

Low-Level Drug Release-Rate Testing of Ocular Implants Using USP Apparatus 4 Dissolution and HPLC End Analysis

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PURPOSE

To develop a method for the determination of drug-release rate in low-level ocular implants using USP Apparatus 4 dissolution and HPLC end analysis.

BACKGROUND

Ocular implants are small, polymer-based discs or cylinders that improve the delivery of therapeutic drugs into the eye (1). Some common types of implants include episcleral implants, which are placed at the equator of the eye, and intravitreal implants, which are surgically placed in the vitreous humor of the eye (2). Figure 1 provides a visual representation of ocular anatomy and the placement of various implants. Ocular implants are used to treat a variety of ailments such as diabetic retinopathy and macular degeneration (1). Apparatus 4 dissolution was chosen as the technique for testing this product because the laminar flow through the sample cell correlates well with the likely in vivo conditions, and the open-loop configuration is effective for providing sink conditions for compounds with low solubility.

DEVELOPMENT GOALS

There were three main goals of this development project. Because of the low levels of drug released by the ocular implant device, the first goal was to optimize the HPLC conditions to obtain a limit of quantitation ≤ 1 ng/mL. Secondly, any potential interferences or adsorption from the USP Apparatus 4 dissolution system needed to be ascertained. The final goal was to generate a dissolution profile for the implant sample using USP Apparatus 4 dissolution with HPLC end-analysis.

METHODOLOGY

Initially, the experiment was performed on a Sotax CE 7 Smart USP Apparatus 4 dissolution system (open loop) with pH 7.4 phosphate buffer as the drug-release medium. The pump was set to the lowest practical flow rate for the instrument (1.5 mL/min) in an attempt to mimic in vivo conditions since flow of vitreous humor in the eye is approximately 2 μ L/min (3). The dosage unit was placed on glass beads in a 22.6-mm flow-through cell. The effluent tubing from the dissolution apparatus was

plumbed directly into the HPLC injector so that sampling could occur on an hourly basis for several days. It was not desirable to sample using a fraction collector or automated sampler because of a known interaction between the compound of interest and some plastics.

To inject directly from the eluent stream of the dissolution system, the outlet tubing from the dissolution apparatus was connected to a length of PEEK tubing through a reducing union and then into an injector valve. In the inject position, the dissolution eluent would flow through the valve and into waste. In the load position, the eluent would fill the sample-injection loop. Valve switching was controlled electronically. The injection valve was connected to a Perkin Elmer Series 200 HPLC system (injector excluded). Because of the low flow rate of 1.5 mL/min from the apparatus, backpressure into the injection loop was minimal and did not compromise the integrity of the system fittings and connections. Samples were analyzed on a 2.1-mm diameter SB-Phenyl HPLC column with an injection volume of 500 μ L to maximize sensitivity. The HPLC flow rate was 0.5 mL/min, and the detection wavelength was 280 nm. The optimized chromatographic parameters were developed to analyze samples at concentrations as low as 1 ng/mL.

An initial evaluation of possible interferences observed from Apparatus 4 revealed that our compound of interest had limited solubility in the pH 7.4 phosphate buffer drug-release medium and that degradation of the compound may have been occurring during the dissolution experiment. The addition of 0.1% sodium dodecyl sulfate (SDS) to the medium significantly increased the solubility and stability of the drug. Additional experimentation demonstrated that adequate solubility and solution stability could also be obtained using sodium chloride in place of SDS. Ultimately, pH 7.4 saline phosphate buffer, prepared as per the *British Pharmacopoeia*, was chosen as the drug-release medium because of its biological relevance as well as its efficacy.

The drug-release medium, a standard at 10 ng/mL, and an LOQ solution at 1 ng/mL were exposed to the dissolution conditions for 24 hours to determine any potential interferences or adsorption from the apparatus. See Figure 2 for a chromatographic overlay of the standard, LOQ, and medium obtained from the Apparatus 4-HPLC interface.

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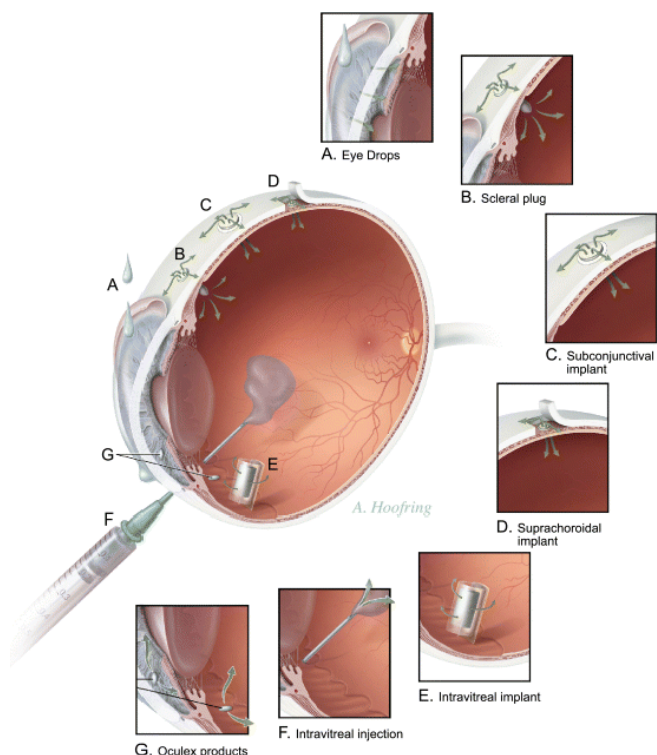


Figure 1. Anatomy of the eye and principal methods of local drug delivery. (Reprinted with permission from ref 1. Copyright 2004 Informa Healthcare USA, Inc.)

Once the instrument parameters had been optimized, a single sample was exposed to the dissolution conditions for 14 days. For the first 24 hours, sampling occurred every hour using a programmable autosampler electronically connected to the HPLC injection valve. From 24 to 72 hours, sampling occurred every two hours, and after 72 hours, sampling occurred every four hours. A drug-elution profile was generated from hourly samplings over the two-week period. See Figure 3 for a plot of the elution

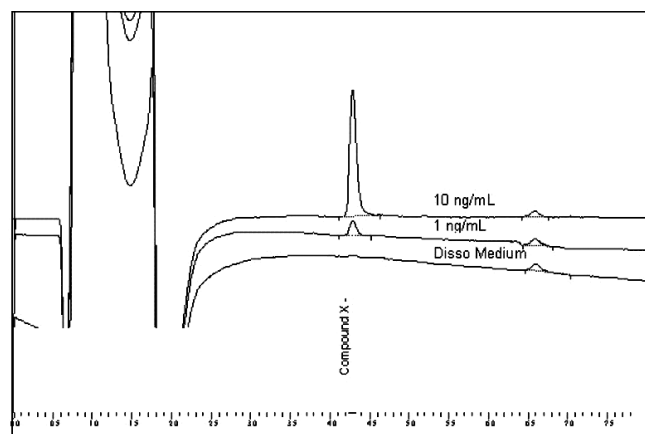


Figure 2. Chromatographic overlay of dissolution medium, LOQ solution, and 10 ng/mL standard.

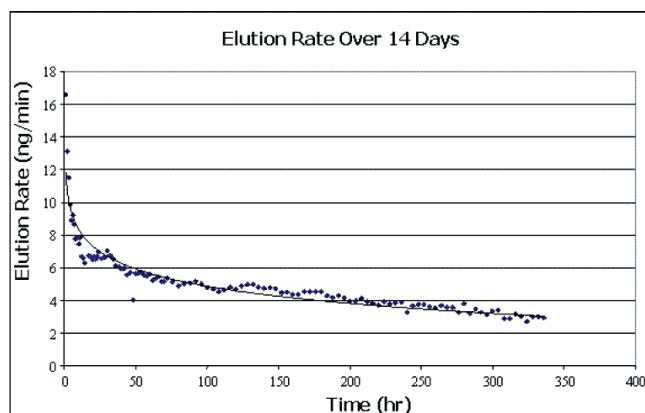


Figure 3. Elution rate for compound X.

rate in ng/min over the 14-day experimental period. See Figure 4 for a plot of the elution profile in amount released per hour.

RESULTS

The optimized conditions provided adequate sensitivity and a retention time of approximately 4.3 min for the compound of interest. No interference was observed from the components of the dissolution apparatus. Recovery at the 1 ng/mL level was high due to poor injection reproducibility. It was determined that insufficient time for rinsing and filling the sample loop was programmed into the autosampler. This resulted in an artificially low standard response and higher assay values. However, the data support the conclusion that no adsorption occurred within the components of the apparatus. In later experiments, injection reproducibility was improved by programming rinse injections into the autosampler and giving the 500- μ L sample loop more time to fill completely. During the 14-day profile experiment, the drug elution rate was initially 17 ng/min, which progressively dropped to 6 ng/min during the first 48 hours. Over the course of the 14-day experiment, the profile leveled off at 3 ng/min. This elution trend was not

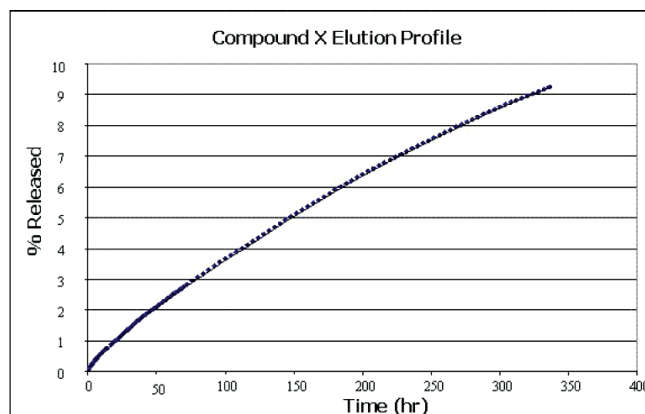


Figure 4. Elution profile for compound X.

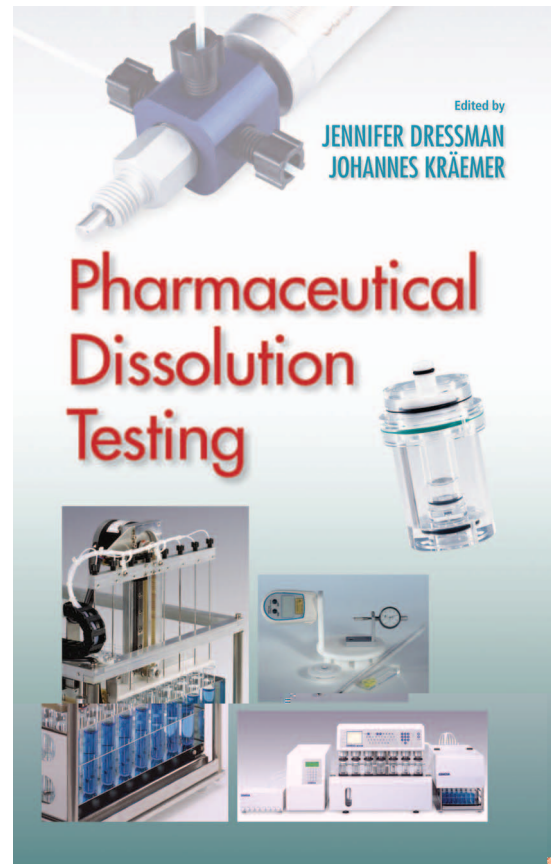
unexpected; drug release for this product likely occurs very slowly over a long period due to the design of the implant.

CONCLUSIONS

A method was developed to determine drug release in ocular implants at the ng/mL level. Using a narrow-bore HPLC column and an injection volume of 500 μ L, a quantitation limit less than 1 ng/mL was achieved to analyze the low level of drug release in ocular implants. The Sotax USP Apparatus 4 system was successfully configured to an HPLC for direct sampling from the eluent stream. No interferences were observed in the dissolution medium exposed to the dissolution conditions, and no adsorption was observed at the 1 ng/mL level. A preliminary dissolution profile was generated for a single ocular implant over the course of two weeks. This method has the potential for wide application correlation with in vivo conditions.

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