# Determination of Mercury in Pharmaceutical Products by Neutron Activation Analysis

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**Abstract** □ A reliable, efficient, and highly specific method is presented for the determination of mercury in pharmaceutical products. Neutron activation analysis is used for the elemental determination of various organomercurials and requires no chemical separation or cleanup. The induced radioactivity of $^{197}Hg$ and $^{203}Hg$ is the basis of this method. Recoveries ranged as follows: neutron activation analysis (14 samples), 90–108% ($^{197}Hg$) and 91–110% ($^{203}Hg$); X-ray fluorescence (10 samples), 91–101%; atomic absorption (nine samples), 84–112%; and cold vapor atomic absorption (four samples), 106–121%.

**Keyphrases** □ Mercury—analysis in pharmaceutical products, neutron activation analysis □ Organomercurials—mercury analysis, neutron activation analysis □ Neutron activation analysis—mercury in pharmaceutical products

Organomercurial chemicals have recently acquired increased importance because of widely publicized hazards associated with their use. Thimerosal (merthiolate), merbromin (mercurochrome), phenylmercuric acetate, and mersalyl are typical organomercurials used clinically as antibacterials or diuretics. They may be formulated as preservatives or active ingredients in pharmaceutical dosage forms. Current official methods of analysis for the mercury content of these chemicals usually require preliminary exhaustive and drastic oxidation to the ionic form, followed by a determinative step such as titrimetry with ammonium thiocyanate, gravimetry as the sulfide, or colorimetry of a dithizonate extract (1–3). Atomic absorption spectroscopy (2) and polarography (1) have also been used, but these tend to be restrictive because they require reference material of high purity qualitatively identical to the samples being analyzed. These various techniques are often cumbersome and tedious and are prone to manipulative losses, particularly in cases such as this where the element of interest is volatile. Moreover, they are easily affected by the chemical nature of the compound containing the mercury, by the presence of various components containing other metals, and by the character of the drug matrix or of the solvent system used in solubilizing it.

The application of nondestructive neutron activation analysis (NAA) for the determination of the presence of specific elements in pharmaceutical chemicals *per se* and as the basis for a presumptive assay in dosage forms has been demonstrated for aluminum (4), zinc (5), and the halogens (6). The technique has been successfully applied to the determination of trace amounts of mercury in environmental samples (although chemical methods were used for separation) as discussed by Weissler (7). The great value of nondestructive (instrumental) NAA in determining the elemental composition of pharmaceutical chemicals is mainly that it obviates the need for separative procedures, thereby eliminating the most common source of losses. This method is free from contamination and is independent of the physicochemical state of mercury and the matrix system normally inherent in drug products. It is simple, reliable, efficient, quantitative, and uniquely specific; therefore, it should prove very useful to drug chemists. From an economic and logistical point of view, NAA is readily accessible for regular laboratory use (8).

NAA consists of placing a known amount of sample (bulk or formulation powder, tablet, capsule, suppository, or solution) into an appropriate container and subjecting it to neutron bombardment, along with a mercury reference material (which need not be of the same chemical identity). The induced radionuclides are uniquely characterized by the energy and half-life of the $\gamma$-ray emitted. Quantitation is obtained by direct comparison of the ratio of activity to the concentration of the element within the sample with that of a reference material.

This study demonstrates the applicability of nondestructive NAA for the qualitative and quantitative determination of mercury in organomercurials and as a presumptive assay method for organomercurials in various pharmaceutical products.

## EXPERIMENTAL

**Apparatus and Equipment**—The nuclear research reactor, pulse-height analyzer, sodium iodide detector, and readout equipment were previously described (4–6). The analyzer had a gain control of 2 kev./channel. In addition, a lithium-drifted germanium [Ge(Li)] detector with a resolution of 6 kev. full-width (half-maximum), an efficiency of about 4% compared to the sodium iodide detector, and a peak-to-Compton ratio of about 15:1 was occasionally employed.

**Standard**—A stock solution of the mercury standard was prepared by dissolving an accurately weighed amount of reagent grade mercuric chloride in a volumetric flask with water. Standards for comparison were prepared by dilution of an aliquot of the stock solution. Pipeted aliquots of these standard solutions were trans-

**Table I**—Pertinent Nuclear Properties of Mercury for Activation with Thermal Neutrons

| Stable Nuclide | Abundance, % | Cross-Section, barns | Radionuclide Formed | Half-Life, $t_{1/2}$ | Major $\gamma$-Ray, Mev. |
|---|---|---|---|---|---|
| $^{196}Hg$[a] | 0.146 | 880 | $^{197}Hg$ | 65 hr. | 0.077 |
| | | 25 | $^{197m}Hg$ | 24 hr. | 0.134, 0.279 |
| $^{198}Hg$ | 10.02 | 0.02 | $^{199m}Hg$ | 43 min. | 0.158, 0.375 |
| $^{199}Hg$ | 16.84 | 2000 | Stable | — | — |
| $^{200}Hg$ | 23.13 | <50 | Stable | — | — |
| $^{201}Hg$ | 13.22 | <50 | Stable | — | — |
| $^{202}Hg$[a] | 29.80 | 4 | $^{203}Hg$ | 47 days | 0.279 |
| $^{204}Hg$ | 6.85 | 0.4 | $^{205}Hg$ | 5.5 months | 0.205 |

[a] Nuclides of interest in this study.

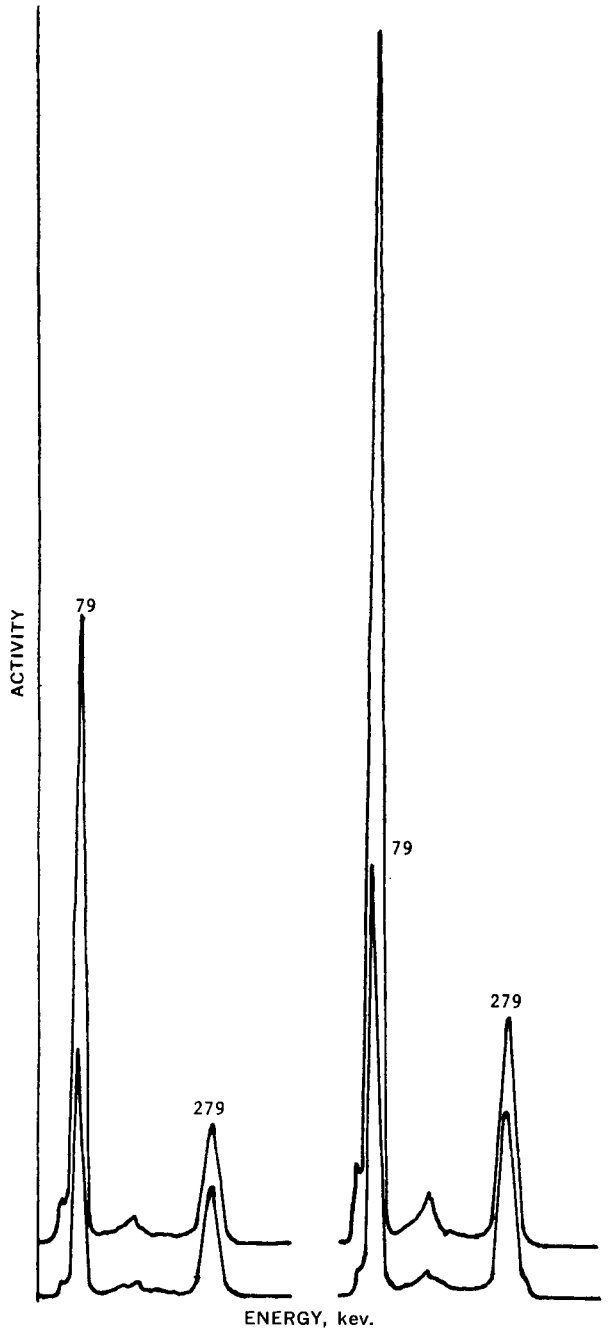


**Figure 1**—*Typical γ-ray spectra of mercuric chloride (standard) (2.519 mg./ml.) and phenylmercuric acetate obtained with NaI(Tl) detector after decay of several days and after 3 weeks (lower curves).*

ferred to polyvials with concentrations ranging from 50 mcg. to 2.5 mg./ml.

**Samples**—Accurately weighed milligram amounts of bulks, 1-ml. aliquots of liquid dosage forms, and whole suppositories were placed "as is" in the polyvials and double-sealed into 1-mil polyethylene bags. The bulk chemicals were readily available from commercial sources and were analyzed as received.

**Procedure**—Four to five polyvials (depending on size), comprising samples and one standard previously ascertained to be at about the same concentration levels, were packaged together into an irradiation bucket and lowered into the high flux exposure tube for a 5-min. irradiation. After a minimal overnight waiting/cooling period, the activity of each specimen was measured from a count of 10 live min. at a minimal distance from the detector so as not to exceed a deadtime of 20%. In this initial test, the activity measurement was repeated several times over a period of days or weeks for verification and/or to improve statistics. The data obtained were analyzed by the Covell method (9) for photopeak measurements to quantitate the mercury activity.

## RESULTS AND DISCUSSION

Elemental mercury is a natural composite of seven stable nuclides whose nuclear parameters and reaction with thermal neutrons are well known and predictable (10, 11) (Table I). The formation and use of $^{197}Hg$ and $^{203}Hg$ are particularly favored in this analysis because of the combined effect of these parameters to produce practical activity levels.

The γ-ray spectra of mercury obtained with the sodium iodide [NaI(Tl)] detector and the Ge(Li) detector are shown in Figs. 1 and 2, respectively. The 79-kev. photopeak of $^{197}Hg$ is initially more intense than the 279-kev. photopeak of $^{203}Hg$. However, after some period of decay, the 279-kev. photopeak begins to dominate (Fig. 2). The mercury contents of several commercial grade organomercurials and several dosage forms containing them were determined by NAA based on both radionuclide photopeaks; the results are shown in Table II. This type of doublecheck is considered by many experts in the field to be as reliable as a confirmatory chemical method and has been used previously in NAA of zinc and bromine (5, 6). The results obtained compare favorably with those obtained by other spectroscopic techniques. However, because of the low concentration of mercury and the interference due to other components and/or the matrix system, these alternative methods were deemed impractical and could not be reliably employed with the dosage forms. Their most salient shortcomings were as follows:

1. In X-ray fluorescence, several samples in solution incurred radiolysis, resulting in the slow precipitation of elemental mercury onto the window of the optical cell so that lower results were obtained.

2. In regular atomic absorption, the samples have to be completely in solution and have to be correlated with a pure organomercury reference material (sometimes hard to obtain) dissolved in an identical solvent system.

3. In the cold vapor atomic absorption method, absorption in the UV region by extraneous volatile substances obtained after pyrolysis causes additive interference. Hence, the presence of any aromatic compound such as phenylmercuric salts, aminoacridine, or hydrocortisone formulated in some of these dosage forms could be of consequence. Moreover, the precision associated with the cold vapor atomic absorption system used for these samples was estimated to be about ±20% (7). On the other hand, in NAA the precision calculated from three separate irradiations of identical samples showed a coefficient of variation of better than 2%, attributed largely to variation in flux and in count statistics. Other factors affecting precision are minimized by irradiating standards

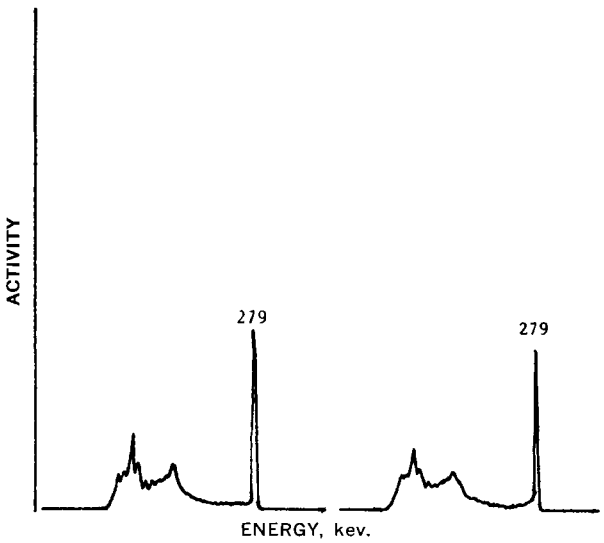


**Figure 2**—*Typical γ-ray spectra of mercuric chloride (standard) and phenylmercuric acetate obtained with Ge(Li) detector after 5 weeks of decay.*

**Table II—Determination of Mercury in Various Drugs**

| Sample | Type | Weight or Label Claim | Percent of Theory or Label Claim — NAA $^{197}Hg$ | NAA $^{203}Hg$ | X-Ray Fluorescence[a] | Atomic Absorption | Cold Vapor Atomic Absorption[b] |
|---|---|---|---|---|---|---|---|
| Sodium 4-chloromercuribenzoate | Bulk | 2.112 mg. | 101 | 104 | 101 | 97 | — |
| Merbromin (mercurochrome) | Bulk | 2.028 mg. | 93 | 95 | 97 | 84[c] | — |
| Sodium mersalyl | Bulk | 2.002 mg. | 100 | 104 | 100 | 110 | 110 |
| Nitromersol | Bulk | 2.110 mg. | 98 | 98 | 94 | 104 | 106 |
| Phenylmercuric acetate[d] | Bulk | 2.084 mg. | 103 | 109 | 91 | 112 | 121 |
| Phenylmercuric nitrate | Bulk | 2.228 mg. | 100 | 102 | 97 | —[c] | — |
| Thimerosal (merthiolate): | | | | | | | |
| Lot 1 | Bulk | 2.322 mg. | 97 | 99 | 93 | 104 | — |
| Lot 2 | Bulk | 2.112 mg. | 95 | 100 | 92 | 105 | — |
| Lot 3 | Bulk | 2.856 mg. | 94 | 96 | 93 | 98 | — |
| Thimerosal (merthiolate) | Solution | 0.1% | 108 | 110 | — | — | — |
| Mersalyl with theophylline | Solution | 100 mg./ml. | 108 | 105 | — | — | — |
| Phenyl mercuric acetate: | | | | | | | |
| Lot 1[e] | Suppository | 3.0 mg. | 96 | 100 | — | — | — |
| Lot 2[e] | Suppository | 3.0 mg. | 93 | 91 | 94 | 104 | 110 |
| Lot 1[f] | Suppository | 3.0 mg. | 90 | 91 | — | — | — |

[a] Some radiolysis of samples. [b] Expected interference. [c] Some insolubles or precipitates. [d] Practical grade. [e] Formulated with tyrothricin, hydrocortisone acetate, and aminacrine hydrochloride. [f] Formulated with tyrothricin, hydrocortisone acetate, and 9-aminoacridine hydrochloride.

and samples simultaneously and by maintaining identical position and geometry in the activity measurements.

Factors affecting accuracy are essentially due to competing nuclear reactions producing $^{197}Hg$ and $^{203}Hg$ and those due to $\gamma$-ray spectral interferences. In the first case, the probability of additive error from accompanying transmutation reactions from fast neutrons producing $^{203}Hg$ from $^{203}Tl$ and/or $^{206}Pb$ through an (n, p) or an (n, $\alpha$) reaction, respectively, can be considered nil. The other type of interference is minimized by employing a Ge(Li) detector, which markedly increases resolution (at the expense of counting efficiency) and, hence, increases the photopeak baseline selectivity for integration. Moreover, an overnight cool/wait period allows the shorter lived radionuclides such as $^{38}Cl$ ($t_{1/2}$ 37 min.), $^{80}Br$ (18 min.), $^{128}I$ (25 min.), $^{199m}Hg$ (43 min.), and $^{205}Hg$ (5.5 min.) to dissipate completely, particularly when the NaI(Tl) detector is used. Major quantities of such elements as the halides, for instance, would normally dominate and interfere with $\gamma$-ray photopeak activity measurements. Further cooling over a period of days or weeks allows even longer lived radionuclides such as $^{24}Na$ (15 hr.) and $^{42}K$ (12 hr.), which are notoriously bothersome in biological systems, to decay out. Thus, the advantages of working with relatively long-lived radionuclides, as in this study, are twofold. First, spectral interferences due to extraneous shorter lived radionuclides can be permitted to dissipate, resulting in lower Compton background corrections and lower analyzer deadtime. Second, corrections due to radioactive decay become negligible when the counting time is short compared to the half-life and are, therefore, omitted. Self-shielding, or the attenuation of the flux through the sample, may be significant in heavy nuclides such as mercury but could be neglected in this case because of the relatively small size of the samples and standards that were correlated at approximately the same concentration level and were irradiated simultaneously so as to receive the same flux for the same duration of time.

NAA measures total mercury regardless of the physicochemical state of the element. To add another degree of specificity to differentiate the organomercurial from inorganic salts or from the elemental form, a suitable solubility test in an organic solvent is a simple and adequate complement.

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