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Quantitative High-Pressure Liquid Chromatographic Determination of Thimerosal in Pharmaceutical Formulations

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Abstract □ A quantitative high-pressure liquid chromatographic method using an anion-exchange resin column and an aqueous perchlorate solution as the mobile phase is employed for the determination of thimerosal in pharmaceutical formulations. With a liquid formulation containing large amounts of edetate disodium, calcium chloride is used for complexation to eliminate the interference from edetate disodium.

Keyphrases □ Thimerosal—high-pressure liquid chromatographic analysis, pharmaceutical formulations □ High-pressure liquid chromatography—analysis, thimerosal in pharmaceutical formulations

Thimerosal is an organomercurial chemical with antibacterial activity. It is used as an antiseptic for applications such as skin disinfection, urethral irrigation, and preservation of ophthalmic formulations (1). Compendial analytical methods using atomic absorption spectroscopy lack specificity (2). Another analytical method employing colorimetry of a dithizone complex (3) is tedious, and the presence of metal ions in the reagents causes interference.

Described here is a high-pressure liquid chromatographic (HPLC) analysis of thimerosal using an anion-exchange resin column with buffered sodium perchlorate as the mobile phase. The method is specific and simple.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1] equipped with a pump (7000 psig maximum), dual-channel UV detectors at 254 and 280 nm, and a 3 × 500-mm stainless steel column packed with anion-exchange resin[2] was used.

Reagents—The mobile phase was 0.35% perchloric acid[3] in 0.001 *M* dibasic sodium phosphate with pH adjusted to 7.0 with 1 *N* sodium hydroxide.

Standard Solution—Dissolve 8, 10, and 12 mg of thimerosal, separately, in purified water and dilute to 1 liter. These solutions represent thimerosal concentrations of 0.0008, 0.001, and 0.0012%, respectively.

Sample Solution—Dilute the sample solutions with purified water to make a final thimerosal concentration of 0.001%.

Chromatographic Separation—The procedure is run at ambient temperature, and the solvent flow is 1.6 ml/min. The UV monitor is set at 254 nm with a sensitivity of 0.02 absorbance unit. The samples and standards are injected with a 30-μl loop. The concentration of thimerosal is calculated from a standard curve using peak area (*i.e.*, height times width at half-height) for quantitation.

[1] Spectra Physics isocratic model 3500B, Santa Clara, Calif.
[2] Vydac, Applied Science Laboratories, State College, Pa.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.

RESULTS AND DISCUSSION

A typical chromatogram of thimerosal is shown in Fig. 1*a*. The chromatogram of one sample was complete in less than 5 min. Areas were used for the calculation of thimerosal concentration. Linear response over the concentration range from 0 to 15 μg/ml was obtained.

Table I shows the results using both the HPLC and the dithizone complex colorimetric methods. A solution containing edetate disodium, a common ingredient in ophthalmic formulations, and thimerosal in the ratio of less than 10:1 was separated and analyzed by this method without further sample treatment. However, an overlap of peaks was observed if a formulation contained edetate disodium and thimerosal in a ratio of more than 10:1. In such cases, calcium chloride was added to the sample solution to suppress the edetate disodium peak. With this step, complete separation and analysis of thimerosal were obtained.

Figure 1*b* illustrates a typical chromatogram of a sample solution containing 0.02% edetate disodium and 0.001% thimerosal. Figure 1*c* shows the chromatogram of the sample solution after treating with calcium chloride (to each 5 ml of sample solution was added 0.1 ml of 2%

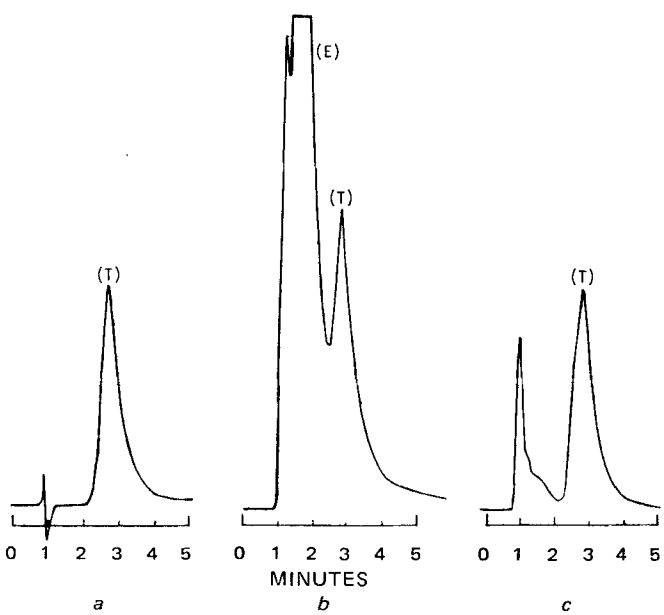

Figure 1—*Typical chromatograms of* (a) *thimerosal standard solution at 0.001%,* (b) *sample solution containing 0.02% edetate disodium and 0.001% thimerosal, and* (c) *0.1 ml of 2% $CaCl_2$ solution with 5 ml of sample solution* (b). *Key: T, thimerosal; and E, edetate disodium.*

Table I—Comparison of Dithizone Colorimetric and HPLC Methods for Thimerosal Determination

Sample	Thimerosal, μg/ml	
	Colorimetric	HPLC
1	11.5	11.9
2	11.5	11.7
3	11.8	12.3

calcium chloride solution). The amount of calcium chloride added to the sample solution depended on the actual amount of edetate disodium present. Since the complexation of edetate disodium with metal ions is pH dependent, one must adjust the sample solution pH to between 6.8 and 7.4 to obtain the best results.

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Sex Differences in Drug Evaluations

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Abstract □ Twenty normal, nonobese subjects (10 male and 10 female) were administered a battery of seven psychomotor tests as well as affect checklists and physiological measurements on 6 alternate days. Subjects performed the entire battery predrug and at 45, 90, and 135 min postdrug. Fenfluramine, dextroamphetamine, two combined doses of the drugs, and a placebo were given in a double-blind, repeated measures design. Findings revealed significant sex-based differences in initial performance on five of the seven psychomotor tasks and in two physiological measures, with males performing at higher levels than females. Additionally, sex differences in postdrug changes were found in three psychomotor and two physiological measures, with females evidencing greater change scores than males.

Keyphrases □ Fenfluramine—alone and combined with dextroamphetamine, sex-based differences in psychomotor and physiological effects, humans □ Dextroamphetamine—alone and combined with fenfluramine, sex-based differences in psychomotor and physiological effects, humans □ Psychomotor effects—fenfluramine and dextroamphetamine, alone and in combination, sex-based differences, humans □ Physiological effects—fenfluramine and dextroamphetamine, alone and in combination, sex-based differences, humans □ Sex-based differences—fenfluramine and dextroamphetamine, alone and in combination, psychomotor and physiological effects, humans

Fenfluramine is an anorexic agent structurally similar to dextroamphetamine; however, it does not produce central nervous system (CNS) activation with respect to measures of sleep (1) or psychomotor performance (2). The mood elevation, euphoria, and lift produced by dextroamphetamine are well documented (3–5); fenfluramine, however, appears to have sedative properties (2, 4, 6). There is some evidence that fenfluramine may block the CNS stimulant effects of dextroamphetamine (7).

As with most psychopharmacological investigations, sex-based differences in drug reactivity were not explored in the studies cited (8). A chance assignment of subjects in a study to be reported elsewhere provided a comparison of pre- and postdrug performance and mood differences between the sexes given identical treatments. Based upon a preliminary analysis, it was hypothesized that males would have higher levels of performance on psychomotor tests than would females and that sex differences would occur in the pattern of drug reactivity.

EXPERIMENTAL

Twenty subjects (10 male and 10 female, age range of 21–30, $\bar{X}$ = 23.7) were recruited by newspaper advertisements and screened by psychiatric interview and psychological test (16 PF) (9). Informed consent was obtained and subjects were paid for their participation. Subjects were asked to abstain from all medication and alcohol and to maintain normal diet and sleeping habits during the 2-week testing period.

Subjects were tested at the same time of day (11:30 am–5:00 pm) on 6 alternate days. The 1st day was primarily for practice and familiarization with the tests, and this day's data were subsequently discarded. On each of the remaining 5 test days, the subject performed the entire test battery of psychomotor, perceptual, physiological, and affect evaluations prior to receiving medication and at 45, 90, and 135 min postmedication (exceptions noted in test descriptions).

Five treatments were used: (*a*) placebo (lactose), (*b*) fenfluramine alone (60 mg), (*c*) dextroamphetamine alone (10 mg), (*d*) a low combination (60 mg of fenfluramine plus 10 mg of dextroamphetamine), and (*e*) a high combination (90 mg of fenfluramine plus 10 mg of dextroamphetamine).

Each of the five conditions appeared equally on each test day, and subjects were randomly assigned to a treatment sequence upon acceptance. Postdrug measures were compared against the predrug baseline for each subject on each test day to minimize practice and potential day-of-week effects. Both the subject and experimenter were unaware of the contents of the identically appearing capsules. In the five treatment groups representing orders of administration of placebo and four drug conditions, males and females appeared in the ratios of 1:1, 0:1, 3:1, and 1:3. This approach provided the opportunity for an evaluation of study results with sex as a main factor.

The test battery included various psychomotor tasks, several physiological measures, and self-report mood evaluative forms. Psychomotor tests have been shown to be sensitive to various drug effects (8, 10) and were used previously in this laboratory (2).

Physiological Measures—Pulse rate, oral temperature, blood pressure, and salivary output were taken daily prior to drug administration and at 200 min postdrug; pupil size was measured predrug and at 45, 90, and 135 min postdrug. Additionally, the Lorr outpatient mood scale