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# Note

## Simultaneous determination of thimerosal and chlorhexidine in solutions for soft contact lenses and its applications in stability studies

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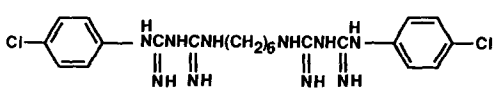
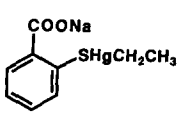
Chlorhexidine, hexamethylenebis[5-(4-chlorophenyl)biguanide] (**I**), is a disinfectant which is effective against a wide range of vegetative Gram-positive and Gram-negative bacteria. Thimerosal, ethyl(sodium *o*-mercaptobenzoato)mercury (**II**), is an effective antibacterial and antifungal agent (Fig. 1). Both drugs are widely used together or alone as preservatives in pharmaceutical formulations, particularly, in liquid formulations such as ophthalmic solutions and storage and rinsing solutions for contact lenses.

Thimerosal is known to be unstable in aqueous solution [1,2]. It has also been reported that the presence of halides can have an adverse influence on the stability of



CHLORHEXIDINE
(I)



THIMEROSAL
(II)

Fig. 1. Structures of thimerosal and chlorhexidine.

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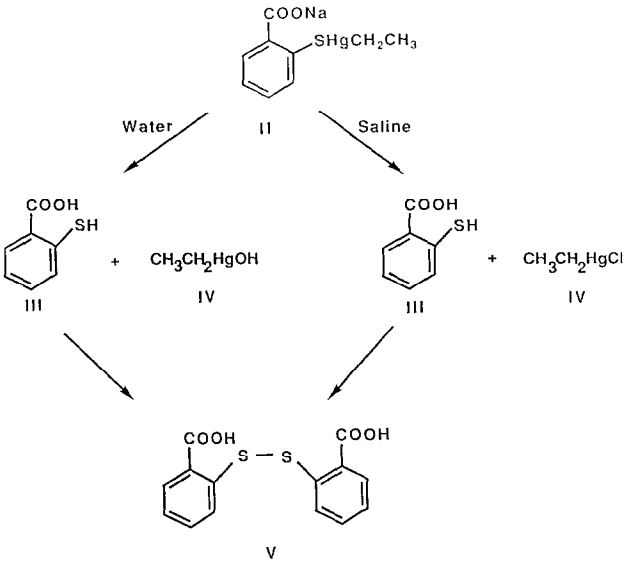


Fig. 2. Reaction scheme of the decomposition of thimerosal in aqueous solution. **II** = Thimerosal; **III** = thiosalicylic acid; **IV** = ethylmercury hydroxide; **V** = 2,2′-dithiosalicylic acid.

thimerosal [3,5], which also can be adsorbed from solutions stored in plastic containers [6]. The decomposition of thimerosal in aqueous solution has been studied [2], and it was shown that the major degradation products were thiosalicylic acid (**III**) and ethylmercury hydroxide (**IV**). Thiosalicylic acid in turn can undergo oxidative degradation irreversibly to 2,2′-dithiosalicylic acid (**V**) [7] (Fig. 2.).

In the past, chlorhexidine and thimerosal in pharmaceutical products have been determined by various conventional methods, such as UV spectrophotometry [8], polarography [9,10] and colorimetry [11,13] for thimerosal and colorimetry [14,15], gas chromatography [16–18] and by catalytic oxidation of [$^{14}$C]chlorhexidine [19] for chlorhexidine. These procedures are often tedious and time consuming.

Although a number of high-performance liquid chromatographic (HPLC) procedures such as the anion-exchange HPLC [20], HPLC with a radial-compression column [21], HPLC with electrochemical detection [22] and HPLC with atomic absorption [23] have been developed for the determination of thimerosal in dosage forms, there was no specific and accurate method available for simultaneous determination of thimerosal and chlorhexidine in soft contact lens storage and rinsing solutions.

This paper describes a stability-indicating method which rapidly and simultaneously determines thimerosal and chlorhexidine gluconate in solutions. The method has been successively applied in a stability study of contact lens storage and rinsing solutions containing thimerosal and chlorhexidine.

## EXPERIMENTAL

### *Apparatus*

The HPLC apparatus consisted of a Model 880-PU pump (JASCO, Tokyo, Japan), a SIC (Tokyo, Japan) Autosampler 23 automatic sampler, a JASCO 870-UV variable-wavelength UV detector and a SIC Chromatocorder 12 integrator. A 7-μm Nucleosil $C_{18}$ column was used. For the column system a precolumn (7-μm Nucleosil $C_{18}$; Inpac International, Taipei, Taiwan) was also used.

### *Chemicals and reagents*

Thimerosal and chlorhexidine (as gluconate) were purchased from E. Merck (Darmstadt, F.R.G.). Softline "Kingdom" storage and rinsing solution (thimerosal 0.0025%, chlorhexidine 0.0125%) was obtained from Kingdom Pharmaceutical (Taipei, Taiwan). All chemicals were of analytical-reagent grade and all solvents were of HPLC grade.

### *Chromatography*

The assays for thimerosal and chlorhexidine were performed using the 7-μm Nucleosil $C_{18}$ column and a mobile phase of 0.1 *M* $KH_2PO_4$ (pH 3.5)–methanol (40:60), prepared as follows. A 13.6-g amount of potassium dihydrogenphosphate was placed in a 1000-ml volumetric flask, 800 ml of distilled water were added and after dissolution, the solution was diluted to volume with distilled water. The final pH was 3.5, adjusted with phosphoric acid. This solution was mixed with 1500 ml of methanol and the mixture was degassed and filtered through a 0.45-μm membrane filter prior to use. A flow-rate of 1.0 ml/min, a UV detector wavelength of 254 nm (0.04 a.u.f.s.), ambient temperature and an injection volume of 20 μl were used in all the assays.

### *Precision, reproducibility, linearity and accuracy*

Thimerosal (500.2 mg) and chlorhexidine gluconate (20%) (1089.9 mg) were weighed and transferred with the aid of *ca.* 30 ml of distilled water into a 100-ml volumetric flask. They were mixed well until all the ingredients had dissolved, then diluted to volume with distilled water. An aliquot of appropriate volume of the thimerosal and chlorhexidine gluconate solution was diluted with distilled water to make a series of diluted solutions covering the ranges 2–150 μg/ml of thimerosal and 2.72–436 μg/ml of chlorhexidine gluconate. A 50-μl volume of methylparaben (3 mg/ml), as internal standard, was added to each 1 ml of sample in the above sample solutions.

### *Stability study*

Samples of the commercial product (Softlite "Kingdom" storage and rinsing solution), which were in their original containers kept in an environmental chamber of 45°C for periods of up to 3 months, were used as analytical samples. A 9.5-ml volume of these samples was transferred into a 10-ml volumetric flask containing 0.5 ml of internal standard solution (methylparaben, 3 mg/ml). The HPLC determinations were conducted at specific time intervals (0, 18, 30, 44, 61, 93 days). The same solution, kept in an environmental chamber at 4°C, was used as a control in the assays.

RESULTS AND DISCUSSION

A typical chromatogram of simultaneously determined thimerosal, thimerosal decomposition products and chlorhexidine gluconate is shown in Fig. 3. Under the

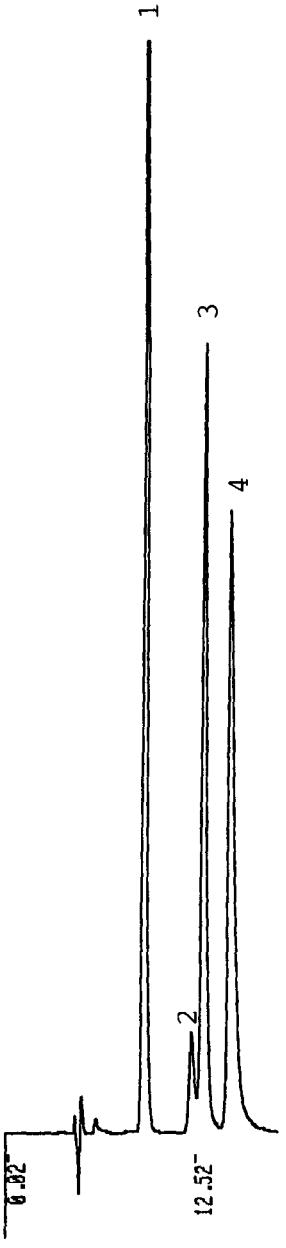


Fig. 3. Chromatogram of methylparaben, thiosalicylic acid, 2,2′-dithiosalicylic acid, thimerosal, and chlorhexidine. Peaks: 1 = methylparaben (3 μg); 2 = thiosalicylic acid (20 ng) and 2,2′-dithiosalicylic acid (20 ng); 3 = thimerosal (0.5 μg); 4 = chlorhexidine gluconate (2.5 μg). Time scale in min.

TABLE I

BETWEEN-DAY ACCURACY AND PRECISION OF THE DETERMINATION OF THIMEROSAL AND CHLORHEXIDINE GLUCONATE IN AQUEOUS SOLUTION BY HPLC

| Compound | Actual concentration ($\mu$g/ml) | Measured concentration (mean $\pm$S.D., $n = 3$) | R.S.D. (%) ($n = 3$) | Concentration determined expressed as % of the standard |
|---|---|---|---|---|
| Thimerosal | 50.0 | 51.5 $\pm$ 0.40 | 0.77 | 103 |
| Chlorhexidine gluconate | 136 | 138 $\pm$ 2.50 | 1.81 | 101 |

assay conditions described, the retention times determined were 11.4 min (thimerosal), 14.9 min (chlorhexidine gluconate), 7.8 min (methylparaben) and 10.9 min (thiosalicylic acid, 2,2'-dithiosalicylic acid). The chromatogram clearly demonstrates that thiosalicylic acid and 2,2'-dithiosalicylic acid can be separated well from thimerosal and chlorhexidine gluconate. The sample was completely eluted in 20 min. The limit of detection of the assay was 0.1 $\mu$g for thimerosal and 0.2 $\mu$g for chlorhexidine gluconate.

Excellent linearity of the calibration graphs was observed over the range 2–150 $\mu$g/ml for thimerosal and 2.72–436 $\mu$g/ml for chlorhexidine gluconate. The within-day ($n = 3$) and between-day ($n = 10$) calibration graphs for chlorhexidine gluconate and thimerosal all had correlation coefficients of 0.999. Table I demonstrates the accuracy and precision of the determination of thimerosal and chlorhexidine gluconate in aqueous solution. The reproducibility of the method was determined by analysing the results of three replicate injections of a solution containing 50 $\mu$g/ml of thimerosal and 136 $\mu$g/ml of chlorhexidine gluconate. Thimerosal, which eluted first, was determined with a relative standard deviation (R.S.D.) of 0.77%. Chlorhexidine gluconate, which had the longest retention time, was determined with an R.S.D. of 1.81%.

In the stability study, the concentrations of thimerosal and chlorhexidine gluconate in the Softlite "Kingdom" storage and rinsing solution with time revealed that their contents in stability test samples at 45°C did not decrease significantly ($P > 0.05$) and no signs of thiosalicylic acid or 2,2'-dithiosalicylic acid were detected.

The proposed procedure offers a rapid and sensitive method for the routine simultaneous determination of thimerosal and chlorhexidine gluconate. Also, after a series of tests, the method was found to be insensitive to small variations of pH, in the ionic strength of the $KH_2PO_4$ solution and hence in minor variations in the composition of the mobile phase.

We conclude that the stability-indicating assay procedure presented here is accurate and precise for the simultaneous determination of thimerosal and chlorhexidine gluconate in solutions, and that the Softline storage and rinsing solution is stable for at least 3 months at 45°C.

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