ANALYSIS OF CLOZAPINE TABLETS USING A NEW ANALYTICAL REACTION TARGETING ITS TERTIARY AMINO GROUP

Blazheyevskiy M. Ye., Moroz V. P., Kryskiv O.S., Shpychak O.S.
National University of Pharmacy,
Kharkiv, Ukraine

Introduction. Clozapine is a tricyclic dibenzodiazepine (8-Chloro-11-(4-methylpiperazin-1-yl)-5H-dibenzo[*b,e*][1,4]diazepine, Fig. 1) that is structurally very similar to Loxapine, classified as an atypical antipsychotic agent. It binds several types of central nervous system receptors, and displays a unique pharmacological profile. Clozapine is a serotonin antagonist, with strong binding to 5-HT 2A/2C receptor subtype. It also displays strong affinity to several dopaminergic receptors, but shows only weak antagonism at the dopamine D2 receptor, a receptor commonly thought to modulate neuroleptic activity. Agranulocytosis is a major adverse effect associated with administration of this agent [1]. It is primarily used to treat people with schizophrenia and schizoaffective disorder who have had an inadequate response to two other antipsychotics or who have been unable to tolerate other drugs due to extrapyramidal side effects. It is also used for the treatment of psychosis in Parkinson's disease.

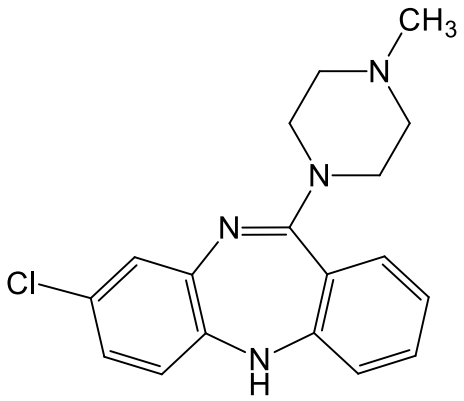

Fig. 1 The structural formula of Clozapine

Clozapine (CZP) is an official drug in USP, EP, BP, and IP. Clozapine (CZP) is available in pale yellow tablets of 25 mg and 100 mg for oral administration. Inactive Ingredients: colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch (corn), and talc.

To date, techniques for the determination of clozapine, including titrimetry [2], HPTLC [3] and HPLC [4, 5], spectrophotometry [6-8], differential pulse voltammetry [9] and other [10]. The official Pharmacopeia of United States monographed a HPLC assay procedure [11] for CZP in tablets.

Nevertheless, the required complex equipments is common disadvantages of these methods. Therefore, the development of a low-cost, simple, sufficiently sensitive and precise titrimetric and/or spectrophotometric method for the determination of clozapine in pharmaceutical formulations is desirable.

There are no data in the literature on the titrimetric and spectrophotometric determination of CZP using potassium hydroperoxomonosulfate, which inspires us to develop these methods.

The aim of the study. To develop two (titrimetric and spectrophotometric) simple, rapid, sensitive and cost-effective methods for the determination of clozapine (CZP) in pharmaceutical dosage forms.

Research methods. The titrimetric method (A) is based on an *N*-oxidation reaction using potassium hydroperoxomonosulfate and subsequent iodometric back titration of the known residual reagent. The spectrophotometric method (B) is based on the stoichiometric *N*-oxidation of CZP with potassium hydroperoxomonosulfate in an alkaline medium, followed by determination of the unreacted oxidizing agent by reaction with excess iodide in an acidic medium to obtain a chromogen (triiodide) with a maximum absorption wavelength at 350 nm.

CZP was oxidized to a CZP *N*-oxide with the aid of potassium hydrogen peroxymonosulfate ($KHSO_5$), a component in the commercial product called Oxone®; Formula of Oxone®: 2KHSO5 ·$KHSO_4$ ·K_2SO_4; CAS Number. 70693-62-8, extra pure, min. 4.5% active oxygen, ACROS Organics™; Its formula weight is 614.78 g/mol. Moreover, it is considered as "green" oxidizing agent because of its non-toxic effect. *Solutions:* $KHSO_5$, 2×10^{-2} mol/L from analytical-grade Oxone. Potassium Iodide, 5 % from analytical-grade potassium iodide. Sulfuric acid, $c(H_2SO_4)$ = 0.5 mol/L, volumetric solution. Sodium thiosulfate standard solution [$c(Na_2S_2O_3{\cdot}5H_2O)$ = 0.1 mol/L]. *Buffer solutions*: 0.2 M solution potassium pyrophosphate with values of 8.6 and 9.3. A stock standard solution of the pure preparation containing 5×10^{-4} mol/L (0.16341 mg/mL) CZP was prepared by dissolving 163.41 mg (exactly weighed) CZP in a 1 L volumetric flask in doubly distilled water and used in a titrimetric method. A standard solution of pure preparation containing 1×10^{-4} mol/L (0.032682 mg/mL) CZP was prepared diluted of the stock standard solution of the pure preparation with double-distilled water exactly 5 time to obtain the working concentration (1×10^{-4} mol/L) in double-distilled water and used in a spectrophotometric method.

Titrimetrimetric assay. An aliquot of the test solution (5.0 – 20.0 ml), containing 0.1 – 3.5 mg of CZP, is added to a 150 ml conical flask with a ground stopper, and 20 ml of a buffer mixture with pH 8.6 is added. With stirring, add a 0.002 mol/L solution of potassium hygrogen peroxymonosulfate with a pipette based on the calculation of 50% excess relative to the amount of CZP being determined and leave for 20 min. Then the solution is acidified with sulfuric acid to pH 3-4 and 1 ml of 5% KI solution is added. The flask is closed, the contents of the flask are stirred for 30 s, and the released iodine is titrated with a 0.01 mol/L solution of sodium thiosulfate in the presence of starch. Then a control experiment is carried out with the same amount of $KHSO_5$ under similar conditions. The CZP content corresponds to the amount of potassium peroxymonosulfate consumed for its oxidation.

Spectrophotometric determination of CZP in tablets Azapin 25 mg: twenty tablets were weighed and pulverized. The equivalent to 25 mg of *CZP* was dissolved

in double distilled water and filtered; the residue was rinsed, volume adjusted to 150 mL, and further diluted with same diluent exactly 5 time to obtain the working concentration (1×10^{-4} mol/L). The prepared aqueous solution of *CZP* (2.00 mL) and 2×10^{-4} mol/L $KHSO_5$ solution (3.00 mL) were pipetted into a 50 mL graduated flask, and subsequent addition of reactants, diluents and buffers as in the above-written spectrophotometric procedure for obtaining results for calibration graph. The prepared solution was made up to 50 mL (after being kept for 60 seconds) and measured absorbance measured; the amount of CZP present in the sample was computed from the calibration curve.

Main results. A kinetic study using iodometric titration showed that the optimal time for quantitative interaction is 20 min at pH = 8.6. The stoichiometry of the reaction was estimated to be 1:1 (CZP : $KHSO_5$). The product of the oxidation reaction of CZP was identified by preparative chemistry as *N*-oxide CZP. Based on the results obtained, the interaction of CCP with potassium hydroperoxomonosulfate can be represented by a Scheme (Fig. 2).

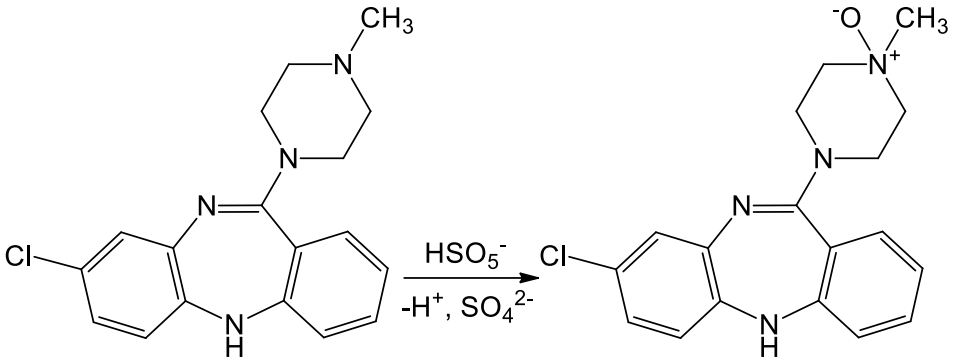

Fig. 2 Scheme reaction of CZP with potassium hydroperoxomonosulfate

For the first time, we have developed methods for the analysis of clozapine tablets using a new analytical reaction aimed at its tertiary amino group. The proposed methods were successfully applied to the determination of clozapine in its dosage forms. The results obtained were in good agreement with those obtained using the B.P. official method. The proposed methods are superior to other published articles that they do not need the use of sophisticated instrumentation, more cost effective, simple and sensitive. Method “A” was applicable over the concentration range of 0.1-3.5 mg to aliquot of the test solution (5.0 – 20.0 ml). In method “B”, Beer's law was obeyed over the concentration range of 0.3-3.5 μg/mL with a molar absorptivity of 24600 L/mol cm. The limits of quantification (10S) were calculated to be 0.05 mg/5 mL (A) and 0.2 μg/mL (B), respectively.

Conclusions. The suggested methods are simple, accurate, selective and sensitive with no significant difference in the precision. Application of the proposed methods to the analysis of clozapine in laboratory prepared mixtures and pharmaceutical formulations shows that neither the degradation product nor the excipients interfere with the determination, indicating that the proposed methods could be applied as stability indicating methods for the determination of clozapine

either in bulk powder or in pharmaceutical formulations. Statistical analysis of the results obtained by the two proposed methods and by the non-aqueous titration method of B.Ph, revealed no significant difference within a probability of 95%. However the proposed methods are far more sensitive than the B.Ph. method. Moreover, the suggested methods are more selective, since the B.P. method does not differentiate between the intact drug and its degradation product, namely the corresponding amine oxide.

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