Table II—Assay Results of Commercial Products

Product	Phenylpropanolamine Hydrochloride		Additional Active Ingredients[a]
	Labeled	Found	
Uncoated tablet	12.5 mg/tablet	13.1	2, 4
Syrup	37.5 mg/5 ml	34.6	4, 5
Film-coated tablet	18.75 mg/tablet	19.9	2, 4
Capsule	25 mg/capsule	25.5	3, 4
Spray	0.2%	0.224	6, 7, 9
Capsule	18 mg/capsule	19.0	1
Uncoated tablet	25 mg/tablet	24.1	2
Sugar-coated tablet	18.75 mg/tablet	17.7	2, 4
Uncoated tablet	25 mg/tablet	24.2	1, 8
Press-coated tablet	25 mg/tablet	25.2	2, 3, 4

[a] 1 = acetaminophen, 2 = aspirin, 3 = caffeine, 4 = chlorpheniramine maleate, 5 = dextromethorphan hydrobromide, 6 = naphazoline, 7 = phenylephrine hydrochloride, 8 = phenyltoloxamine citrate, and 9 = pyrilamine maleate.

synthetic samples (Table I). One set contained phenylpropanolamine at the level of interest while the other two sets contained levels 20% above and below that level.

To demonstrate the utility of the proposed method, several commercially available products were assayed (Table II). An attempt was made to select products representing a wide variety of dosage form types as well as accompanying active ingredients. Duplicate analyses were carried out on two individual unit dose samples or, with liquids, on two aliquots containing between 12 and 25 mg of phenylpropanolamine hydrochloride. The object was merely to illustrate the applicability of the assay and not to establish accurate phenylpropanolamine hydrochloride levels for the various products.

For dosage forms where phenylpropanolamine is the only primary amine present, this method provides a convenient, specific, sensitive, and easily automated alternative to existing methods. It has been employed routinely and successfully in this laboratory for more than 2 years.

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Thimerosal Determination by High-Pressure Liquid Chromatography

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Abstract □ A sensitive and useful high-pressure liquid chromatographic method for the determination of intact thimerosal was developed. This method is extremely fast and reliable, and its inherent specificity makes it a breakthrough over other common wet chemical methods.

Keyphrases □ Thimerosal—high-pressure liquid chromatographic analysis in aqueous pharmaceutical preparations □ High-pressure liquid chromatography—analysis, thimerosal in aqueous pharmaceutical preparations □ Anti-infectives, topical—thimerosal, high-pressure liquid chromatographic analysis in aqueous pharmaceutical preparations

Thimerosal, ethyl (sodium *o*-mercaptobenzoato)mercury (I), is a relatively stable organomercurial that has long been used as both a topical antiseptic and a preservative. It is the preservative of choice for soft contact lens care solutions. Its stability, compatibility, and low toxicity account for its wide use.

BACKGROUND

The low concentrations of thimerosal typically used (10–50 ppm) have led to difficulties in development of suitable assays. Early analysis methods for organic mercurials involved the decomposition of the metallo-organic compounds (1) and detection with a complexing agent such as diphenylthiocarbazone (dithizone) or diphenylcarbazone (2, 3). More specificity was obtained by the use of better extraction procedures (4) or column chromatography (5). More sophisticated analytical methods such as atomic absorption (6), neutron activation (7), and GLC (8) detect degradation products as well as intact thimerosal.

Thimerosal degrades in aqueous solution to form ethylmercury salts and thiosalicylic acid (9, 10). Therefore, techniques based on either total mercury or total organic mercury do not reflect accurately the amount of intact thimerosal present in solution. Since some degradation products may have a higher toxicity potential than the original thimerosal (11, 12), accurate analytical techniques are essential.

Quantitative high-pressure liquid chromatography (HPLC) has been suggested as a simple, specific method that meets these objectives (13).

The HPLC method described here is a rapid, specific determination of intact thimerosal at 5–25 ppm (μg/g) in aqueous samples. In many cases, no sample cleanup is needed before determination. However, interferences are sometimes encountered because of components of the formulation. In such cases, a simple extraction procedure can be used.

To facilitate the handling of large numbers of samples, the assay is designed for the smallest retention time consistent with good chromatography. Thimerosal content of several commercial soft contact lens formulations was determined using this method to verify the usefulness of the procedure.

EXPERIMENTAL

Apparatus—The HPLC system consisted of a pump[1], a UV detector[2], and an automatic sampler[3].

Reagents—All reagents were analytical reagent grade unless noted otherwise. The mobile phase was 0.1 *M* ammonium carbonate with the pH adjusted to 7.9 with acetic acid. To prepare pH 4 acetate buffer, 410

[1] Waters Associates model 6000 pump.
[2] Chromatonix model 260 UV detector (254 nm).
[3] DuPont Instruments model 834 autoinjector with a 50-μl loop.

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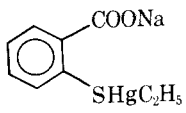

I

ml of Solution A (11.55 ml of concentrated acetic acid/liter of water) and 90 ml of Solution B (2.72 g of sodium acetate trihydrate/100 ml of water) were mixed with 500 ml of water. To prepare pH 10 carbonate buffer, 27.5 ml of Solution C (2.12 g of sodium carbonate/100 ml of water) and 22.5 ml of Solution D (1.68 g of sodium bicarbonate/100 ml of water) were mixed and diluted to 200 ml with water.

Standards—A stock solution was prepared by dissolving 200 mg of thimerosal in 100 ml of distilled deionized water. The working standard (20 ppm or 0.0020%) was a 1:100 dilution of the stock solution.

Chromatography—A 3.9 × 500-mm, Vydac C-18 reversed-phase 30–44-μm column was equilibrated with acetonitrile by overnight conditioning at a low flow rate. The next morning, the mobile phase was pumped through the system followed by five to 10 injections of the stock standard to saturate the column. Reproducibility usually was obtained with a few injections of a 20-ppm standard solution.

Polymers such as polysorbate 80 possess no UV absorption that can interfere. However, these and other compounds do build up slowly on the column; slowly decreasing retention times with increasing peak heights are observed as the compound coats the column packing. This problem can be alleviated by flushing the column with acetonitrile.

The chromatographic conditions were as follows: flow rate, 2 ml/min; pressure, ~2000 psi; temperature, ambient; detector wavelength, 254 nm; detector sensitivity, 0.08 aufs; and chart speed, 20 cm/hr.

Sample Preparation—Many formulations can be injected on the column, and thimerosal can be determined directly. Samples containing certain surfactants, polymers, and other excipients that interfere with the assay can be cleaned up with a simple extraction procedure. A 5-ml sample (20 ppm of thimerosal) is added to 50 ml of pH 4 acetate buffer and extracted with 50 ml of chloroform. The chloroform is then extracted with 5 ml of pH 10 carbonate buffer. The carbonate buffer is then chromatographed as any other sample. The initial sample volume can be altered appropriately so that the final thimerosal concentration is within the range of the standard.

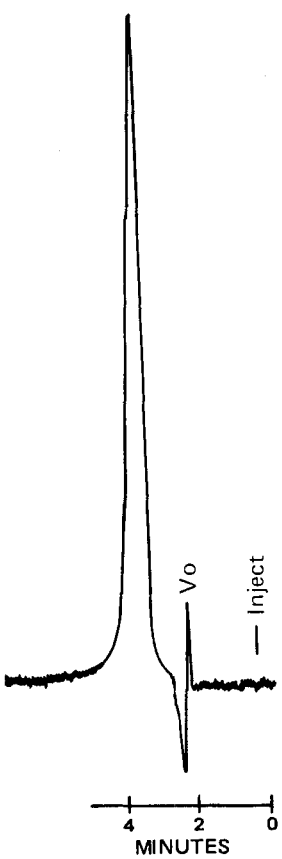

Figure 1—*Chromatogram of thimerosal.*

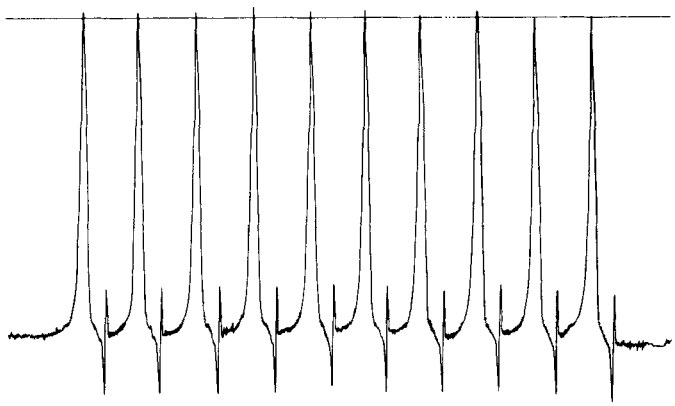

Figure 2—*Typical reproducibility of thimerosal.*

Calculation—The results are calculated using:

$$\text{concentration of thimerosal (sample)} = \frac{\text{peak height (sample)}}{\text{peak height (standard)}} \times \text{concentration of thimerosal (standard)} \quad \text{(Eq. 1)}$$

Test Solutions—Several soft lens products containing thimerosal formulated in this laboratory were determined using the method. In addition, several solutions labeled as containing thimerosal were bought from commercial sources both outside and within the United States. All laboratory samples were within their shelflife expiration date, and one commercial sample was past it. Exact age could not always be determined. When extraction cleanup was necessary, a sample of the product was spiked with thimerosal and tested immediately.

RESULTS

As seen in Fig. 1, thimerosal eluted as a sharp peak soon after the baseline had been reestablished following the initial injection spike (V_0). This behavior allows for an analysis time of approximately 4 min/sample.

The calibration curve of thimerosal concentration *versus* peak height was linear over the 0–25-ppm range and passed through the origin (r = 0.99994). Replicate injections of a standard solution had a relative

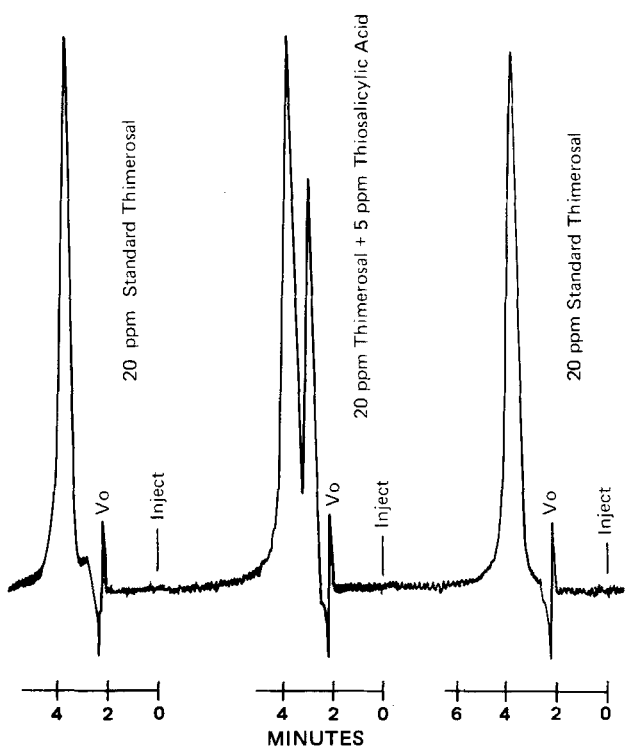

Figure 3—*Separation of thimerosal and thiosalicylic acid.*

Table I—Thimerosal Assay of Soft Contact Lens Products

Product/ Major Ingredients	Sample	Expiration Date	Date of Assay	Age at Assay, months	Method	Percent of Label Found	Spike Recovery, %
Product A: 0.001% thimerosal, 0.01%	1	6/77	12/76	18	Direct injection	122	—
ethylenediaminetetraacetic acid (II) and 0.85% sodium	2	10/78	6/77	6	Direct injection	122	—
chloride (III) boric acid	3	12/78	4/77	6	Direct injection	110	—
Product B: 0.002% thimerosal and 0.03%	1	4/77	3/77	20	Direct injection	97	—
alkyltriethanolammonium chloride	2	1/78	3/77	12	Direct injection	101	—
	3	9/78	3/77	4	Direct injection	106	—
Product C: 0.001% thimerosal, 0.005% chlorhexidine, 0.1%	1	5/77	10/76	9	Extraction	<5	102
II, 0.9% III, and povidone (IV)	2	12/77	2/77	6	Extraction	16	—
	3	1/78	3/77	2	Extraction	41	—
	4	9/77	10/76	5	Extraction	<5	—
Product D: 0.001% thimerosal, 0.1% II, and 0.005%	1	10/78	2/77	4	Extraction	101	103
chlorhexidine	2	12/78	3/77	3	Extraction	114	—
Product E: 0.001% thimerosal, 0.1% II, and 0.7% III	1	1/78	2/77	13	Extraction	65	94
Product F: 0.001% thimerosal, 0.1% II, and 0.7% III	1	5/78	2/77	9	Extraction	113	103
Product G: 0.001% thimerosal, 0.1% II, and 0.7% III	1	11/76	2/77	26	Extraction	52	102
	2	11/78	2/77	3	Extraction	112	—
	3	1/79	2/77	2	Extraction	113	—
	4	11/78	2/77	3	Extraction	100	—

standard deviation of ±0.91% (Fig. 2). Replicate injections of samples yielded slightly higher relative standard deviations of ±1–2%. The minimum detectable concentration of thimerosal is less than 0.5 ppm with a 50-μl sample loop.

One degradation product of thimerosal is thiosalicylic acid. Its peak appears before that of thimerosal, however, so it does not interfere with the assay (Fig. 3). Ethylenediaminetetraacetic acid interferes at concentrations greater than approximately 0.02% but can be eliminated by the extraction cleanup procedure.

DISCUSSION

The data in Table I indicate the utility of the method. All samples tested, except G_1, were within their expiration dates. However, one-third of the 18 products tested contained significantly less than the labeled amount of thimerosal, with two showing virtually none present at all. When these products were spiked with thimerosal, the assay did detect appropriate amounts, showing that there was no interference with the assay.

The fact that all four samples of Product C showed inadequate amounts of thimerosal suggests that the expiration data for the product may be based on an inadequate assay and that the thimerosal may not be stable in the formulation. Sample C_3 was only 2 months old but contained only 41% of its labeled thimerosal in the intact form. Samples C_1 and C_4, at 9 and 5 months, respectively, had virtually no intact thimerosal present.

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