Diclofenac Sodium Injection Sterilized by Autoclave and the Occurrence of Cyclic Reaction Producing a Small Amount of Impurity

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ABSTRACT: A known impurity is formed in the production of a parenteral dosage form of diclofenac sodium if terminally sterilized by autoclave. This impurity has been detected as 1-(2,6-diclorophenyl) indolin-2-one, which is also an intermediate from which diclofenac sodium is generally synthesized. It is only the condition of the autoclave method (i.e., $123 \pm 2^{\circ}C$) that enforces the intramolecular cyclic reaction of diclofenac sodium forming the indolinone derivative and sodium hydroxide. The formation of this impurity has been found to depend on the initial pH of the formulation. The reaction follows first-order kinetics, and the energy of activation is 5.34 kcal/mol. The other excipients in the formulation do not have a role in this reaction. The concentration of the impurity in the resultant product in the ampule goes beyond the limit of the raw materials in the pharmacopoeias. It is thus preferable to use an alternative sterilization method; that is, an aseptic filtration method in which the formation of this impurity can be avoided.
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Keywords: diclofenac sodium; injection; sterilization; impurity; reaction kinetics; intramolecular cyclization

INTRODUCTION

Diclofenac sodium [2-(2,6-dichloroanilino) phenyl] acetate (Figure 1), a commonly used nonsteroidal antiinflammatory drug, is manufactured via a stable intermediate, 1-(2,6-dichlorophenyl) indolin-2-one, which is commonly known as the indolinone derivative, by heating in the presence of sodium hydroxide. The impurity level of the indolinone derivative in diclofenac sodium raw material is limited to only 0.2% mentioned in the British Pharmacopoeia (BP).[1] Among the various dosage forms of diclofenac sodium, the parenteral dosage form is also very popular. In general, injection in the ampule form is sterilized either by the aseptic filtration method or by terminal autoclaving under heat and pressure, provided the product is stable under the autoclaving conditions. The validated terminal sterilization processes achieve a high degree of sterility assurance (1 in 10^{-6}) compared with aseptic processing methods (1 in 10^{-3}).[2] Thus, a natural tendency of the pharmaceutical companies is to go for the most assured terminal autoclave method. Regarding heat stability of diclofenac sodium in solution, there is one report[3] in which a solution of diclofenac sodium had been kept at 71°C for 201 days and 75.3% of the diclofenac sodium degraded. The recommended storage conditions for the parenteral dosage form of diclofenac sodium stated[4] to protect ampules for injection from heat and light. To our knowledge, there are no reports on the stability of diclofenac sodium under autoclaving conditions. In the case of parenteral dosage form of diclofenac sodium, both sterilization procedures (i.e., term-

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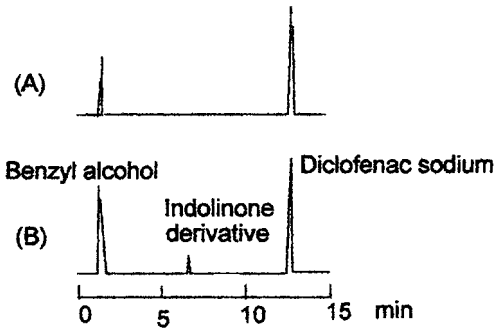


Figure 1. High performance liquid chromatograms of (A) typical parenteral formulation before autoclave and (B) after autoclave. Column: Zorbex ODS (4.6 × 250 mm); Mobile phase: methanol: phosphate buffer (66:34); Flow rate: 1.5/mL/min, UV 254 nm; Chart speed: 0.5 cm/min

inal autoclave or aseptic filtration method) are practiced in Bangladesh. In the Bangladesh drug market, this parenteral form of diclofenac sodium is available under different brand names. Screening the marketed products revealed the presence of indolinone as an impurity in some brands, but some brands were free of this impurity.

The objective of this work, therefore, was to study the sources of this indolinone impurity in the injectable ampule form. This analytical study was carried out using a typical formulation, according to the Scheme 1.

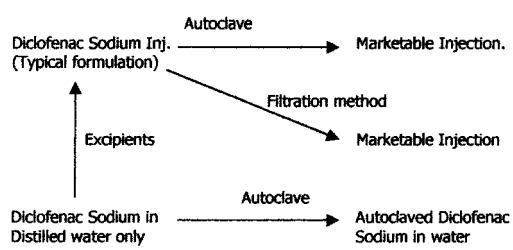
EXPERIMENTAL SECTION

Materials

The sample of diclofenac sodium with potency 99.3% was supplied by the Chemicals Division of Square Pharmaceuticals Ltd. All the other excipients were of BP grade. HPLC grade methanol was obtained from E. Merck. Double-distilled water was used throughout the work.

Typical Formulation

Diclofenac sodium injection (75 mg/3 mL) was formulated using diclofenac sodium BP (75 mg).



Scheme 1.

The excipients included benzyl alcohol (0.12 mL), ethanol 96% (0.15 mL), propylene glycol (0.75 mL), sodium metabisulfite (9.00 mg), mannitol (18.00 mg), disodium edetate (0.3 mg), sodium hydroxide (3.15 mg), and water for injection (quantity required to make 3 mL).

Autoclaving Conditions

The autoclaving conditions were 123 ± 2°C at 1.1 kg/cm^2 pressure for 25 to 30 min.

Effect of Initial pH on the Formation of the Indolinone Derivative

The effect of the initial pH of the typical formulation of the diclofenac sodium injection on the extent of formation of the indolinone derivative was detemined at pH 8, 9, 10, 11, and 12.

Reaction Kinetics Studies

A typical formulated diclofenac sodium injection was used for the determination of the specific reaction rate constant at different temperatures and reaction activation energy. The ampules thus prepared were placed at different temperatures using an oven WC HERAEUS HANAU, Type FTU340, with temperature range 30–220°C. The experimental temperatures included 103, 123, 133, and 143°C for 0 to 83 min duration.

Different specific reaction constants at various elevated temperatures were obtained by plotting the log of the concentration of indolinone derivatives against time. Then, the logarithms of the specific rates of formation of indolinone derivatives were plotted against the reciprocals of the absolute temperatures. From the slope of the best-fitted straight line of the Arrhenius plot, the activation energy (E_a) was determined.

Analysis

Reversed-phase high-performance liquid chromatography (HPLC) analysis of diclofenac sodium and its different formulations were carried out using a Shimadzu LC-10 as pump, a Shimadzu SPD-10A UV detector, a Shimadzu C-R 6A integrator, and a Rheodyne-7125 injector. A reversed-phase column (Zorbex ODS, 4.6 mm i.d. × 250 mm) was used for analysis. The mobile phase consisted of 66% (v/v) methanol and 34% phosphate buffer of pH 2.5, adjusted by adding acetic acid. The overall analysis was conducted according to the BP.[1]

RESULTS AND DISCUSSION

Diclofenac sodium powder used in this study was analyzed by HPLC and found to be free from indolinone impurity and had 99.3% potency. Typical formulated diclofenac sodium injection was initially (before autoclave) free from any impurity, including indolinone derivative; however, after autoclave, 1.7% indolinone derivative was detected. On the other hand, no such impurity was detected in the same formulated injection when the injectables were sterilized by aseptic filtration processes. Typical HPLC chromatograms are shown in Figure 1.

To exclude any role of excipients in the formation of indolinone derivative while autoclaving, a solution of diclofenac sodium formulated only in distilled water was prepared and analyzed before and after autoclaving. The HPLC analysis again indicated the presence of indolinone in the later sample. When autoclaving was done at relatively lower temperature[5] (i.e., at $116 \pm 1^{\circ}C$ for 30 min, the formation of indolinone derivative was reduced to 0.7%, as expected. On the other hand, there was no detectable degradation of diclofenac sodium to indolinone derivative impurity after heating at 83°C for 100 min.

Based on these results, we can reasonably propose that the intramolecular cyclic reaction of diclofenac sodium under autoclaving condition (i.e., heat) produces the indolinone derivative according to the probable mechanism depicted in Figure 2. The reaction shown in Figure 2 also indicates that the intramolecular cyclization of diclofenac sodium also produces NaOH; therefore, the pH of the resulting solution after autoclaving should be higher than that of initial solution before autoclaving. In fact, this difference was noted. The diclofenac sodium in distilled water with pH 7.94 changed to pH 10.89 after autoclaving. In addition, when the autoclaving was done

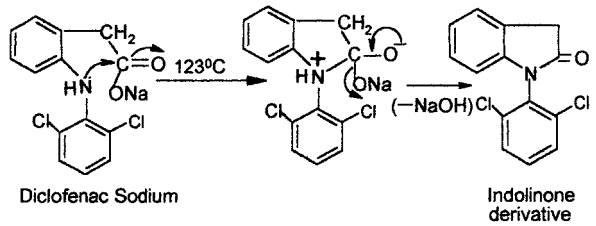


Figure 2. Formation of indolinone derivative from diciofenac sodium.

two times consecutively, the amount of indolinone derivative formed was increased compared with the amount formed in single autoclave. At the same time, pH of the resultant solution of the doubled autoclaving was increased compared with the pH of single autoclave (Table 1).

It appears from the results in Table 1 that the increase of pH as well as formation of indolinone derivative occurred to a lesser extent in the typical parenteral formulation of diclofenac sodium, than in the formulation of diclofenac sodium in water only. This difference is because the typical injectable formulation contained NaOH and the pH was higher to start with: thus, the formation of more NaOH as well as the production of indolinone derivative was inhibited. In fact, the concentration of indolinone derivative formed during autoclaving decreases as the initial pH of the formulation increase (Figure 3). It may be noted that the typical diclofenac sodium injection formulations are not possible at lower pH (i.e., at < 7) because of the conversion of diclofenac sodium to insoluble diclofenac acid.

The intramolecular cyclization reaction of diclofenac sodium producing the indolinone derivative under the autoclaving condition (i.e., at 123°C) follows first-order kinetics, and the activation energy in typical injection formulation at pH 8.41 is 5.34 kcal/mol. The formation of indolinone derivative in the typical diclofenac sodium injection formulation at different temperatures as a

Table 1. Change of pH and Indolinone Derivative Impurity on Autoclaving

Sample	pH before autoclave (conc. of indolinone derivative)	pH after single autoclave (conc. of indolinone derivative)	pH after double autoclave (conc. of indolinone derivative)
Diclofenac sodium injection in typical formulation, 75 mg/3 mL	8.24, clear solution (nil)	8.30, clear solution (1.7%)	—
Diclofenac sodium injection in water only, 75 mg/2 mL	8.06, clear solution (nil)	10.70, turbid solution (3.3%)	—
Diclofenac sodium injection in water only, 75 mg/3 mL	7.94, clear solution (nil)	10.89, turbid solution (3.9%)	11.13, turbid solution (5.2%)

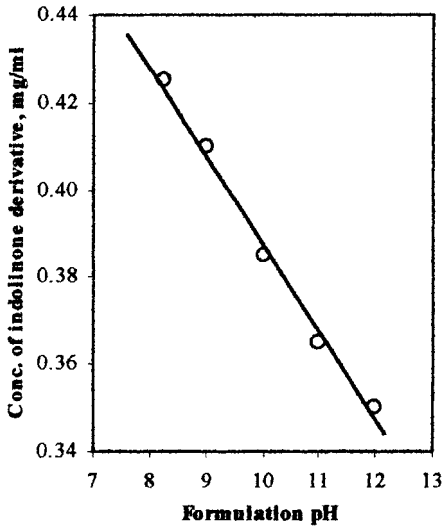


Figure 3. Plot showing the effect of initial pH on the formation of indolinone derivation.

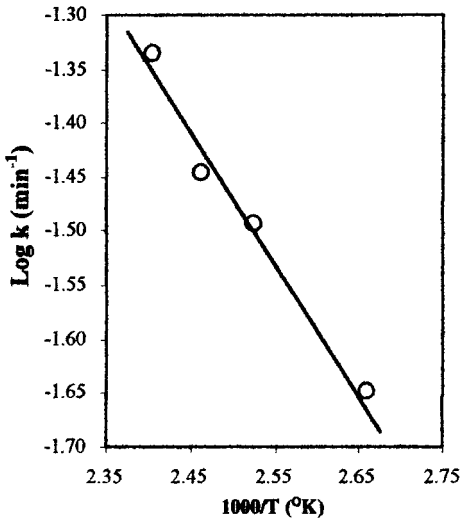


Figure 5. Arrhenious plot showing temperature dependence of reaction rates of indolinone derivative formation.

function of time is shown in Figure 4, and the corresponding Arrhenius plot showing temperature dependence of reaction rates of indolinone derivative formation is given in Figure 5. The relatively low energy of activation found in the formation of indolinone derivative from diclofenac sodium suggests that the reaction follows the most favorable intramolecular substitution nucleophilic cyclization mechanisms, as shown in Figure 2.

CONCLUSION

The indolinone derivative impurity in some samples of marketed diclofenac sodium injection can be formed if the ampule for injection is sterilized terminally by the autoclave method. As long as the level of impurity conforms to the specifications of the pharmacopoeia, there is no problem in using the autoclave method for sterilizing ampules for injection of diclofenac sodium. However, the marketed samples that were monitored contained, impurifies at levels 4 to 9 times higher than the individual impurity level mentioned in the diclofenac sodium raw material in BP. Thus, the aseptic filtration method is preferable because it does not cause the formation of the indolinone derivative impurity at all. The future edition of BP or United States Pharmacopoeia (USP) should include the specifications and the allowable limits of impurities for the ampule form of diclofenac sodium.

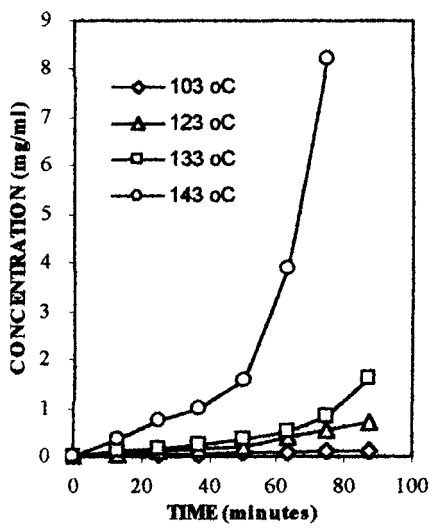


Figure 4. Formation of indolinone derivative in diclofenac sodium injection at different temperature as a function of time.

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