

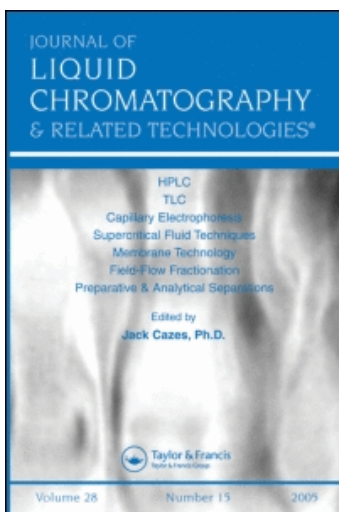
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Analysis of Mercury-Containing Drugs by High Performance Liquid Chromatography with Atomic Absorption Detection

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**ANALYSIS OF MERCURY-CONTAINING
DRUGS BY HIGH PERFORMANCE LIQUID
CHROMATOGRAPHY WITH ATOMIC
ABSORPTION DETECTION**

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ABSTRACT

A simple and specific high performance liquid chromatographic method has been developed for the determination of mercury-containing drugs. In most cases, an aliquot of the diluted sample is injected directly into a Zorbax ODS column and eluted with methanol-0.05 N ammonium acetate (3+2), pH 5.7, containing 0.01% 2-mercaptoethanol. Detection is accomplished by atomic absorption spectrophotometry with the aid of a specially designed apparatus for the generation of mercury vapor.

INTRODUCTION

Existing methods for the analysis of mercury-containing drugs are generally based on the determination of total mercury. In the older methods (1) the determinative steps consisted of either a gravimetric precipitation of mercury as mercuric sulfide or an indirect iodometric titration. More modern methods are based on vapor-phase or flame atomic absorption spectrophotometry (2,3). In general, these methods are non specific since they fail to distinguish between the mercury derived from the mercury-containing compound and that from other components of the sample.

We have recently interfaced a high performance liquid chromatograph (HPLC) with an atomic absorption spectrophotometer (AAS) for the determination of methylmercury in fish (4). Mercury vapor was generated using a specially constructed apparatus. This analytical system has now been adapted to the specific analysis of mercury in mercury-containing drugs.

EXPERIMENTAL

Apparatus.--The high performance liquid chromatograph and the atomic absorption interface used were those described earlier (4). A Perkin-Elmer Model 5000 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) was used. The light source was a mercury hollow cathode lamp set at a wavelength of 253.7 nm. Separations were accomplished on a 25 cm x 4.5 mm i.d. 5 μ m Zorbax ODS column (Du Pont Co., Wilmington, DE). The mobile phase was methanol-

0.05 N ammonium acetate (3+2) (final pH 5.7), containing 0.01% 2-mercaptoethanol.

Reagents.-- Thimerosal, phenylmercuric borate, mersalyl and mercuric chloride were of analytical reagent grade. Dilute working solutions containing (5 $\mu\text{g/ml}$ Hg) were prepared daily from aqueous stock solutions (1000 $\mu\text{g/mL}$ Hg).

Sample Preparation and Analysis.--Tinctures, solutions and eye drops were diluted with water to about 5 $\mu\text{g/mL}$ Hg, as necessary. Ointment of ammoniated mercury was treated as follows: To 0.1 μg well-mixed sample, accurately weighed into a 50 mL beaker, 5 mL 5N HCl was added. The beaker was covered with watch glass and heated on steam for 30 min with occasional mixing. The mixture was cooled to room temperature, the fat allowed to coagulate, and the solution was decanted into a 100 mL volumetric flask. The beaker and its content were washed with several portions of water, washings were added to the 100 mL volumetric flask, and the contents diluted to volume. An aliquot of this solution was adjusted to pH 5.7 with ammonium hydride and acetic acid, and further diluted to contain 5 $\mu\text{g/mL}$ Hg. The sample solutions were injected into the HPLC followed by the corresponding standard, except for ammoniated mercury, where a mercuric chloride standard was used. Calculations were based on either peak heights or peak areas.

RESULTS AND DISCUSSION

Flame ASS is a relatively insensitive technique for detecting mercury. Improvement of the sensitivity by direct interfacing of HPLC to a flame AAS is impractical due to peak broadening. However, greater sensitivity can be achieved by using a HPLC/AAS interface

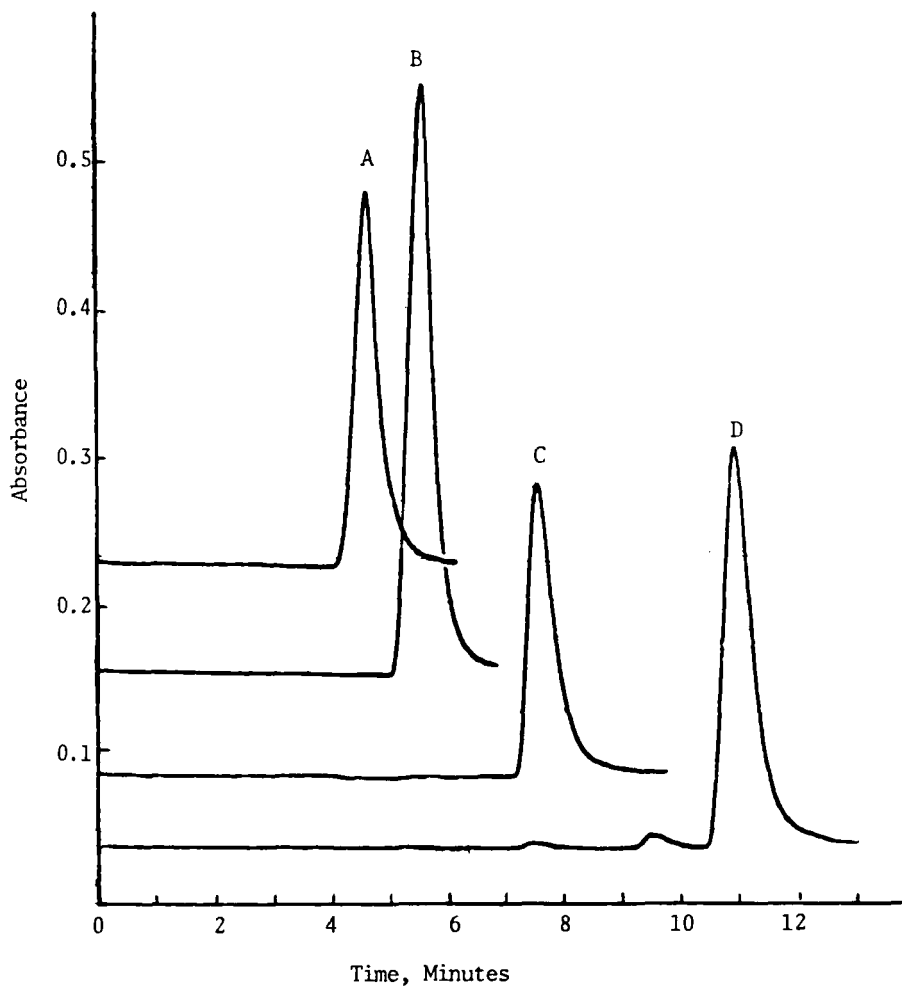


Figure 1. HPLC/AAS chromatograms of mercury - containing compounds. A, mersalyl; B, Hg^{++} ; C, thimerosal; C, phenylmercuric borate.

Table 1. Results of analysis of samples by the proposed HPLC/AAS and direct AAS (s) methods.

Sample ^a	Found	
	HPLC/AAS	AAS
Thimerosal eye drops, 0.001%	0.00098% ^b	0.00097%
Thimerosal tincture, 0.1%	0.098%	0.099%
Phenylmercuric borate solution, 0.01%	0.0097%	0.0101%
Mersalyl injection, 100 mg/mL	100.4 mg/mL	100.2 mg/mL
Ammoniated mercury ointment, 5%	4.92% ^c	5.02%

^aMersalyl injection and ammoniated mercury ointment were commercial samples, others were synthetics.

^bAverage of six determinations, SD 0.0000217, CV, 2.2%

^cCalculated using peaks areas.

which is designed to generate mercury vapor. Figure 1 shows typical signals and retention times obtained for mersalyl, Hg⁺⁺, thimerosal and phenylmercuric borate standards by the proposed method. Inorganic mercury is eluted from the reverse phase column because of the presence of 2-mercaptoethanol in the mobile phase. This reagent combines with mercuric ion to form a neutral compound, which can then be determined along with the organo-mercurials.

A number of synthetic as well as commercial samples were analyzed by both the proposed HPLC/AAS method and by direct AAS (3). The

results shown in Table 1 indicate that a good agreement exists between the two methods. The HPLC/AAS method, however, has a higher specificity for mercury-containing compounds due to the chromatographic separation and to the mode of detection. Thus, other compounds eluting simultaneously with the organo-mercurials will not interfere, since they are not detectable.

Either peak heights or peak areas may be used for quantitation purposes provided that the compounds in the standard and sample preparations elute similarly. However, when matrix differences exist between samples and standards, the assay results will be dependent on the choice of approach. For example, in the case of ammoniated mercury ointment, only peak areas under the peaks were proportional to the quantity of mercury. Alternatively, the method of standard additions may be used.

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