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SUMMARY AND CONCLUSIONS

3.0 SUMMARY AND CONCLUSION:

This investigation was envisaged to develop conventional as well as improved ophthalmic delivery systems for a promising nonsteroidal anti-inflammatory agent, namely ketorolac tromethamine, which has recently proved its preference over other nonsteroidal and steroidal anti-inflammatory agents for use in the eye.

Extensive literature survey provided useful information which assisted in the selection of drug candidate as well as logical development and evaluation of the dosage forms.

Ketorolac tromethamine, used in the study complied with the USP-23 pharmacopoeial specifications. The development work was commenced by carrying out various important preformulation studies including development of stability-indicating HPLC and HPTLC analytical methods for the drug, evaluation of aqueous stability of ketorolac tromethamine, compatibility studies of drug with additives, closures, containers and, selection of suitable sterilization techniques for the drug powder as well as for the formulations. Compatibility studies were carried out at 60°C, 45°C and 4°C for 3 months.

The developed HPLC and HPTLC analytical methods, were able to analyse 0.1 % of degradation/impurities from the drug and also from the ophthalmic formulations. However, HPTLC method was used for final formulation stability studies, because it was rapid and economical compared to HPLC method.

Compatibility of the drug with additives, closures and containers were studied by keeping in accelerated temperature conditions for 3 months. The parameters evaluated during compatibility studies included drug analysis by HPLC, clarity, pH, change in UV-spectra of the drug. All the compatibility studies were carried out with 0.5% w/v ketorolac tromethamine aqueous solution.

Among the tonicity modifiers studied, sodium chloride, mannitol, sorbitol, propylene glycol and glycerine were found to be compatible with ketorolac tromethamine.

To improve the corneal residence time of conventional solution, and to formulate long-acting aqueous gels, compatibility study of drug in aqueous solution with various polymers were carried out, and it was found that, Carbopol 940, Carbopol 971, HPMC E4M and Poloxamer-407, were compatible with the drug.

In case of preservatives, benzalkonium chloride, thiomersal and parabens were found to be compatible with the drug solution. To improve the stability of ketorolac tromethamine aqueous solution, compatibility of drug with commonly used stabilizers was studied. The results revealed that nonionic surfactants such as Cremophor-EL and Brij-35, a chelating agent di-sodium EDTA and a complex forming agent HPβCD were found to be compatible with the drug. However, ascorbic acid and sodium metabisulfite were found to be incompatible with the drug. HPβCD improved the stability of the drug and nonionic surfactants enhanced the clarity of the solution.

Results of evaluation of glass containers suggested that amber coloured vials were necessary for preventing light degradation of

aqueous solution of ketorolac tromethamine.

Amongst the various sterilization techniques studied, membrane filtration and ethylene oxide sterilization methods were found to be the most suitable, whereas, autoclaving and dry-heat under vacuum methods were found to degrade ketorolac tromethamine. Sterilization by Ethylene oxide was studied to sterilize the drug in powder form, since the objective of this study also included development of ophthalmic ointment dosage form of ketorolac tromethamine.

Based on the objective of the study, first, stable conventional ketorolac tromethamine ophthalmic drops were developed. To achieve this, various prototype formulations were prepared and evaluated for stability at accelerated conditions, of temperatures and light, for 3 months. The formulation containing nonionic surfactants were found to be stable with maximum degradation products of less than 1.0 % after 3 months at 45°C. However, a very stable formulation was obtained with the inclusion of HPβCD. A final formulation of 1 litre batch size was prepared and evaluated.

Once the stable conventional formulation was developed, viscous solutions and long-acting gels were prepared by incorporating suitable polymers. In case of viscous solution, HPMC E4M (0.5% w/w) was selected, and various prototype formulations were prepared using stabilizers such as nonionic surfactants and HPβCD. Different concentrations of HPMC E4M were also evaluated. It was found that even in case of viscous solutions, HPβCD improved the stability of the formulation significantly.

Two types of gels namely, preformed and thermoreversible gels were prepared. Carbopol 940 at concentrations between 1.0 and 2.0% w/w was evaluated to prepare preformed gel. Poloxamer-407,

a polymer which is liquid at refrigerated conditions and turns semisolid at body temperature, was utilized to prepare *in situ* gel systems. Different concentrations of Poloxamer-407 were evaluated, and it was found that 20 % w/w was the most suitable one. Prototype formulations of thermoreversible gels were prepared with and without stabilizers and were filled into amber coloured vials as well as into lacquered aluminium collapsible tubes.

Since, ketorolac tromethamine ophthalmic ointment is not commercially available, a technology was developed for the same by utilizing simple petrolatum base.

All the prepared formulations were evaluated for initial drug content, pH, *in-vitro* drug release profile, clarity, stability, antimicrobial preservative effectiveness, sterility, eye-irritation in rabbits, efficacy and pharmacokinetic parameters.

Accelerated stability studies were carried out at 4°C, 25°C, 37°C and 45°C for 6 months for all the formulations in their final package. In addition, the ointment was subjected to 75% RH at 37°C also. Light stability studies in room light were carried out for 6 months at ambient temperature. It was found that ketorolac tromethamine conventional solution, viscous solution and Poloxamer based gel required a stabilizer such as HPβCD. The Carbopol based gel as well as the ointment were stable even without any stabilizer. Nonionic surfactants improved the clarity of the conventional as well as the viscous solutions of ketorolac tromethamine. Hence all the formulations were found to be stable and a shelf life of 18 months could be assigned to them.

In-vitro drug release studies suggested that both Carbopol based and Poloxamer based gel released the drug in a similar fashion. The drug release followed Higuchi kinetics, with non-Fickian diffusion. The drug release profile was found to be the same after 6 months of stability studies.

All the formulations were tested for sterility by the USP XXII membrane filtration method and were found to be sterile. The preservative effectiveness study was carried out as per USP XXII guidelines. It was found that in all the formulations the count of viable microorganisms decreased significantly during the 28 day test period, and hence passed the official test for preservative efficacy.

Eye-irritation studies, conducted in rabbits according to the guidelines of Draize test, suggested that all the stable formulations were non-irritating.

To study the efficacy of the conventional formulation, an alkali induced ulcer model was selected, because it provided many parameters, such as, protein concentration in aqueous humor, PMN's in aqueous humor and tears, and vascularization of the cornea, to evaluate the formulation. The innovator's product was used as the standard, whereas normal saline was used as the control. The prepared conventional formulation was found to be as effective as that of the innovator's product.
Since, the efficacy studies were time consuming and required large number of rabbits, it was decided to study the aqueous humor drug concentration-time profile for other formulations, by comparing with the conventional formulation. Different studies conducted were; i) comparison of single dose of conventional formulation with the innovator's product. ii) comparison of

single dose of conventional formulation with single dose of viscous solution, ointment and gels. iii) comparison of multiple dose of conventional formulation with single dose of gels. The parameters evaluated included AUC, C_{max} and t_{max}.

Conventional ophthalmic solutions of ketorolac tromethamine were found to be bioequivalent, when compared with its marketed counterpart. The results of the single dose study of conventional drops, viscous solution, ointment and gels showed that the was in the following order, Carbopol 940 gel > Poloxamer-407 gel > viscous solution > ointment > conventional solution. The C_{max} achieved with multiple dose of conventional solution was much as less compared to a single dose of Carbopol 940 gel, but it was more than Poloxamer 407 gel. The prepared gel formulations retained in the eye for longer time as compared to conventional solution, and hence improved the bioavailability. These gel formulations can reduce the frequency of administration and also reduce the risk of side effects.

In conclusion, two approaches adopted to optimize ophthalmic delivery systems for ketorolac tromethamine, included improvisation of the stability of the conventional dosage form as well as developing a technology for ointment dosage form and long acting delivery systems, which could improve patient compliance and overcome limitations of the conventional solution.

The study provided an opportunity to develop and evaluate some prototypes and final delivery systems of the drug for ophthalmic use, as well as, to develop and standardize some new analytical methods and evaluation techniques for ensuring stability, efficacy, safety and acceptability of the systems.