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Determination of Trace Amounts of Selenium in Pharmaceuticals

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Abstract ☐ A quantitative method for the determination of less than 30 p.p.m. selenium in organic sulfur-containing drugs has been developed. The procedure utilizes oxygen flask combustion for decomposition of the organic matter and colorimetric measurement of selenium after reacting with 2,3-diaminonaphthalene, a specific complexing agent for selenium. At the level of 30 p.p.m., better than 90% recovery is obtained. The method is rapid, specific, and free from interferences.

Keyphrases ☐ Selenium determination—trace amounts ☐ Oxygen flask combustion, organic material—selenium determination ☐ Colorimetric analysis—spectrophotometer ☐ 2,3-Diaminonaphthalene—color reagent

The heavy demand for sulfur has resulted in a downgrading of the quality of the sulfur used with the result that some sulfur can contain up to 0.5% selenium. Consequently, drugs which contain sulfur, or where sulfur-containing compounds are used in the manufacture, may be contaminated with selenium. In addition some drugs require selenium or its compounds in the manufacturing process. Selenium is a toxic element with the same order of toxicity as arsenic, however some investigators (1, 2) suggest that it is essential for some animals under defined conditions. The necessity of controlling the quantity of selenium in drugs is evident.

Many methods are reported in the literature for the determination of selenium in organic and biological materials (2). For compendial purposes it was necessary to select a procedure that would have a wide range of applicability and which would not require unusual equipment. X-ray fluorescence (3) was considered to be out of the realm of most laboratories at the present, as is atomic absorption (4–6). It was therefore necessary to select the best procedures for digestion of the sample and colorimetric measurement, and then to apply the selected procedure to the determination of selenium in those compounds which will be official in USP XVIII and NF XIII which might be contaminated with selenium.

PROCEDURES

Digestion—Wet ashing techniques have been widely used for destruction of the organic material prior to trace element determination. A study of wet digestion procedures prior to selenium analysis was carried out by Gorsuch (7) using isotope tracers to determine selenium recovery. The oxidation mixtures examined were (*a*) nitric and perchloric acids; (*b*) nitric, perchloric, and sulfuric acids; and (*c*) nitric and sulfuric acids. Wet digestion using nitric and perchloric acids was found to be most suitable to prevent losses of the element. Difficulties in the recovery of selenium after the destruction of organic material by wet ashing have been reported; Fogg and Wilkinson (8) reported losses using nitric and sulfuric acids but were able to obtain complete recovery using perchloric acid. Klein (9) recommended a partial oxidation with mercuric oxide present to "fix" the selenium. Grant (10), Cousins (11), and Watkinson (12) have all reported the successful use of nitric-perchloric acid mixtures for digestions of various biological tissues. A mixture of nitric and perchloric acids, and hydrogen peroxide is used by the Food Chemicals Codex (FCC) (13) for the digestion of several organic and inorganic chemicals. However, in all work reported with acid hydrolysis of the sample, losses are prevented only if the conditions of the digestion are carefully controlled and the sample is heated slowly without charring until all selenium has been converted to selenite. The recovery of selenium is low if the perchloric acid is concentrated to the stage when it becomes yellow through autodecomposition, which at this point is accelerated by even 0.5 mcg. of selenium. This is complicated by the fact that the yellow color of incompletely oxidized samples after removal of nitric acid can be confused with the yellow decomposition products of the acid.

The difficultly oxidizable nature of the organic sulfur-containing drugs precludes the use of wet digestion techniques as the organic material cannot be completely removed until the temperature of digestion is raised to a point where excessive losses of selenium occur.

PROCEDURES

Combustion—Combustion in a closed system to eliminate volatilization losses of selenium was first reported by Gutenmann and Lisk (14) who determined selenium in oats using oxygen flask combustion for destruction of the organic material. The oxygen flask technique has since been studied and/or improved by other workers. Dye *et al.* (15) studied oxygen flask combustion for a variety of plant and animal tissues, determining the selenium recovery by radiotracer techniques and using water, HCl, and NaOH as absorbing media. Allaway and Cary (16), Watkinson (17), Cukor *et al.* (18), and Tausky *et al.* (19) have reported successfully on the use of the oxygen flask technique.

The advantages of oxygen flask combustion over the classical methods of wet digestion, particularly in the analysis of selenium, are clear. (a) A small sample, 100–200 mg., is oxidized in a 1-l. flask. This is much more convenient and rapid than the digestion of a large sample in acid media. (b) As mentioned above, complete oxidation without loss of selenium is impossible for many of the compounds under study by wet digestion methods. Every compound tested was completely oxidized in a matter of minutes by the oxygen flask technique. Those compounds which do not burn cleanly may be made to do so with the addition of magnesium oxide prior to combustion. (c) Interferences from contaminants or from the digestion mixture itself are removed easily and effectively with the oxygen flask technique. (d) The time of analysis is materially decreased.

COLORIMETRIC METHODS FOR DETERMINATION OF SELENIUM

Colorimetric methods of measuring selenium include the original method of Robinson (20) who estimated the red color of elemental selenium, which has been used by others after separation of the selenium and reduction to the elemental state, generally with ascorbic acid (21). The elemental selenium is estimated visually either colorimetrically or nephelometrically.

The discovery of 3,3′-diaminobenzidine (DAB) by Hoste and Gillis (22) as a specific complexing agent for selenium has led to the sensitive spectrophotometric measurement of selenium traces. The analytical method developed by Cheng (23) using DAB for fluorometric measurement has been the most widely used technique for the determination of trace quantities of selenium. This reagent suffers from several disadvantages. (a) It contains two pairs of *o*-diamine groups only one of which reacts with selenium under the conditions of the test, so that the resulting piazselenol complex is basic and can only be extracted from aqueous solution into organic solvents by increasing the pH of the solution. This complicates the analytical procedure and necessitates the addition of a suitable complexing agent for the removal of interfering substances, especially metals whose hydroxides are precipitated in neutral or alkaline media. (b) The compound DAB has been reported to have carcinogenic properties (24) and its use should not be indiscriminate.

Parker and Harvey (25) investigated the reaction of selenous acid with a number of aromatic *o*-diamines, and found 2,3-diaminonaphthalene (DAN) to form a piazselenol which exhibits a higher fluorescence efficiency and extinction coefficient than DAB. In addition DAN will react with selenium in acid solution and can be extracted from the acid solution into organic solvents with no difficulty. While the reaction time is increased with DAN rather than DAB, no manipulations are necessary after the reaction, and this, along with the increased sensitivity has led the authors to choose DAN for the determination.

EXPERIMENTAL

Standard Selenium Solution—Transfer 120.0 mg. of powdered metallic selenium to a 1000-ml. volumetric flask, add 100 ml. of dilute nitric acid (1 in 2), warm gently on a steam bath to effect solution, and dilute to volume with distilled water. Dilute 5.0 ml. of this solution to 200.0 ml. with distilled water, and mix. Each milliliter of this solution contains 3 mcg. of selenium.

DAN Reagent—2,3-Diaminonaphthalene, 0.1% (Aldrich Chemical Co.) in 0.1 *N* HCl containing 0.5% hydroxylamine hydrochloride, without using heat to effect solution. This solution must be freshly prepared. Hydroxylamine hydrochloride is added to prevent the oxidation of 2,3-diaminonaphthalene. It may be safely used as it is not a strong enough reducing agent to reduce the selenium (26).

Cyclohexane—Spectrograde cyclohexane is used without purification.

Apparatus—Schöniger flask, 1-l. capacity, Thomas-Ogg modification.[1] Rinse with dilute nitric acid (1:1) followed by distilled water before use. Platinum sample carrier, black ignition papers, cellophane 0.003-cm. (0.001-in.) thick for wrapping samples or a suitable pellet press, and a Thomas-Ogg remote Infrared Safety Igniter[1] (or other suitable ignition device).

Spectrophotometric measurements were made using either a manual[2] or recording[3] spectrophotometer.

[1] Available from Arthur H. Thomas Co.
[2] Beckman DU.
[3] Cary model 14.

Procedure—Using the sample size specified for the individual compound, either 200 or 100 mg., suitably wrap the sample and place on a paper fuse in the platinum basket. Place 25 ml. of 0.5 *N* HNO_3 in the combustion flask, and with the stopper and basket beside the flask, direct a rapid stream of oxygen into the flask while swirling gently to aid in the removal of all air. Quickly insert the stopper, with sample carrier, into the flask and seal with a few drops of distilled water placed on the rim of the flask, then clamp the stopper in place. Ignite the sample and when combustion is complete agitate the flask vigorously and intermittently until all combustion gases have been absorbed. Transfer the liquid to a 150-ml. beaker rinsing the flask with about 25 ml. of distilled water. Place the beaker on a hot plate and warm gently to the boiling point. Digest for 10 min., then remove from heat and allow the solution to cool to room temperature.

Transfer 2.0 ml. of standard selenium solution to a 150-ml. beaker and dilute with 50 ml. of 0.25 *N* HNO_3.

Adjust the pH of the sample, standard, and of a reagent blank consisting of 50 ml. of 0.25 *N* HNO_3, to 2.0 ± 0.2 with diluted ammonium hydroxide (1 in 2). Add 200 mg. hydroxylamine hydrochloride to each beaker, swirl gently until dissolved, and then, without delay, add 5 ml. of DAN reagent to each beaker, mix well, cover the beaker with a watchglass, and allow to stand for 100 min. at room temperature. Transfer each solution to a separator, rinse each beaker with about 10 ml. of water, and add the rinsings to the separators. Add 5.0 ml. cyclohexane to each separator and extract with vigorous shaking for 2 min. Allow the layers to separate, discard the aqueous phase, transfer the cyclohexane extract to a glass-stoppered centrifuge tube, and centrifuge to remove all traces of water. Transfer the extract to a 1-cm. cell and determine the absorbance at the peak of maximum absorbance at about 380 mμ against a cyclohexane blank (see Fig. 1).

RESULTS

The drugs tested are listed in Table I with the conditions of combustion. No errors, due to interferences were found in any of the samples. In all cases less than 3 p.p.m. selenium were found. However it must be pointed out that the samples analyzed were only from one manufacturer, and only one lot was tested so that the results obtained are not necessarily indicative of contamination levels.

Calibration standards were run repeatedly over several months. The absorbance peak (see Fig. 1) was found to be linear with concentration in the range of 1–12 mcg. selenium, as the piazselenol, in 5.0 ml. of cyclohexane.

Recovery of Added Selenium—At the level of 30 p.p.m. better than 90% of added selenium is recovered. Prior to combustion 6 mcg. of selenium were added to a few of the drugs, selected at random, and recoveries obtained were as follows:

Compound	Recovery of Added Selenium, %
Acetazolamide USP	99
Calcium cyclamate NF	100
Menadione sodium bisulfite NF	97
Methapyrilene hydrochloride NF	91
Methdilazine NF	98
Sodium saccharin NF	101
Sodium sulfoxone USP	100
Sodium thiopental USP	104
Sulfadiazine USP	92

Precision—The accuracy of the method was tested on seven replicate analyses of a mixture of acetazolamide which had been spiked with 30 p.p.m. selenium. The average recovery obtained was 99.6% with a $\pm 8.4\%$ *SD* (± 2.5 p.p.m.). Using a sample of methdilazine, combusted with magnesium oxide, to which 30 p.p.m. selenium were added, an average recovery of 99.7% with a $\pm 7.5\%$ *SD* (± 2.3 p.p.m.) was obtained on seven replicate analyses.

DISCUSSION

For oxygen flask combustion in a 1-l. flask a sample size of 200 mg. was chosen. While this was found suitable for most of the compounds, difficulty in obtaining complete combustion was encoun-

Table I—Conditions of Combustion for Drugs Tested

Compound	Sample Size, mg.	Amount MgO if Added, mg.
Acetazolamide USP	200	
Acetohexamide NF	100	100
Acetylsulfisoxazole NF	200	
Amaranth USP	200	
Bendroflumethiazide NF	100	100
Benzthiazide NF	100	100
Calcium cyclamate NF	200	
Calcium saccharin NF	200	
Chlorothiazide NF	200	
Chlorpropamide USP	200	
Cyclothiazide NF	200	
Dapsone USP	100	100
Dichlorphenamide USP	100	100
Dimercaprol USP[a]	100	100
Evans blue USP	200	
Hydrochlorothiazide USP	200	
Hydroflumethiazide NF	200	
Hydroxystilbamadine isethionate USP	200	
Menadione sodium bisulfite NF	200	
Mercaptopurine USP	100	100
Methapyrilene HCl NF	100	100
Methazolamide USP	200	
Methdilazine NF	100	100
Methdilazine hydrochloride NF	100	100
Methimazole USP	200	
Methionine NF	200	
Methotrimeprazine NF	100	100
Methylthiouracil NF	200	
Phthalylsulfathiazole USP	200	
Polythiazide NF	200	
Potassium guaiacolsulfonate NF	200	
Probenecid USP	100	100
Prochlorperazine edisylate USP	100	100
Promazine hydrochloride NF	100	200
Propylthiouracil USP	200	
Saccharin USP	100	100
Sodum acetazolamide USP	200	
Sodium cyclamate NF	200	
Sodium glucosulfone injection USP[b]	200	
Sodium indigotindisulfonate USP	100	100
Sodium methiodal NF	200	
Sodium saccharin NF	200	
Sod um sulfacetamide USP	200	
Sodium sulfadiazine USP	200	
Sodium sulfamerazine NF	200	
Sodium sulfoxone USP	200	
Sodium suramin USP	200	
Sodium thiamylal NF	200	
Sodium thiopental USP	200	
Stibophen USP	200	
Succinylsulfathiazole USP	200	
Sulfacetamide, NF	200	
Sulfadiazine USP	200	
Sulfadimethoxine NF	200	
Sulfaethiodole NF	200	
Sulfamerazine USP	200	
Sulfamethazine USP	200	
Sulfamethizole NF	200	
Sulfamethoxazole NF	200	
Sulfamethoxypyridazine USP	200	
Sulfapyridine USP	200	
Sulfisoxazole USP	200	
Thiethylperazine maleate NF	100	100
Thioguanine NF	200	
Thiotepa USP	200	
Tolbutamide USP	100	100
Trichlormethiazide NF	200	
Trimethaphan camsylate USP	100	100
Trimethidinium methosulfate NF	200	

[a] This sample is liquid and must be triturated with MgO to evenly distribute the sample. Capsule combustion cannot be used. [b] This sample, an injection, is not subjected to combustion but is run directly. No interferences are encountered.

tered with some of the samples. In these cases the use of a 100-mg. sample mixed with magnesium oxide was found to produce a clean burn. Unfortunately, it cannot be predicted when magnesium oxide will be required. While the oxide could be used in all cases, it

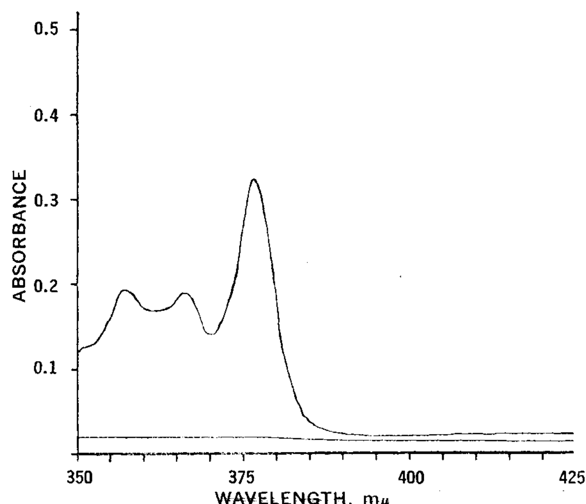
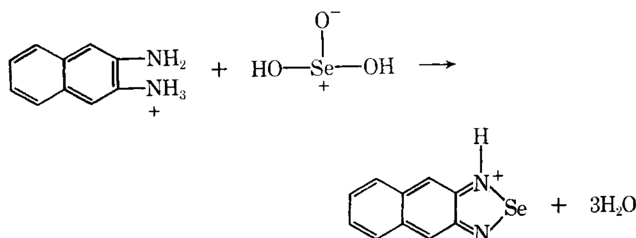


Figure 1—*Absorption spectrum of selenium standard: (a) standard containing 6 mcg. selenium as the piazselenol, in 5 ml. cyclohexane; (b) reagent blank.*

is not routinely recommended because it somewhat reduces the sensitivity of the method. Compounds which contain water of hydration or more than 1% moisture will often, depending on the crystalline structure, not burn smoothly and tend to spatter bits of unburned material on the sides of the flask. To obtain a smoother combustion these materials should be powdered and dried at 140° for 2 hr. to remove all moisture prior to combustion. With a limit of 30 p.p.m. the standard for a 200-mg. sample produces an absorbance of about 0.350 at the peak.

It must be stressed that a clean combustion is necessary in order to obtain complete recovery of selenium. Despite results obtained by Allaway and Cary (16) which indicate that bits of unburned material do not retain selenium, presumably because the temperature of combustion is high enough to cause volatilization of selenium, the best recovery obtainable in these circumstances has been about 70%.

Previous workers using the oxygen flask technique have employed HCl as the absorbing liquid, and in this study this was tried at first. However recovery of added selenium was found to range from 10–50% with many of these drugs. It is probable that this is due to large amounts of sulfur dioxide formed in the combustion gases which may serve to reduce the selenium. As shown by the work of Cukor and Lott (26), in their study of the kinetics and mechanism of the reaction between selenium and the DNA reagent, the reaction takes place between the monoprotonated 2,3-diaminonaphthalene and selenous acid as:



The nitric acid treatment is included to insure that all selenium present will be in the proper oxidation state to react and thus be detected.

This was first shown when a selenium contaminated sample known to contain between 350–450 p.p.m. Se was run using HCl as the absorbing liquid. A recovery of 100 p.p.m. or about 25% was obtained. When nitric acid was used as the absorbing liquid 414 p.p.m. were detected. However, the nitric acid media after absorption of combustion gases possesses a strong oxidizing nature and may oxidize the DAN reagent, leading to a high background absorbance in combusted samples. In order to minimize the possibility of high blank readings hydroxylamine hydrochloride is added to the solution before the addition of the reagent.

Commercially available DAN was suitable for use without further purification. Due to its sensitivity to light the precaution of prepar-

ing the solution freshly each day is observed. The decomposition which occurs after several hours exposure to light is observed as a yellowing of the solution and is due to the formation of a polymer of DAN. The formation of this polymer would decrease the concentration of DAN available for reaction. In analytical work where the stoichiometric excess of DAN is large, such slight changes in concentration as would occur in 8 hr. do not affect the results.

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DRUG STANDARDS

Quantitative Determination of Furazolidone and Nifuroxime in a Water-Soluble Suppository Base

RICHARD BOICE, MURRY SEIDMAN, and B. C. SOUTHWORTH*

Abstract □ A thin-layer chromatographic procedure has been developed for the analysis of furazolidone and nifuroxime in suppositories. The sample is dissolved in a suitable solvent and chromatographed. The separated nitrofurans are then compared to respective standards densitometrically. The coefficient variation is 2.4% for furazolidone and 2.7% for nifuroxime. Evidence is presented to indicate that only the intact compounds are measured.

Keyphrases □ Furazolidone and nifuroxime suppositories—analysis □ TLC—separation □ Spectrodensitometry—TLC spot analysis □ IR spectrophotometry—identity

The present official method (1) for the determination of furazolidone and nifuroxime in a suppository base Furazolidone and Nifuroxime Suppositories (NF[1]) is hampered by the variability of the montmorillonite used for the chromatographic separation of the nitrofurans. As much as 20% of the furazolidone may be irreversibly adsorbed onto the column packing. Although this adsorption may be compensated for by running standards under identical conditions, results are somewhat variable.

Both paper chromatography (2–6, 12) and TLC (2, 7–11) have been used for the detection of furazolidone. Recently Zoni and Lauria (13) and Bortoletti and Perlotto (14) have reported the use of TLC for both the detection and the determination of furazolidone and nifuroxime in admixture. Following plate development the above authors eluted the nitrofurans from the silica gel and determined their concentrations spectrophotometrically. The relative ease with which nitrofuran derivatives may be isolated, and recent developments in instrumentation

[1] Tricofuron Vaginal Suppository, Eaton Laboratories, Division of The Norwich Pharmacal Co.