DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 17(4), 519-530 (1991)

THIMEROSAL ANALYSIS IN KETOROLAC TROMETHAMINE OPHTHALMIC SOLUTION. COMPARING HPLC AND COLORIMETRIC TECHNIQUES

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ABSTRACT

Ketorolac tromethamine, a pyrrolopyrrole, is a nonsteroidal anti-inflammatory drug under development as a topical agent for treating ocular disorders associated with inflammation. Thimerosal is a commonly-used antimicrobial preservative in ophthalmic solutions. This report describes both stability-specific (HPLC) and non-specific (colorimetric) methodology for determining thimerosal stability in ketorolac ophthalmic solution.

The HPLC technique used a reverse-phase Whatman RAC II (C8) column (5 micron particle size, 10 cm X 4.6 mm I.D.) with a 30:67:3 by volume mixture of methanol:10mM acetate buffer (pH 4.5) and tetrahydrofuran as the mobile phase. Detection was at 254 nm.

Thimerosal peak purity, in thermally stressed ketorolac ophthalmic solution is confirmed using absorbance ratio techniques. Accuracy and linearity data are presented.

In addition, a colorimetric (dithizone) technique for quantifying total organic mercury in solution is described. Both the HPLC and colorimetric

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techniques were used to evaluate thimerosol stability in ketorolac ophthalmic solution samples exposed to both thermal and photochemical stress. Results indicated that a stability specific HPLC technique does not reflect accurately the total mercury content in ophthalmic solution. Mercury, in other forms than thimerosal, may contribute to the antimicrobial efficacy of thimerosal in ophthalmic solutions.

Keywords: Thimerosal (Thiomersal); high-performance liquid chromatography; dithizone; stability; organomercury.

INTRODUCTION

Ketorolac tromethamine ((±)-5-(benzoyl)-3H-1,2-dihydropyrrolo-[1,2a]-pyrrole-1-carboxylic acid,2-amino-2-(hydroxymethyl)-1,3-propanediol salt) is a nonsteroidal anti-inflammatory drug under development as a topical agent for ocular disorders associated with inflammation. It has been formulated into an isotonic ophthalmic solution and preserved with thimerosal (sodium ethylmercurithiosalicylate), an antimicrobial agent commonly used in ophthalmic formulations.

The literature reports various analytical procedures for quantifying thimerosal, including: polarography [1,2], atomic absorption [3], colorimetry [4,5,6], and HPLC with UV [7,8,9] and electrochemical [10] detection. Only the HPLC techniques offer stability specificity with respect to thimerosal and its degradation products. Additionally, two of the reported HPLC procedures either featured poor peak shape [8] or proved difficult to reproduce [9] in our laboratory. None of the above techniques demonstrated thimerosal quantitation in the presence of potentially interfering drug substances.

This paper describes a stability specific HPLC procedure for determining thimerosal stability in ketorolac tromethamine ophthalmic solution. Thimerosal peak purity in thermally stressed samples was confirmed using wavelength scanning techniques. For comparison, a non-specific colorimetric procedure, using dithizone to complex mercury, is also described.

Analysis of both thermally and photochemically stressed ketorolac ophthalmic solution samples indicated that the non-specific colorimetric

technique might more accurately reflect the preservative efficacy of thimerosal.

EXPERIMENTAL

Chemicals and Reagents

Dithizone, sodium acetate and glacial acetic acid were Analytical Reagent grade and obtained from Mallinckrodt, Inc. (Paris, KY, USA). HPLC grade methanol was also obtained from Mallinckrodt. HPLC grade methylene chloride was obtained from Burdick and Jackson (Muskegon, MI, USA). Thimerosal reference standard was obtained from the USP (Rockville, MD, USA). Thiosalicylic acid (2-mercaptobenzoic acid) and 2,2'-dithiosalicylic acid (2,2'-dithiobenzoic acid) were obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Ketorolac tromethamine ophthalmic solution was supplied by Syntex Research (Palo Alto, CA, USA). Chromatographic quality water obtained from a Barnstead Nanopure water purification system (Millipore Corp., Boston, MA, USA) was used at all times.

HPLC Procedure

(i) Chromatographic conditions. The system components included a high-pressure pump (ConstaMetric, Model IIG, Laboratory Data Control, Riviera Beach, FL, USA), an automatic sample injector (WISP model 710B, Waters Assoc., Milbourn, MA, USA), a variable wavelength detector (Model 770R, Kratos Analytical Instruments, Westwood, NJ, USA), and a computing integrator (Model SP4100, Spectra-Physics, San Jose, CA, USA).

The analytical column (100 x 4.6 mm i.d.) was packed with 5 μm C_8 Partisil (Rac II, Whatman, Inc., Clifton, NJ, USA). A 3 cm replaceable guard cartridge (RP-8 Spheri-5, Brownlee Labs, Santa Clara, CA, USA) was used at all times. Mobile phase consisting of methanol:10 mM acetate buffer (pH 4.5): tetrahydrofuran (30:67:3 v/v) was filtered and de-aerated. The flow rate was maintained at 1.5 mL/min and thimerosal was quantified at 254 nm.

(ii) Standard solutions. Approximately 20 mg, 25 mg and 30 mg of thimerosal were weighed into three separate 100 mL volumetric flasks and

diluted to volume with water. A 5.0 mL aliquot of each standard stock solution was then pipetted into separate 100 mL volumetric flasks and diluted to volume with water.

(iii) Sample preparation and assay. A 5.0 mL aliquot of ketorolac tromethamine ophthalmic solution was pipetted into a 10 mL volumetric flask and diluted to volume with water. The samples were quantified by injecting 50 µL of each sample and standard solution (ii) into the HPLC.

(iv) Peak spectral purity experiments. Spectral purity of thimerosal chromatographic peaks in thermally stressed samples were confirmed using a linear diode array spectrophotometric HPLC detector (Model 1040A, Hewlett-Packard, Palo Alto, CA, USA).

Dithizone Procedure

(i) Standard preparation. Approximately 25 mg of thimerosal was accurately weighed into a 100 mL volumetric flask and diluted to volume with water. Calibration standard solutions were obtained by pipetting 8.0 mL, 10 mL, and 12 mL aliquots of the stock solution into separate 100 mL volumetric flasks and diluting to volume with water.

(ii) Reference blank preparation. Approximately 40 mL of methylene chloride, 20 mL of 0.33 M acetate buffer (pH 4.5) and 2 mL of ophthalmic placebo solution (ketorolac tromethamine ophthalmic solution without thimerosal) were pipetted into a 125 mL separating funnel and shaken for 2 min. The phases were allowed to separate and the organic phase was transferred into a 50 mL test tube and capped. This solution was used in the spectrophotometer reference cell.

(iii) Sample preparation and assay. Approximately 20 mg of dithizone was accurately weighed into a 100 mL volumetric flask and diluted to volume with methylene chloride. A 5 mL aliquot of this solution was pipetted into a 500 mL volumetric flask and diluted to volume with methylene chloride. A 40 mL aliquot of the resultant dithizone solution, 20 mL of 0.33 M acetate buffer (pH 4.5), and 2 mL ketorolac tromethamine ophthalmic solution were added to a 125 mL separatory funnel. Three standard solutions were similarly

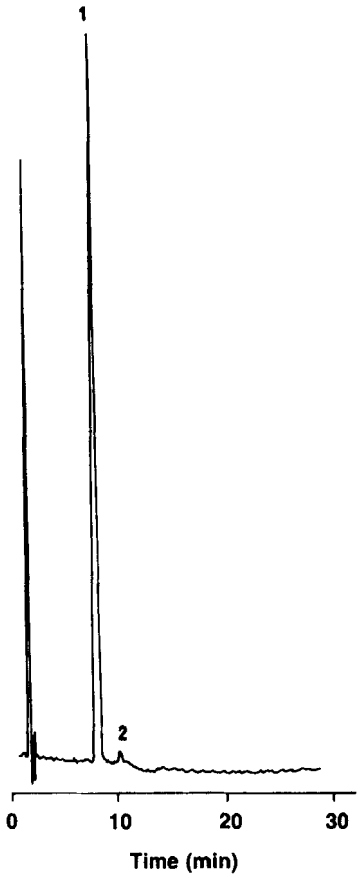

FIGURE 1

Chromatogram of USP thimerosol reference standard in water: (1) thimerosol; (2) 2,2'-dithiosalicylic acid.

prepared by pipetting 2 mL of each standard solution prepared above (i) (instead of ophthalmic solution) into three separating funnels. A 2 mL aliquot of ophthalmic solution placebo was also added to the standard separating funnels. Both "sample" and "standard" separating funnels were shaken for 2 min. After the phases separated, the organic layer was transferred into a test tube, which was then capped. The absorbance of each sample and standard solution was measured versus the reference blank (ii) at 605 nm.

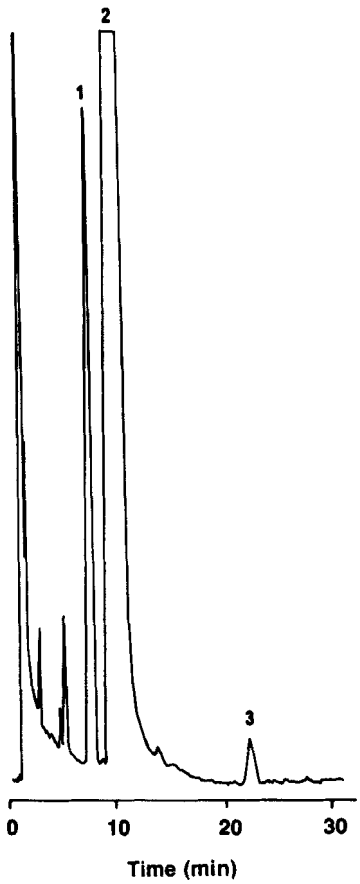

FIGURE 2

Chromatogram of ketorolac tromethamine ophthalmic solution: (1) thimerosol; (2) ketorolac tromethamine; (3) foreign related substance of ketorolac tromethamine.

RESULTS AND DISCUSSION

Previous authors reported that in isotonic solution, thimerosal hydrolyzes primarily to ethylmercury chloride, thiosalicylic acid and 2,2'-dithiosalicylic acid [7,11]. Figure 1 shows a chromatogram of a USP standard thimerosal solution. No interference was noted with 2,2'-dithiosalicylic acid. Thiosalicylic acid eluted in the solvent front. Ethylmercury chloride was not detectable at 254 nm. Figure 2 illustrates a chromatogram of a ketorolac tromethamine ophthalmic solution sample demonstrating that ketorolac

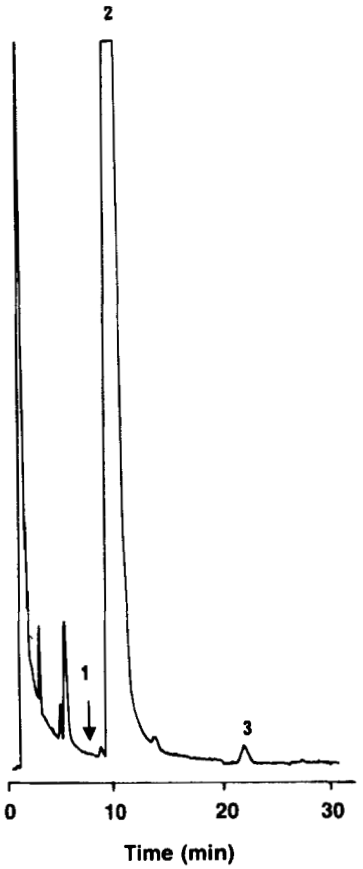

FIGURE 3

Chromatogram of ophthalmic solution thimerosol placebo demonstrating no interference at thimerosol retention time. Key same as Figure 2.

tromethamine (0.5% w/v) did not interfere with thimerosal (0.0025% w/v). The HPLC method for thimerosal was both accurate and linear. A linearity plot, showing milligrams thimerosal added (spiked placebo) versus milligrams thimerosal found, gave a slope of 0.9833 and an intercept of 1.5 $\pm$ 2.8. The correlation coefficient was 0.99886 ($P<0.05$). Placebo (Figure 3) (no thimerosal) and thermally degraded placebo (maintained 1 mo at 80°C) (Figure 4) chromatograms confirmed no interferences at the thimerosal retention time.

FIGURE 4

Chromatogram of thermally degraded (1 mo at 80°C) thimerosol placebo demonstrating no interference at thimerosol retention time. Key same as Figure 2.

Method specificity was further confirmed by determining the spectral purity of the thimerosal peak in a thermally stressed ketorolac solution sample. Figure 5 shows a chromatogram of ketorolac tromethamine ophthalmic solution maintained for 1 mo at 80°C (53% thimerosal remaining). Spectral overlays of the beginning, apex, and tail end of the thimerosal peak (Figure 6) were superimposable, demonstrating that there were no interferences from ketorolac tromethamine.

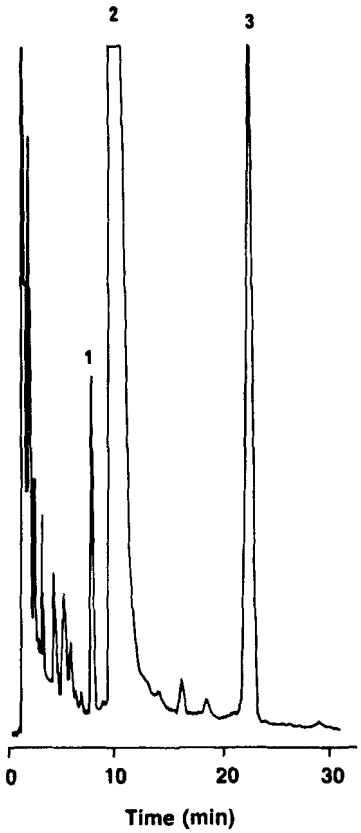

FIGURE 5

Chromatogram of thermally degraded (1 mo at 80°C) ophthalmic solution sample demonstrating 53% thimerosol remaining. Key same as Figure 2.

The dithizone procedure quantified total mercury in solution. Method linearity is shown in Figure 7. This calibration curve exhibited excellent linearity in the range tested with slope of -0.00454, intercept 0.303, a correlation coefficient 0.99565.

Ketorolac ophthalmic solution samples packaged in various low-density polyethylene (LDPE) and polypropylene bottles were thermally stressed (40°C). After 3.5 mo, the thermally stressed samples were quantitated by the HPLC and dithizone procedures.

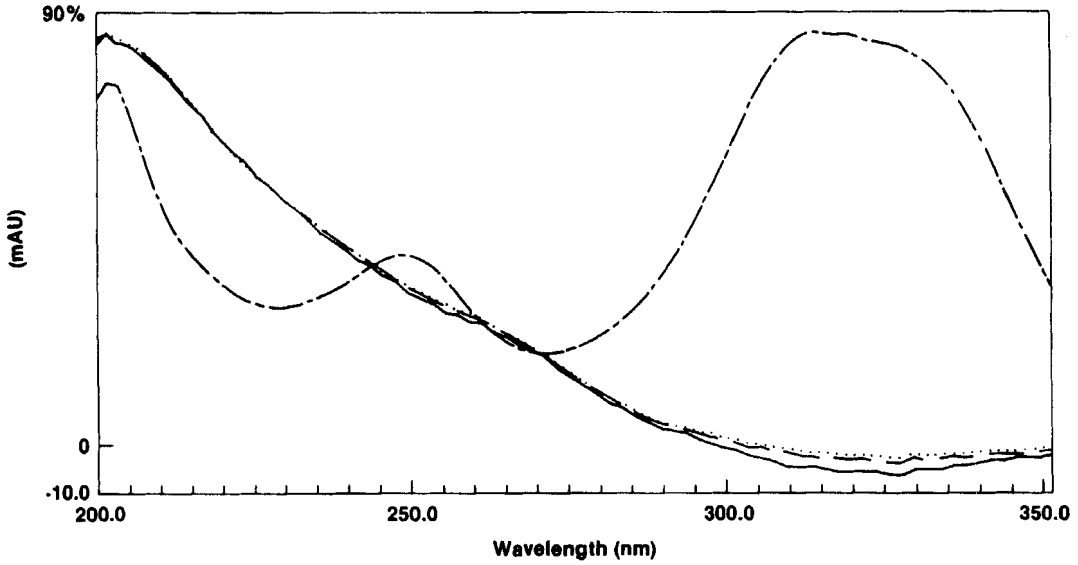

FIGURE 6

Spectral overlay plot of thermally degraded thimerosol peak shown in Figure 5. No interference from ketorolac tromethamine (__ _____ __) noted at beginning (_____), apex (. . .) and tail (__ __) of thimerosol peak.

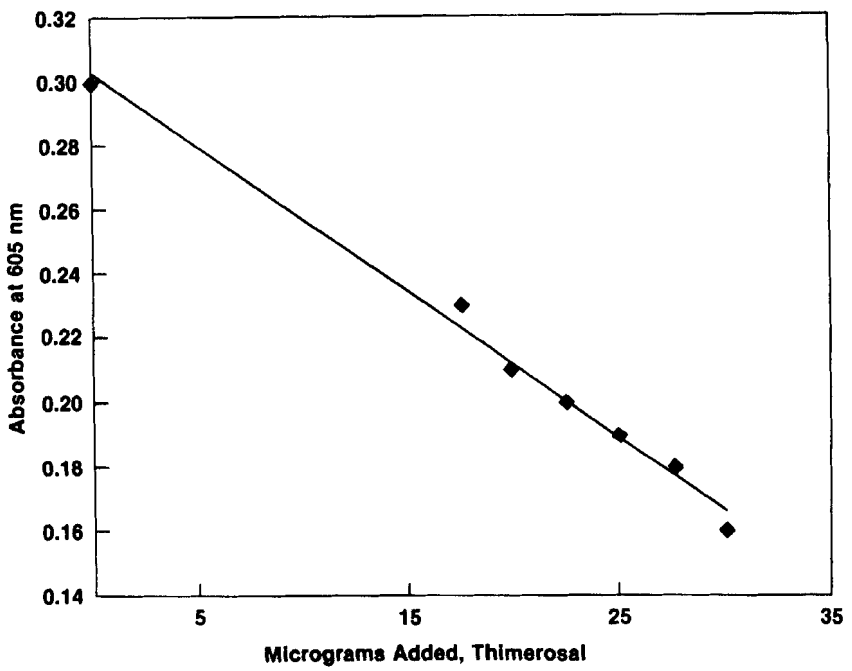

FIGURE 7

Plot demonstrating linearity of dithizone procedure for thimerosol quantitation.

Table 1

Thimerosal Stability in Various Low Density Polyethylene (LDPE) and Polypropylene Bottles

Bottle[a]	Temp. °C	% Thimerosal Remaining at Time, Mo. Initial[b]	3.5[b]	3.5[c]
A	40		27,28	56,56
	25	101,103	41,48	86,86
B	40		31,36	84,88
	25	101,103	46,49	104,109
C	40		23,31	64,68
	25	101,103	41,44	91,95
D	40		27,31	68,68
	25	101,103	41,41	91,95
E	40		26,31	64,68
	25	101,103	41,41	95,95
F	40		37,38	76,80
	25	101,103	40,41	100,104

[a]Bottle B is polypropylene, others are LDPE.
[b]HPLC quantitation.
[c]Colorimetric quantitation (dithizone).

Table 1 shows that all samples quantitated by the HPLC technique demonstrated greater than 70% thimerosal degradation after 3.5 mo at 40°C. Similar low thimerosal values were found in samples maintained 3.5 mo at room temperature. Alternately, identical samples quantitated using the non-specific dithizone technique demonstrated higher thimerosal values.

Therefore, based on the dithizone quantitation results, it may be more appropriate to correlate the non-specific colorimetric procedure rather than a stability-specific HPLC procedure with the antimicrobial activity of thimerosal. This is supported by observations that degraded thimerosal solutions may demonstrate more effective antimicrobial properties than non-degraded thimerosal [12].

ACKNOWLEDGEMENTS

The authors wish to thank Dr. R. Fu and Ms. C. Pulsipher for preparing ketorolac tromethamine ophthalmic solution and Dr. R. Kenley for editorial and technical assistance.

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