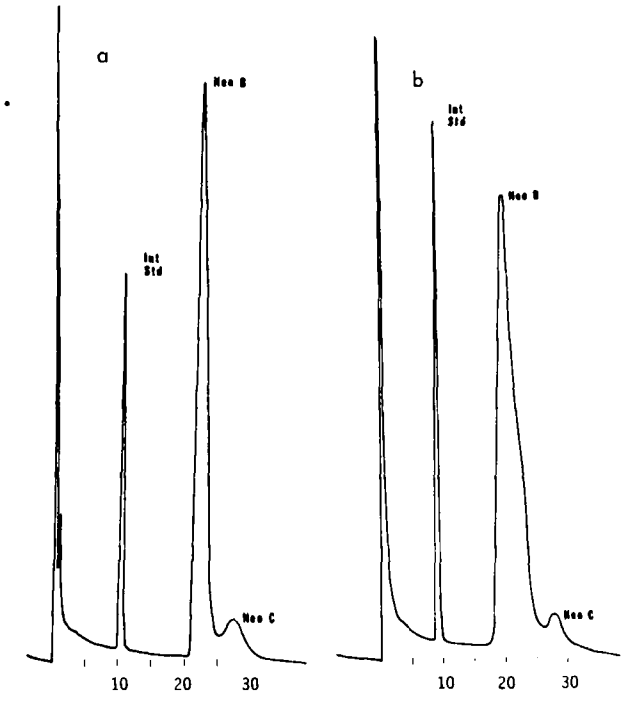

Figure 2—*Chromatogram of trimethylsilyl derivative of neomycin with: (a) proper amount of trimethylsilyldiethylamine, and (b) excess trimethylsilyldiethylamine.*

DISCUSSION

Because of the many potential sources of difficulties, strict adherence to the reported procedure (2, 3) is recommended to obtain reliable results. These problems are enumerated in Table II for convenience; and although each one is of material significance, the following are emphasized:

1. The column must be properly sample conditioned immediately before actual analysis.
2. The amount of trimethylsilyldiethylamine reagent added is critical in the formation of the derivative, as are the temperature and duration of the reaction (3). When the derivative is properly prepared, the neomycin B peak is symmetrical (Fig. 2).
3. Samples should be chromatographed as soon as possible after derivatization or kept refrigerated minimally until time of analysis so as to retain optimal stability. These neomycin derivatives degrade readily (3).

In summary, with proper techniques and caution, the GLC method for analysis of the neomycins can be successfully employed. This method has already been shown to be more specific and reliable than the microbiological method (4, 5). Even with GLC instruments offering poor linearity in recovery, satisfactory results can be obtained when they are correlated with authentic or reference standard calibration mixtures approximating the concentration level to be determined. This type of discussion might prove beneficial in similar analyses of high molecular weight oligosaccharides.

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Determination of Copper and Manganese in Vitamin-Mineral Tablets by Atomic Absorption Spectrophotometry

YOUNG SOON CHAE, JAMES P. VACIK, and WILLIAM H. SHELVER▲

Abstract □ Atomic absorption spectrophotometry was utilized in the determination of copper and manganese in five different combination multiple-vitamin mineral tablets. The analysis of each tablet by direct determination was compared with analysis by the method of additions, and some small but statistically significant differences were noted. No molecular absorption was found in the analysis of manganese utilizing the nonresonance line of lead. The direct analysis of manganese and copper was simple and precise.

Keyphrases □ Copper—determination in multiple-vitamin mineral tablets by atomic absorption spectrophotometry □ Manganese—determination in multiple-vitamin-mineral tablets by atomic absorption spectrophotometry □ Atomic absorption spectrophotometry—determination of copper and manganese in vitamin-mineral tablets □ Vitamin-mineral tablets—determination of copper and manganese by atomic absorption spectrophotometry □ Minerals, in multiple-vitamin tablets—determination of copper and manganese by atomic absorption spectrophotometry

Because of their complex nature, multiple-vitamin mineral tablets present a formidable problem in analytical chemistry. The analysis of specific metals in the presence of numerous other minerals and a complicated organic matrix requires either complex separation schemes or an extremely specific analytical method to

Table I—Instrument Parameters

Item	Multielement Lamp	Lead Lamp
Wavelength	324.7 nm., copper; 279.5 nm., manganese	280.3 nm.
Hollow cathode lamp current	30 ma.	35 ma.
Fuel	Lean acetylene (flowmeter at 27)	Lean acetylene (flowmeter at 27)
Oxidizer	Air (flowmeter at 55)	Air (flowmeter at 55)
Aspiration rate	6.3 ml./min.	6.3 ml./min.
Slit	No. 3 (2 nm.)	No. 3 (2 nm.)
Digital readout	100 average mode	100 average mode

obtain meaningful results. Atomic absorption spectrophotometry promises to be a simple and an extremely specific analytical technique with sufficient precision for pharmaceutical analysis. Despite these desirable qualities, atomic absorption spectrophotometry has not been widely utilized in pharmaceutical analysis (1). Calcium (2), copper (3), iron (4), and cobalt (5) have been analyzed in pharmaceutical preparations by atomic absorption spectrophotometry, but none of these metals has been determined in multiple-vitamin–mineral tablets by this technique.

Copper has been analyzed (3) by atomic absorption spectrophotometry in ointments and in a tablet containing copper, cobalt, and iron with good precision and no indication of interferences. The effects of fuel mixtures and of mineral acids on the atomic absorption analysis of copper and manganese have been carefully studied (6). The extension of these studies to the analysis of copper and manganese in multiple-vitamin–mineral tablets seems feasible, although the analysis of manganese apparently has not been reported by atomic absorption spectrophotometry in any pharmaceutical products.

EXPERIMENTAL

Instrument—A double-beam spectrophotometer[1], equipped with a multielement (Mn–Cu–Co Cr–Ni) hollow cathode lamp and a single-slot burner head, was used for all atomic absorption measurements except for the two-line method where a single-element lead lamp was utilized. The instrument parameters are given in Table I.

Reagents—Standard stock solutions of manganese[2] and copper[2] contained 1000 and 10,000 p.p.m., respectively. All other reagents were ACS or USP grade. Distilled water was deionized by being passed through two mixed-bed resin columns, and it was used to make all solutions and to rinse all glassware.

Direct Method—Standard curves (Figs. 1 and 2) were obtained as follows. Manganese or copper solutions were prepared for calibration by diluting the standard stock solution to obtain concentrations of 0.5, 1.00, 2.00, 3.00, and 3.50 p.p.m. or 1.00, 2.00, 3.00, 4.00, and 5.00 p.p.m. respectively. Both standards were made up to contain 1% (w/v) hydrochloric acid. The instrument was optimized as to lamp position and the position of the burner head. The absorbances of the standard solutions of the element were determined in the 100 average mode with a minimum of three such readings. The regression line of the absorbance–concentration curves through zero was determined using a calculator[3], and the calibration curve was plotted. The calibration curves were determined each day that analytical runs were made by the direct method.

Twenty tablets of each preparation were accurately weighed to determine the average tablet weight. The tablets were crushed and ground to a fine powder with a mortar and pestle. A portion of each tablet powder equivalent to sufficient metal to give an absorbance between 0.100 and 0.300 was accurately weighed, transferred into a 100-ml. beaker, and dissolved in sufficient 10% (w/v) hydrochloric acid to yield a final concentration of 1% (w/v) when diluted. Each solution was filtered through filter paper[4] into a 100- or 200-ml. volumetric flask and diluted to the mark with deionized distilled water through the filter paper. Four separately weighed powdered samples were prepared from each different batch of tablets. The instrument was warmed for a minimum of 4 hr., and the blank solution [1% (w/v) hydrochloric acid solution] was aspirated for a minimum of 15 min. prior to any analytical procedure.

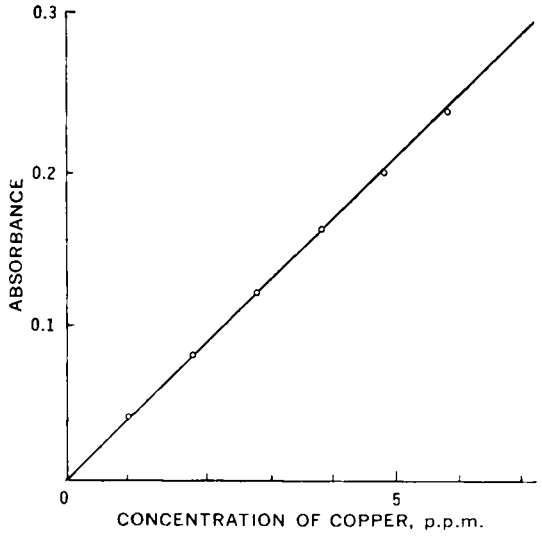

Figure 1—*Typical concentration–absorbance relationship for copper standard at 324.7 nm. Slope of the line = 0.0405 ± 0.0020. Sensitivity = 0.107 mcg./ml. The analysis was carried out on a spectrophotometer (see text) with a slit of 0.2 nm. and the readout in the 100 average mode.*

After aspirating the blank solution and setting the instrument to zero by means of the autozero function, the solution to be analyzed was aspirated and the absorbance was determined using the 100 average function. The first reading was discarded, and at least four successive readings were taken. The instrument was zeroed by aspirating blank solution prior to reading the next solution. The calibration curve and the analytical readings for all samples were taken for one element before changing the wavelength and carrying out similar analyses for the second element. Because of the different metal contents of the tablets and different instrument responses to manganese and copper, some samples required more dilute analytical solutions for manganese. The calculation of tablet content was carried out using the following equation:

$$\text{tablet content} = \frac{\text{absorbance} \times \text{volume analytical solution} \times \text{average tablet weight} \times \text{dilution factor}}{\text{slope of calibration line} \times 1000 \times \text{sample weight}} \quad \text{(Eq. 1)}$$

The mean and standard deviation (Tables II and III) for each tablet were calculated with a programmable calculator[5].

Method of Additions—The same analytical solutions and standard stock solutions used in the direct method were used in preparing solutions for analysis by the method of additions. Three solutions were used for each analysis, containing the same quantity (10 ml.) of tablet analytical solution and 0, 10, or 20 ml. of 10 p.p.m. copper standard solution or 0, 5, or 15 ml. of 10 p.p.m. manganese standard solution. The resulting solutions were diluted to 50.0 ml. with 1% (w/v) hydrochloric acid solution. The quantity of tablet analytical solution provided maximum absorbance for the solution containing the highest quantity of metal without exceeding the linear portion of the calibration curve (3.5 p.p.m. for manganese and 6 p.p.m.

[1] Perkin-Elmer model 403.
[2] Fisher certified reagents.
[3] Hewlett-Packard model 10.
[4] Whatman No. 2.
[5] Hewlett-Packard model 10 equipped with a statistical ROM.

Table II—Analysis of Tablets for Copper Content

Tablet	Amount of Copper Declared, mg.	Amount of Copper Found by Direct Determination[a] mg.	%	% SD	Amount of Copper Found by Method of Additions[b] mg.	%	% SD	F
1	1.0	1.032	103.2	2.8	1.107	110.7	6.6	4.391
2	2.0	2.090	104.5	4.2	2.244	112.2	5.4	5.101
3	1.0	0.936	93.6	5.6	0.964	96.4	5.8	0.478
4	1.0	1.084	108.4	1.8	1.140	114.0	7.9	1.902
5	0.75	0.747	99.6	0.5	0.773	103.0	1.2	29.039[c]

[a] The results represent an average of four determinations. [b] The results represent an average of four determinations, each one consisting of the extrapolation of the least-squares line to the *X*-intercept at zero absorbance. The least-squares line was derived from absorbance measurements at three different copper concentrations. [c] Significant at the 0.01 level.

Table III—Analysis of Tablets for Manganese Content

Tablet	Amount of Manganese Declared, mg.	Amount of Manganese Found by Direct Determination[a] mg.	%	% SD	Amount of Manganese Found by Method of Additions[b] mg.	%	% SD	F
1	1.0	1.040	104.0	2.9	1.075	107.5	0.5	4.101
2	1.0	1.244	124.4	3.4	1.213	121.3	6.9	0.561
3	1.5	1.377	91.8	1.7	1.456	96.7	3.5	7.303[c]
4	1.0	0.991	99.1	1.3	1.066	106.6	2.1	33.836[d]
5	1.25	1.456	116.5	0.7	1.400	112.0	1.6	18.759[d]

[a] The results represent an average of four determinations. [b] The results represent an average of four determinations, each one consisting of the extrapolation of the least-squares line to the *X*-intercept at zero absorbance. The least-squares line was derived from absorbance measurements at three different manganese concentrations. [c] Significant at the 0.05 level. [d] Significant at the 0.01 level.

for copper). The absorbance of each solution was read as described under the *Direct Method*. The content of the original solution was calculated by determining the regression line for the three solutions using the absorbance as the *Y*-value and the parts per million of the metal added for the *X*-coordinate. The negative of the *Y*-intercept divided by the slope gave the content in parts per million for the original solution. The tablet content can thus be calculated by Eq. 2:

$$\text{tablet content} = \frac{\text{p.p.m. metal} \times \text{volume analytical solution} \times \text{average tablet weight}}{1000 \times \text{sample weight}} \qquad \text{(Eq. 2)}$$

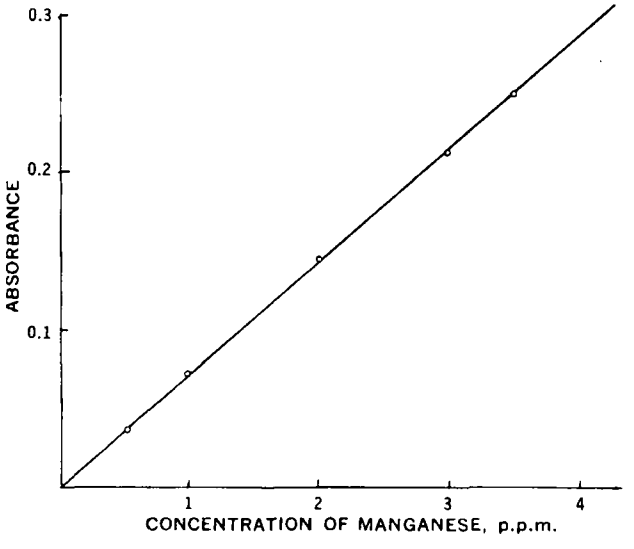

Figure 2—*Typical concentration–absorbance relationship for manganese standard at 279.5 nm. Slope of the line = 0.0713 ± 0.0012. Sensitivity = 0.061 mcg./ml. The analysis was carried out on a spectrophotometer (see text) with a slit of 0.2 nm. and the readout in the 100 average mode.*

The mean and the standard deviation for each tablet were determined as already described.

Two-Line Method—To examine the problem of possible molecular absorbance, a nonresonance line near the analytical wavelength was needed. Lead has a nonabsorbing line at 280.3 nm. near the analytical wavelength of manganese (279.5 nm.). Measurement of the absorbance of all standard solutions and of all analytical solutions using the lead line revealed no solutions with absorbances greater than 0.001 unit. Since absorbances this low would not have appreciable effects on the calculated results, the experiment was terminated.

Recovery Experiments—The recovery of copper and manganese was found by taking an aliquot of the tablet solution, diluting, and reading and then taking the same aliquot of the tablet solution, adding standard solutions of copper and manganese, diluting, and reading. The difference between the readings was divided by the slope of the calibration line for the respective element, and the resulting parts per million (measured) was divided by the amount added (theoretical) and multiplied by 100 to obtain the percent recovery (Table IV).

RESULTS AND DISCUSSION

Since there exists the possibility of matrix effects in the analysis of copper and manganese in the complex mixture produced by a multiple-vitamin tablet, three independent atomic absorbance techniques were selected to check the validity of the analysis: direct analysis, the method of additions, and the use of a nonresonance line adjacent to the manganese line.

The use of the nonresonance line of lead at 280.3 nm. near the analytical line for manganese at 279.5 nm. resulted in a maximum absorbance of 0.001 absorbance unit. The negligible absorbance in

Table IV—Recovery of Metals from Analysis of Multiple-Vitamin–Mineral Tablets

	Mean[a]	SD	Recovery, %
Copper	1.044	0.042	104.4
Manganese	1.018	0.006	101.8

[a] Each value represents the mean of 10 determinations.

all samples indicated that molecular absorbance was not interfering with the analysis of manganese in this procedure. The possibility of molecular absorption occurring at the analytical wavelength of copper seems small since the copper analytical wavelength is not far from the manganese line. No calculations are reported for the two-line method since the results would not differ from the results of the direct method.

A typical standard curve for copper (Fig. 1) reveals good linearity up to 6 p.p.m. The slope of the line gives a sensitivity of 0.107 mcg./ml., in agreement with reported (7) values, and the standard deviation of the slope indicated suitable accuracy. The results of the analysis of the tablets for copper are shown in Table II. Both the direct method (3% average *SD*) and the method of additions (5% average *SD*) show reasonable precision. Probably some error is produced by difficulties in obtaining homogeneous samples of the vitamin tablets, and this is supported by the differences in precision among the different tablets. The ability to obtain a homogeneous sample would depend upon the particular formulation of the tablet. Some tablets were extremely difficult to reduce to a fine powder while others were powdered with comparative ease. This method of sampling in the analysis of iron has been reported to give a higher variation than an ashing procedure (4). The increased error in the method of additions can be anticipated because of the larger number of pipetings and the extrapolation procedure required. Statistical comparisons of the two methods indicate that Tablet 5 showed a significant difference in the two analytical methods. Inspection of Table II shows that the significance was produced by the unusually small standard deviations associated with this tablet. Other workers (2) found a greater variance produced by the method of additions. The slightly higher results from the method of additions for the analysis of copper cannot be presently explained.

A typical standard curve for manganese (Fig. 2) was linear up to 3.5 p.p.m., and the sensitivity (0.061 mcg./ml.) agrees well with reported (7) values. The standard deviation of the slope is within acceptable limits. The results of the analysis of manganese in multiple-vitamin–mineral tablets (Table III) are more precise than the copper analysis. The average errors in the direct determination and in the method of additions (2 and 3% average *SD*, respectively), are extremely satisfactory. Unlike the copper analysis, there seems no direction in the differences in the two methods (Tablets 2 and 5 are lower by the method of additions and Tablets 1, 3, and 4 give higher results by the method of additions). The existence of the small, but significant, differences in the results of the two methods of determination might indicate a small matrix effect. Since the differences between the methods are small, the more convenient direct method can be used for all but the most crucial applications.

At the beginning of the project, the decision was made to produce the analytical solution by grinding the tablet and filtering the resulting suspension, thus avoiding ashing or digesting of the sample. This procedure obviously introduced some variation into the results; but from the results of Tables II and III, this effect cannot exceed 3% and is probably much less. By avoiding digestion or ashing, a solution is produced that can be utilized in the analysis of the vitamins soluble in 1% acid media, minimizing the number of solutions needed for the quality control of the multiple vitamins. The results (Table IV) indicate acceptable recovery for both manganese and copper and again demonstrate the smaller variability of the manganese analysis. The determination of manganese and, to a slightly lesser degree, copper by direct atomic absorption spectrophotometry is a simple and precise method of analyzing these metals in multiple-vitamin–mineral tablets.

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Determination of Chloramphenicol Palmitate in Pharmaceutical Suspensions

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Abstract □ Three simple procedures for the quantitative determination of chloramphenicol palmitate in commercially available chloramphenicol palmitate oral suspensions are presented. One method is a modification of the USP method in which the suspension residue is washed with water prior to the USP specified steps of dissolving the residue in chloroform and determining the quantity spectrophotometrically. The second method takes advantage of the presence of two chlorine atoms in the side chain of chloramphenicol which can be converted into alkali chloride to be estimated potentiometrically with a standard silver nitrate solution. The third method is based on the liberation of chloramphenicol from chloramphenicol palmitate by treatment with dilute alkali at room temperature and microbiological assay of chloramphenicol using *Escherichia coli* (ATCC 10536). The potentiometric and microbiological assay methods were equally suitable for determination of chloramphenicol palmitate in all of the different suspensions tested. The microbiological assay method was more specific for the active antibiotic, was less cumbersome, and took less time.

Keyphrases □ Chloramphenicol palmitate—three methods of analysis in pharmaceutical suspensions □ Suspensions, chloramphenicol palmitate—three methods of analysis □ Antibiotics—analysis of chloramphenicol palmitate in pharmaceutical suspensions

In chloramphenicol palmitate oral suspensions containing 0.5% vanillin as the flavoring agent, the potency of chloramphenicol palmitate, as determined by the USP spectrophotometric method (1, 2), was always high. The results varied between 116 and 133% of the labeled content. However, the USP method was suitable