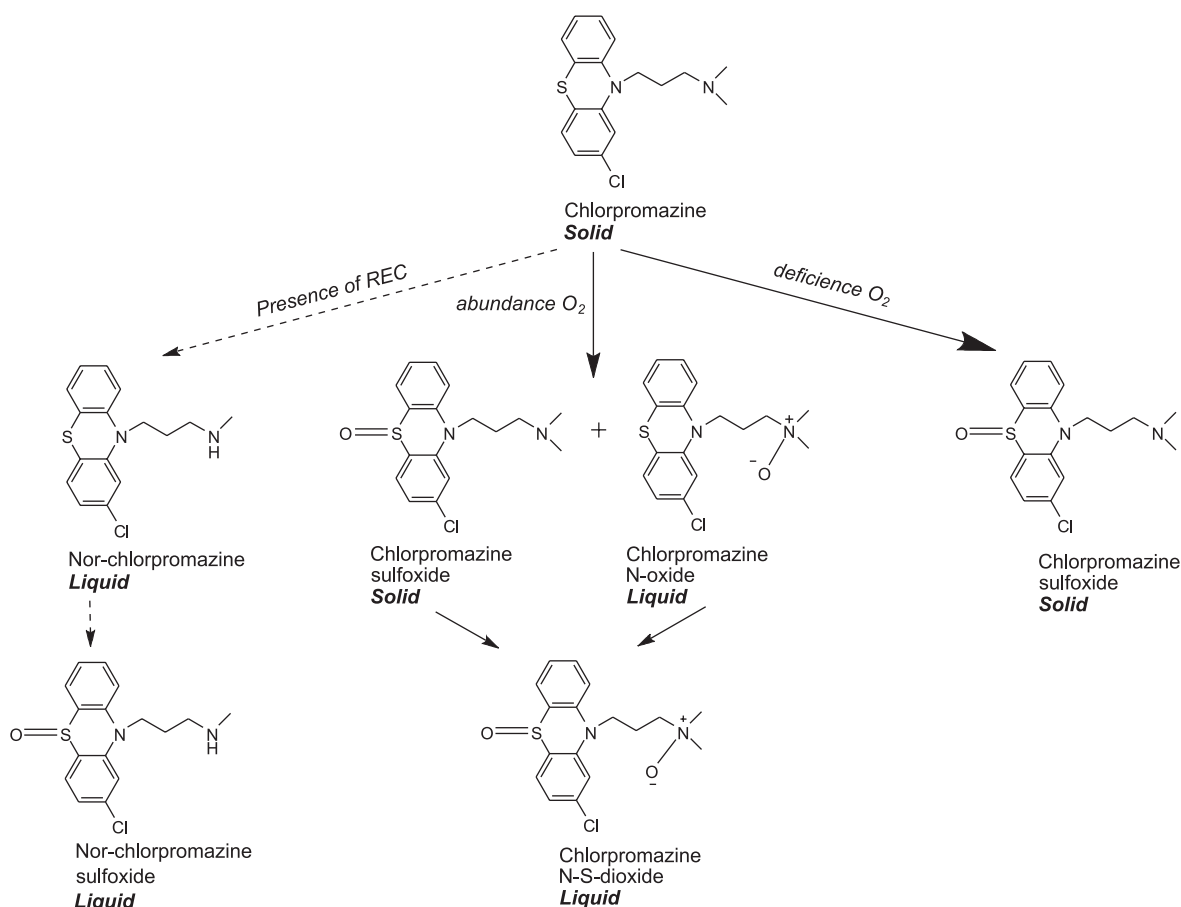


Fig. 7. The ways of degradation of chlorpromazine hydrochloride in an aqueous solution.

in the solution have no time to interact with oxygen) chlorpromazine being oxidized forms simultaneously chlorpromazine sulfoxide and chlorpromazine N-oxide, which can then be oxidized to chlorpromazine N-S-dioxide, and the presence of unused reducing agents in the solution provides a parallel process of formation of nor-chlorpromazine and the corresponding sulfoxide. Since chlorpromazine N-oxide, chlorpromazine N-S-dioxide, nor-chlorpromazine and nor-chlorpromazine sulfoxide are amorphous substances that are poorly soluble in water, the two-phase heterogeneous system is formed in the solution, and it is the cause of opalescence. Impurities of the chlorpromazine substance (for example, chlorphenothiazine) can also be oxidized to sulfoxides; however, since the Pharmacopoeia normalizes the content of impurities in the range of 0.15-0.3%, the contribution of their derivatives to the overall picture of the profile of impurities of the drug is insignificant.

The results obtained are in good agreement with the work of the British scientists [8], in which the principal possibility of formation of the impurities analyzed is shown.

Based on the research results mentioned above one can judge about the nature of possible degradation products; it, in turn, has allowed to develop a number of measures that make possible to obtain the drug, which fully complies with the requirements of

Pharmacopoeia. Thus, it has been proposed to perform the exposure of the solution prepared before its sterilization during the manufacturing process, it will allow to remove dissolved oxygen most fully from the solution. It has been also suggested to optimize the process of removing oxygen from the solution of the drug by increasing the purging time and toughening of requirements to the purity of the nitrogen used. However, changes in the technological process is not the only way to provide the quality of the drug prepared. Quite effective way is to optimize the composition of the drug. Therefore, it has been proposed to increase the concentration of substances absorbing oxygen in the composition of the drug, it will reduce the risk of its opalescence in formation of oxidation products of chlorpromazine hydrochloride.

Conclusions

As the result of the research conducted the chromatographic method for detection of impurities using liquid chromatography/mass spectrometry has been developed; the main ways of degradation of chlorpromazine hydrochloride in aqueous solutions have been determined; the recommendations for improving the process of drug manufacture based on the specified substance have been developed, and measures for optimizing the composition of drugs based on chlorpromazine have been proposed.

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