Determination of Mercury-Containing Pharmaceuticals by Vapor Phase Atomic Absorption Spectroscopy

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Abstract □ A procedure was developed for the determination of mercurials of pharmaceutical interest. Protic acid cleavage of the compound was followed by reduction of the resulting mercuric ion and vapor phase atomic absorption spectroscopy. This procedure was applied to 11 different mercurial compounds in various pharmaceutical preparations and offers excellent sensitivity with respect to presently used compendial assays. Comparative analytical data between this procedure and compendial methodology are presented.

Keyphrases □ Mercurials—determination by vapor phase atomic absorption spectroscopy, compared to compendial methods □ Atomic absorption spectroscopy, vapor phase—determination, mercury-containing pharmaceuticals, compared to compendial methods

Pharmaceutical compounds containing the element mercury, either organically combined or as the inorganic form, have found widespread medicinal use as diuretics, anti-infectives, and bacteriostatic agents. Mercury-containing pharmaceuticals have been analyzed by numerous analytical approaches. Many presently used procedures (1–3) involve conversion of mercurial mercury to mercuric ion followed by classical thiocyanate titrimetry or precipitation of the insoluble mercuric sulfide and gravimetric measurement. Theimer and Arnow (4) applied the thiocyanate titration procedure to injectable organomercurial diuretics.

A titration procedure similar in sensitivity to the official methods involves the acetolysis of the compound in the presence of methylamine hydrochloride followed by nonaqueous titration of the liberated strong base with perchloric acid (5). A more sensitive and convenient approach, utilizing coulometric titration of mercuric ion with electrogenerated sulfhydryl reagent, was developed (6) and applied to marketed dosage forms. Mercaptomerin sodium, which contains a mercury–sulfur bond, could not be analyzed by this method. However, the determination can be carried out in the presence of chloride ion, a species incompatible with both thiocyanate titration and acetolysis procedures.

Direct current polarography has been used to analyze thimerosal (7, 8). Unsubstituted phenylmercuric compounds and mersalyl and meralluride were investigated also (8).

More recently, several sensitive instrumental approaches have been used in mercurial compound analysis. The principle of isotopic exchange utilizing ^{203}Hg was employed in the determination of 13 different mercurials of pharmaceutical or agricultural interest, including three commercial preparations (9). Margosis and Tanner (10) applied neutron activation analysis to the determination of seven different organomercurial compounds, three in pharmaceutical dosage forms. Their results, obtained from the ^{197}Hg- and ^{203}Hg-induced radioactivity, were compared to those obtained by X-ray fluorescence and atomic absorption techniques. The X-ray fluorescence results generally were lower than those achieved with the neutron activation procedure; the atomic absorption results obtained by direct and vapor phase techniques, although incomplete, generally were higher. The poor correlation obtained with the vapor phase technique was attributed to the possible presence of volatile aromatic-type compounds.

The introduction of atomic absorption spectroscopy as a sensitive and specific means for metal analysis has resulted in numerous applications for the determination of mercury, primarily from the environmental impact of the presence of this element (11–13). To date, only a few studies have employed this approach in the analysis of mercury-containing pharmaceuticals. Leaton (14) described a direct atomic absorption procedure for mercuric iodide in ointments, and similar methodology for the direct analysis of phenylmercuric nitrate in ophthalmic preparations was proposed (15). More recently, vapor phase atomic absorption was applied to the analysis of mercurial bacteriostatic agents (16, 17), including phenylmercuric acetate, phenylmercuric nitrate, and thimerosal (17).

The purpose of this study was to determine the feasibility of vapor phase atomic absorption spectroscopy for the analysis of mercurial compounds of pharmaceutical interest and their commercial preparations. The described procedure is convenient, sensitive, and accurate with respect to the official methods.

EXPERIMENTAL

The developed procedure is based on "protodemercuration" of the mercurial compound with hydrochloric acid or a hydrochloric acid–nitric acid mixture under various heating conditions followed by reduction of the resulting mercuric ion to elemental mercury with subsequent detection and quantitation by vapor phase (*e.g.*, flameless, cold vapor) atomic absorption spectroscopy. The final determinative step is in accordance with the reduction procedure of Hatch and Ott (18) as modified by Munns and Holland (19).

Table I—Analysis of Mercurial Compounds[a]

Compound	Heating Condition[b]	Atomic Absorption Method		Official Method	
		Recovery, %	Range, %	Recovery, %	Range, %
Thimerosal	C	100.1	0.2	100.0[c]	0.0
Mersalyl	A	103.1	0.2	103.5[d]	0.4
Meralluride	A	100.0	0.9	99.8[e]	0.1
Mercaptomerin sodium	C	100.9	0.6	99.5[e]	0.1
Phenylmercuric acetate	B	100.4	0.3	100.2[c]	0.1
Chlormerodrin	A	100.5	0.6	100.1[c]	0.0
Merbromin	A	94.6	0.1	94.8[f]	0.6
Nitromersol	B	99.5	1.0	99.8[c]	0.2
Phenylmercuric nitrate	B	99.2	1.5	98.4[c]	0.7
Mercuric oxide, yellow	D	100.8	0.5	100.0[c]	0.1
Ammoniated mercury	D	99.4	0.0	98.8[e]	0.0

[a] Mean of duplicate determinations. [b] See *Experimental*. [c] NF XIII. [d] NF XI. [e] USP XVIII. [f] NF XII.

Eleven mercurial compounds and the respective commercial preparations representing six different types of products were analyzed using the proposed methodology.

Apparatus—An atomic absorption spectrophotometer[1] equipped with a mercury hollow cathode lamp[2], a hydrogen continuum lamp[2] to provide background correction capability, and a cylindrical flow-through cell (2.1-cm i.d. × 10.0-cm) with quartz windows was used. Absorbance measurements were monitored with a 0–100-mv strip-chart recorder[3]. Mercury analysis was performed under the following conditions: wavelength, 253.7 nm; hollow cathode lamp current, 3 mamp; scale, 0.5; circulating pump flow rate, 4.8 liters/min; slit width, 160 μm; and chart speed, 2.5 cm (1 in.)/min.

The reduction apparatus was essentially as described previously (19) with the following modifications. The reduction vessel consisted of a 250-ml two-necked distilling flask with a vertical side neck and ⅋ 24/40 joints. The reducing solution was added *via* a 100-ml cylindrical separator attached to the side neck of the reduction vessel. All connections within the system were of Tygon tubing (5.0-mm i.d.).

Glassware was thoroughly rinsed with hot nitric acid (50% v/v) followed by distilled water prior to use. Dilutions of samples, pure compounds, and mercury standards were carried out in the presence of hydrochloric acid to minimize adsorption of mercuric ion on glassware surfaces.

Materials and Reagents—Powdered portions of thimerosal[4], mersalyl[5], meralluride[6], mercaptomerin sodium[7], phenylmercuric acetate[8], chlormerodrin[6], merbromin[9], nitromersol[10], phenylmercuric nitrate[11], yellow mercuric oxide[12], and ammoniated mercury[12] (mercury amide chloride) were used. Pharmaceutical preparations containing each mercurial compound were obtained through commercial sources. Crystalline mercuric chloride[12] prepared in an acidic medium was employed as the standard mercury solution. All other chemicals and reagents were reagent grade, commercially available materials and were used without further purification.

Standard Mercury Solution—A standard stock solution was prepared by dissolving 0.1354 g of crystalline mercuric chloride in 1 *N* HCl and diluting to 100.0 ml with the same solvent. This solution was prepared fresh on a biweekly basis. Suitable dilutions were prepared in 0.05 *N* HCl to provide a working standard solution having a concentration of 0.25 μg of mercury/ml. These dilutions were carried out daily and just prior to the quantitative reduction step.

General Procedure—Accurately weighed portions of solid materials or measured aliquots of the liquid forms representing the various mercurial compounds or respective products were treated under one of the following heating conditions with occasional swirling of the mixture:

A. Heated for 1 hr on a steam bath with 75 ml of concentrated hydrochloric acid in a 1-liter volumetric flask.

B. Boiled gently for 25 min on a hotplate with 75 ml of concentrated hydrochloric acid in a 300-ml erlenmeyer flask containing five glass beads and fitted with a 6.5-cm diameter powder funnel. Care was taken to prevent the solution from going to dryness by addition of 5–10 ml of the acid when necessary.

C. Heated for 1 hr on a steam bath with 75 ml of diluted hydrochloric acid–nitric acid mixture[13] in a 1-liter volumetric flask.

D. Heated for 15 min on a steam bath with 80 ml of dilute hydrochloric acid (10% v/v) in a 1-liter volumetric flask.

The required heating condition for each mercurial compound and the respective commercial preparation is noted in Table I. Upon completion of the heating step, the 1-liter flask and contents were cooled under tap water to room temperature and the acidic solution was diluted to volume with distilled water. When heating condition B was employed, the cooled acidic solution was quantitatively transferred to a 1-liter volumetric flask with distilled water and diluted to volume.

Further dilutions were prepared with 0.05 *N* HCl to provide an assay solution having a final concentration of approximately 0.25 μg of mercury/ml. Three milliliters of this assay solution was transferred to the reduction vessel, and the published procedure (19) was followed. The system was normally equilibrated with several standard determinations prior to the reduction of sample aliquots. A reagent blank consisting of the appropriate acid solution was carried through the entire procedure in a similar manner to the samples. Quantitation was accomplished by direct comparison of the sample absorbance value to the absorbance obtained with 3.0 ml of the working standard solution of mercury.

Bulk Drug Substances or Reference Compounds—An accurately weighed portion of the powdered material, equivalent to 60 mg of mercury, was taken for analysis. After completion of the appropriate heating step and dilution of the acid solution to 1 liter with distilled water, an assay solution was prepared by further dilution of 2.0 ml to 500.0 ml with 0.05 *N* HCl. A 3.0-ml aliquot of the assay solution was transferred to the reduction vessel for quantitative purposes.

Commercial Preparations—The quantities of mercury taken for the analysis of the various products are given in Table II. The amount of mercury present in the sample portions ranged from 2.5 to 82.5 mg, depending on the particular formulation with the exception of the product containing phenylmercuric nitrate[14]. Therefore, specified dilutions are not given due to the diversity in concentrations of mercury in the preparations.

Tablets and Gels—An accurately weighed portion of a powdered tablet composite or well-mixed gel preparation was taken for the analysis. The general procedure was followed.

Solutions and Tinctures—An accurately measured aliquot was evaporated to dryness under a current of air on a steam bath, the

[1] Model 353, Instrumentation Laboratory, Lexington, Mass.
[2] Instrumentation Laboratory, Lexington, Mass.
[3] Model 194, Honeywell Electronik.
[4] Eli Lilly and Co., Indianapolis, Ind., and NF reference standard.
[5] Winthrop Laboratories, Rensselaer, N.Y.
[6] Lakeside Laboratories, Milwaukee, Wis.
[7] Wyeth Laboratories, Philadelphia, Pa.
[8] Holland-Rantos Co., Trenton, N.J.
[9] Hynson, Westcott and Dunning, Baltimore, Md.
[10] Abbott Laboratories, North Chicago, Ill.
[11] Eastman Kodak Co., Rochester, N.Y.
[12] Mallinckrodt Chemical Works, St. Louis, Mo.

[13] Prepared by mixing 10 parts of water, eight parts of hydrochloric acid, and four parts of nitric acid by volume just prior to use.
[14] A 4.0-g sample portion equivalent to 0.24 mg of mercury was taken for the analysis.

Table II—Analysis of Commercial Products

Mercurial Compound	Type of Product	Declared Amount	Sample Size, Milligrams of Mercury Taken for Atomic Absorption Analysis	Percent of Declared Amount Found[a]			
				Atomic Absorption Method	Range	Official Method[b]	Range
Thimerosal	Tincture	1:1000	2.5	101.4	0.8	104.0	0.0
Mersalyl with theophylline	Injection	100 mg/ml	80	101.7	0.0	102.2	0.2
Meralluride	Injection	39 mg/ml[c]	78	100.2	0.5	100.8	0.1
Mercaptomerin sodium	Injection	125 mg/ml	82.5	100.7	0.0	99.0	0.0
Phenylmercuric acetate	Solution	1:500	6	110.2	0.5	107.2[d]	1.5
Chlormerodrin	Tablet	18.3 mg/tablet	10	97.8	0.0	102.4	0.7
Merbromin	Solution	2%	26.7	90.7	0.5	89.5	1.0
Nitromersol	Tincture	1:200	14.2	89.2	0.2	87.0	0.4
Phenylmercuric nitrate	Gel	0.01%	0.24	77.4	0.9	—[e]	—
Mercuric oxide, yellow	Ointment	2%[f]	32.4	100.5	1.0	102.0	0.0
Ammoniated mercury	Ointment	10%[g]	80	103.6	0.6	102.5	0.0

[a] Mean of duplicate determinations. [b] See Table I. [c] Label claim: 39 mg of mercury in organic combination and 48 mg of theophylline/ml. [d] Product assayed by the NF XIII procedure for phenylmercuric acetate; 250.0 ml was evaporated to dryness on a steambath and the NF XIII procedure was followed beginning with "add 15 ml. water, and 5 ml. of formic acid." [e] Official method not available. [f] Twice the NF strength. [g] Twice the USP strength.

required acid was added, and heating condition A, B, or C was followed (*General Procedure*).

Injectables—An accurately measured aliquot was taken directly for the analysis as described under *General Procedure*.

Ointments—An accurately weighed portion of the well-mixed product was transferred to a 125-ml separator and dispersed by shaking with 50 ml of ether. The ethereal dispersion was extracted four times with 20-ml volumes of dilute hydrochloric acid (10% v/v), and the acidic extracts were drained into a 1-liter volumetric flask. The general procedure was followed, beginning with heating condition D. This condition was employed to facilitate the removal of the residual ether present.

Calculations—The amount in milligrams of the mercurial compound present in the weighed portion or aliquot was calculated in the following manner:

$$\text{mg} = \frac{A_u}{A_s} \times C \times D \times \frac{E}{200.59} \times \frac{1\text{ mg}}{1000\ \mu\text{g}} \qquad \text{(Eq. 1)}$$

where A_u and A_s represent the absorbance values obtained for the reduced sample and standard aliquots, respectively (corrected for any reagent blank absorbance); C is the concentration in micrograms of mercury per milliliter in the final diluted mercuric chloride standard solution; D is the appropriate dilution factor; E is the molecular weight of the mercurial compound; and the value[15] 200.59 represents the atomic weight of mercury. Calculation of the quantity of the mercurial compound per dosage unit or labeled amount was achieved by introduction of the appropriate factors into Eq. 1.

Linearity—Under the assay conditions described, a linear relationship between absorbance and micrograms of mercury was obtained over a 0–1.4-μg range. A quantity of mercury equal to 0.75 μg was selected for the reduction step of the general procedure.

Official Methods—For comparative purposes, each pure compound and pharmaceutical preparation, except the product containing phenylmercuric nitrate, was assayed in duplicate by compendial methodology (1, 2).

RESULTS AND DISCUSSION

Preliminary experiments were conducted to determine the most suitable acid medium for the protolysis of the mercurial compounds in this study, since several different chemical bonds associated with mercury were apparent. Adaptation of a sulfuric acid–nitric acid digestion medium utilized for marine products (19) proved to be both time consuming and cumbersome. Modification of similar procedures (11, 12) under nonreflux conditions with respect to time and temperature was also investigated, but the results obtained were erratic due to the possible loss of mercury from volatilization. Evidence regarding volatility losses of mercury employing such digestion procedures for the total destruction of organic material was reported previously (11, 20).

It was concluded from these initial experiments that a heating step under acidic conditions was necessary to effect cleavage of mercury from the compounds in most cases. The inorganic mercurials, yellow mercuric oxide and ammoniated mercury, were easily cleaved at room temperature; compounds such as phenylmercuric acetate and mercaptomerin sodium containing the mercury–carbon and mercury–sulfur bond, respectively, required more drastic reaction conditions. Organomercurial compounds such as mersalyl and meralluride containing the mercury–nitrogen linkage were intermediate with respect to ease of cleavage. These observations were in agreement with the relative stabilities of such bonds discussed by Makarova and Nesmeyanov (21).

The use of hydrochloric acid to facilitate the cleavage of mercury–carbon bonds in addition to mercury in combination with oxygen or nitrogen has been described (6). The importance of bond type associated with mercury in analytical studies has also been noted (5). A study of the protolysis of the mercury–carbon bond with concentrated hydrochloric acid has been carried out (22). Based on these reported observations, hydrochloric acid was investigated for use as a protolytic solvent under varying time and temperature conditions to cleave mercury from the compounds considered in this study. Many difficulties associated with the total digestion procedures were overcome with hydrochloric acid. Moreover, total mercury could be successfully determined using hydrochloric acid for all compounds with the exception of mercaptomerin sodium and thimerosal, both of which contain the mercury–sulfur bond. The mercury present in these two compounds was effectively cleaved by a hydrochloric acid–nitric acid medium.

The results obtained for the bulk drug substances and reference compounds by both the described atomic absorption procedure and official methodology are summarized in Table I. The comparative data based on the amount of mercury found illustrate that the correlation between these two procedures is very good.

The comparative results for the analysis of the mercurial compounds in the pharmaceutical preparations by the atomic absorption and official procedures are presented in Table II. The atomic absorption procedure produced slightly different results for the product containing chlormerodrin, in which the mean percent of declared was 4.6% lower with the atomic absorption procedure.

[15] Twice this value was employed for the calculation of phenylmercuric nitrate, an equimolar combination of phenylmercuric nitrate and phenylmercuric hydroxide having an assigned molecular weight of 634.40.

The reason for this difference is not readily apparent from the limited analytical data available. In addition, mean differences of 2.6 and 3.0% of the percent of the declared amount were obtained for the products containing thimerosal and phenylmercuric acetate, respectively.

The product containing phenylmercuric nitrate, which gave a mean value of 77.4% of the declared amount by the atomic absorption procedure, could not be analyzed by adaptation of official methodology due to the low concentration of this ingredient. Additional atomic absorption analysis of this product utilizing heating condition C gave a mean value of 78.5% of the declared amount. Approximately 13 months after the initial analyses were performed, the product was reassayed by both the proposed atomic absorption method and a total digestion procedure (19). The results obtained from single determinations were 60.7 and 60.8% of the declared amount, respectively. These observations provide corroborative evidence that adsorption of this compound by the container material (polyethylene in this case) may occur over an extended period (16). A simulated preparation of the product was formulated in this laboratory and subjected to atomic absorption analysis utilizing heating condition B. The mean recovery for the phenylmercuric nitrate based on duplicate results was 99.6% with a range of 0.8%.

An orange precipitate was formed during the protolysis of the merbromin reference standard, which, upon isolation and total digestion under reflux conditions (19), revealed the absence of mercury. The product containing merbromin showed a similar precipitate during this stage of the analysis.

Recovery experiments employing heating condition B (hotplate) with mercuric chloride standards representing quantities of mercury in the range encountered with the commercial preparations yielded values between 98.7 and 100.4%. This finding indicated that loss of mercury due to volatilization was negligible.

At the sensitivity level employed in the atomic absorption procedure, the use of the background correction mode indicated the absence of interferences due to nonspecific absorption.

In general, the results obtained indicate the overall applicability of the described atomic absorption procedure or modifications thereof to the analysis of various mercurial compounds present in bulk form, as reference compounds, or in pharmaceutical mixtures. The method also should be adaptable to products containing *o*-hydroxyphenylmercuric chloride and various other preparations containing thimerosal.

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GLC Determination of Hexadiphane in Pharmaceutical Preparations

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Abstract □ A specific, rapid, and sensitive GLC method for purity control of hexadiphane and its determination in pharmaceutical preparations is described. The method utilizes an extraction of the free base, followed by GLC on a 0.5% OV-17 column at isothermal temperature for 6 min and then the temperature was programmed. Results from this method and from a titrimetric method were compared, and no significant differences were found.

Keyphrases □ Hexadiphane—GLC analysis in pharmaceutical formulations □ GLC—analysis, hexadiphane in pharmaceutical formulations

Hexadiphane, 1,1-diphenyl-3-hexamethyleneiminopropane (I), is a papaverine-like compound with weak anticholinergic effects. It is widely employed as an antispasmodic (1–5).

Quantitative determination of hexadiphane is essentially based on the chemistry of the imine moiety. The methods employed are those used for basic nitrogen compounds, primarily titration in nonaqueous