Vitamin Analysis Comparison Study

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• PURPOSE: **We compared and analyzed the concentrations of vitamin C, vitamin E, zinc, and copper in both national and regional brands of dietary supplements recommended for patients who are at risk for macular degeneration.**
• DESIGN: **Prospective cross-sectional study.**
• METHODS: **National brand name and generic multivitamin formulations for age-related macular degeneration were obtained. Comparative analysis of the vitamin C and vitamin E content was performed by gas chromatography-mass spectrometry and the zinc and copper content was analyzed by atomic absorption spectroscopy in an institutional chemistry laboratory.**
• RESULTS: **All national brand name vitamins, both tablet and gel capsule formulations, and generic brands in tablet form were relatively accurate in their product labeling. For most of the samples tested, the measured quantities of vitamin C, vitamin E, zinc, and copper were slightly higher than labeled but not to an amount that would cause any systemic toxicity if taken at the recommended dosages.**
• CONCLUSIONS: **Physicians may recommend national brand name vitamins and generic brands in tablet form to their patients with some confidence; however, the content may have some inaccuracies regarding labeling. (Am J Ophthalmol 2021;222:202–205. © 2020 Elsevier Inc. All rights reserved.)**

AGE-RELATED MACULAR DEGENERATION (AMD) IS A leading cause of blindness in the United States.[1] While effective treatment for exudative AMD exists, there is no effective treatment for dry AMD. The Age-Related Eye Disease Study (AREDS) demonstrated that daily high-dose multivitamin supplements containing vitamins and minerals are beneficial in reducing the risk of vision loss in patients with high-risk AMD, and a follow-up study of the AREDS2 demonstrated the beneficial effect of lutein and zeaxanthine.[2–5]

Accepted for publication Aug 13, 2020.

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In the United States, vitamins are regulated by the Food and Drug Administration (FDA) as dietary supplements. A dietary supplement is a product taken by mouth that is intended to supplement the diet and that contains ≥1 "dietary ingredient." Dietary supplements are classified as foods with regard to FDA regulation, and their manufacturers and distributors are not required to obtain approval from the FDA before marketing dietary supplements. The Dietary Supplement Health and Education Act of 1994 and the Current Good Manufacturing Practice established in 2007 provide regulatory framework for the safety and labeling of dietary supplements, but manufacturers are expected to self-regulate. In 2012, an estimated 70% of manufacturers were considered noncompliant with "good manufacturing practices."[6]

The purpose of our study was to compare and analyze the concentrations of vitamin C, vitamin E, zinc, and copper in national brands of dietary supplements that are recommended for patients who are at risk for macular degeneration and to determine if the labeling of vitamin content for each supplement was accurate.

METHODS

NATIONAL BRAND NAME AND GENERIC MULTIVITAMIN formulations for AMD were obtained and comparative analysis was performed by gas chromatography-mass spectrometry (GC-MS) and atomic absorption spectroscopy (AAS), where GC-MS was used to measure vitamin C and vitamin E content and AAS was used to measure zinc and copper content.

• **GC-MS SAMPLE PREPARATION:** Four vitamin brands (2 national brand names and 2 generic formulations) in tablet format and 1 in gel format were tested for comparative analysis. For name brands we used PreserVision AREDS 2 formula minigels (Bausch and Lomb, Bridgewater, New Jersey, USA), for Ocuvite the AREDS formula (Bausch and Lomb), and for I-Caps the AREDS formula (Alcon, Fort Worth, Texas, USA); for the generic brands, we assessed Kroger VisionShield (AREDS2 formula; Kroger, Cincinnati, Ohio, USA) and Walgreens Advanced Eye Health (Walgreens, Deerfield, Illinois, USA). Three additional generic gel formulation multivitamins were unable to be analyzed for comparative analysis because of a lack of labeling. Five tablets and 8 gels were randomly selected from each vitamin brand. After weighing, each sample was dissolved in 100% methanol. A larger number of gel caps

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2020.08.028

TABLE 1. Concentration of Vitamin C in Select Dietary Supplements

Brand	Labeled Milligrams	Measured Milligrams ± SD
PreserVision (N1)	250	259.7 ± 8.1
Ocuvite (N2)	200	292.5 ± 7.01
I-Caps (N3)	200	256.9 ± 13.72
Walgreens (G1)	200	260.6 ± 13.87
Kroger (G2)	200	254.8 ± 30.74

G = generic; N = national brand; SD = standard deviation.
All trade names are property of their respective owners.

TABLE 2. Concentration of Vitamin E in Select Dietary Supplements

Brand	Labeled IU	Measured IU ± SD
PreserVision (N1)	200	190.4 ± 4.28
Ocuvite (N2)	60	73.2 ± 2.42
I-Caps (N3)	75	70.0 ± 5.30
Walgreens (G1)	60	62.5 ± 3.37
Kroger (G2)	60	62.3 ± 2.36

G = generic; IU = international units; N = national brand; SD = standard deviation.
All trade names are property of their respective owners.

was used because the gel had to be extracted from the capsule to obtain a similar volume to the tablets. The mixture was homogenized until the tablet or the content in the gel was completely dissolved. After 5 minutes in a centrifuge at 15,000 rpm, 20 μL of supernatant was collected. The supernatant was further diluted twice with methanol (20 μL supernatant was mixed with 180 μL of methanol, 80 μL of the diluted supernatant was further mixed with 170 μL of methanol). One hundred microliters of diluted supernatant was transferred to a glass vial and dried in a SpeedVac (ThermoFisher Scientific, Waltham, MA, USA). The dried sample was dissolved in 100 μL of solvent acetonitrile and N-methyl-N-(trimethylsilyl) trifluoroacetamide (1:1 volume) for derivatization at 70 Centigrade for 30 minutes. One microliter of the derivatized sample was injected into a GC-MS system for measurement.

• **GC-MS ANALYSIS:** The concentrations of vitamin C and vitamin E for all of the samples were measured on a TRACE 1310 gas chromatograph and an ITQ 1100 Ion Trap MS system (both from ThermoFisher Scientific). The column was a 30 m × 0.25 mm ${}^{1}d_{c}$ × 0.25 μm ${}^{1}d_{f}$, DB-17MS GC capillary column (phenyl arylene polymer virtually equivalent to [5%-phenyl]-methylpolysiloxane). The column was obtained from Agilent Technologies (Santa Clara, CA, USA). The flow rate of helium carrier gas (99.999% purity) was set to 1.0 mL/min at a corrected constant flow via pressure ramps. The inlet temperature was set to 280 C. The column temperature was programmed with an initial temperature of 80 C for vitamin C and 200 C for vitamin E and then ramped at 40 C/min to 280 C and maintained at 280 C for 8 minutes. The mass range was set as 29 to 800 m/z. The ion source chamber was set to 230 C with the transfer line temperature of 280 C, and electron energy was 70 eV. The concentration of each type of vitamins in a sample was respectively calculated using corresponding calibration curves.

• **SAMPLE PREPARATION FOR ATOMIC ABSORPTION ANALYSIS:** Four tablets were randomly selected from each brand. Each sample was digested using 15 mL of 6 M hydrogen chloride (HCl) overnight. The dissolved mixture was transferred into a 50-mL volume flask, and 0.1 M HCl was added to bring the total volume to 50 mL. One mL of the solution was centrifuged for 10 minutes at 15,000 rpm. The supernatant was diluted 20× or 10× with 0.1 M HCl. The diluted sample was analyzed by AAS to measure the amount of copper. The sample was further diluted 50× or 80× using 0.1 M HCl and used to measure the content of zinc in the solution. The concentrations of the copper and zinc in the sample were then respectively calculated using corresponding calibration curves.

• **AAS:** Each sample was measured on a Thermo Solaar S4 atomic absorption spectrometer (Triad Scientific, Manasquan, New Jersey, USA). The concentrations of zinc and copper were calculated from a calibration curve constructed by AAS response of a set of zinc and copper standard solutions. By this assay, total zinc and copper in the sample are measured and expressed as milligrams per tablet or gel.

RESULTS

FOR THE RESULTS, THE NATIONAL BRANDS ARE LABELED N1 (gel), N2 (tablet), and N3 (tablet); generic brands are labeled G1 (tablet) and G2 (tablet). Table 1 lists the labeled values and measured values for vitamin C. Table 2 lists the labeled values and measured values for vitamin E, and Tables 3 and 4 list the levels of zinc and copper, respectively. All values are per tablet or per gel capsule.

The average percentage change for the vitamins was 27.3% for vitamin C, 3.7% for vitamin E, 17.5% for zinc, and 17% for copper. Preservision (N1) had the lowest skew for all vitamins, while Ocuvite (N2) had the highest

TABLE 3. Concentration of Zinc in Select Dietary Supplements

Brand	Labeled mg	Measured mg ± SD
PreserVision (N1)	40	44.3 ± 1.8
Ocuvite (N2)	40	48.1 ± 1.04
I-Caps (N3)	30	37.2 ± 0.96
Walgreens (G1)	40	46.4 ± 0.72
Kroger (G2)	40	46.5 ± 0.73

G = generic; N = national brand; SD = standard deviation.
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TABLE 4. Concentration of Copper in Select Dietary Supplements

Brand	Labeled mg	Measured mg ± SD
PreserVision (N1)	1	1.1 ± 0.04
Ocuvite (N2)	2	2.5 ± 0.25
I-Caps (N3)	2	2.4 ± 0.07
Walgreens (G1)	2	2.3 ± 0.10
Kroger (G2)	2	2.3 ± 0.07

G = generic; N = national brand; SD = standard deviation.
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skew for 3 of 4 vitamins tested. The generic brands G2 and G1 were second and third places in error, respectively, for 3 of 4 vitamins tested. The skew of N3 was variable across all vitamins tested.

DISCUSSION

THE AREDS2 GROUP HAS RECOMMENDED A DAILY DOSAGE of 500 mg vitamin C, 400 IU vitamin E, 80 mg zinc oxide, 10 mg lutein, 2 mg zeaxanthine, and 2 mg copper oxide.[5] Four of the 5 products we tested had a lower dosage than the recommendations of the AREDS2 group. Although the actual dosage found when testing was in accordance with the product information, the dosage of these products is not suitable to treat patients with higher risk factors for AMD. We are also aware that the brands we tested are not singular products, and there are several different preparations with the same name that may lead to confusion among patients and health care providers. For example, there are 6 different forms of Preservison.

All national brand name vitamins, both tablet and gel capsule formulations, and generic brands in tablet form were relatively accurate in their product labeling, although some had lower concentrations than the recommended AREDS2 dosage. For the majority of the samples tested, the measured quantities of vitamin C, vitamin E, zinc, and copper were slightly higher than labeled but not to an amount that would cause any systemic toxicity if taken at the recommended dosages. The upper limit or the largest amount of a nutrient that most adults can ingest daily without the risk of adverse effects for vitamin C is 2000 mg, for vitamin E is 1000 mg, for zinc is 40 mg, and for copper is in the range of 5 to 10 mg.[7]

The recommended daily allowance of zinc for adults is 15 mg compared with 3 to 5 mg for infants. Acute zinc toxicity after oral ingestion causes nausea, vomiting, and fever. With chronic gastrointestinal exposures, bone marrow and neurologic effects can also manifest. Symptoms usually do not become evident until ingestions exceed approximately 1 to 2 g of zinc.[8] The estimated safe amount of zinc is 0.15 mg/kg. Reduced erythrocyte superoxide dismutase activity can develop in women who are given daily supplements of zinc 50 mg as zinc gluconate for 10 weeks.[8] Therefore, the accuracy of zinc in the compound is highly important, although the skew for it was relatively low. However, because those patients are receiving supplemental copper as well, the copper deficiency associated with high zinc intakes was eliminated.

Because of the lack of labeling on some generic gel capsules, comparative analysis for labeling accuracy was unable to be performed. Therefore, no comment can be made on the accuracy of vitamin and mineral contents for some generic gel capsule formulations of vitamins for AMD. Although the brand names were different, the generic vitamins that we sampled were manufactured by the same company, were relatively similar in their contents, and were in second and third places with regard to accuracy of the labeled content.

Seventy three percent of dietary supplement manufacturers inspected by the FDA failed to adhere to ≥1 regulation.[9] Because manufacturers set their own standards, the same product from different manufacturers may not be equivalent in composition, strength, or bioavailability.[10] Manufacturers are not required to confirm the identity of all ingredients supplied to them, and following Current Good Manufacturing Practice guidelines does not guarantee the absence of all contaminants.[10]

In our study, we did not check for contaminants or bioavailability, which may alter the effectiveness and potential toxicity of the supplements. Moreover, patients with gastrointestinal conditions may have altered vitamin absorption, which may also alter the effectiveness of the supplementation. Other limitations are that we could not test for xanthophylls or omega-3 fatty acids, and we did not test all available vitamin formulations on the market. However, our findings suggest that there is no major

difference in the constituents of the tablets whether generic or branded.

In conclusion, physicians may recommend national brand name vitamins and generic brands in tablet form to their patients with some confidence. We were unable to accurately analyze or compare the general gel capsules and therefore cannot comment on the vitamin and mineral content of these specific formulations.

KEY POINTS

Question: Are there differences in the labeling and constituents of generic and nongeneric Age-Related Eye Disease Study 2 vitamins?
Findings: In this original study, generic and nongeneric Age-Related Eye Disease Study 2 vitamin content was elevated by 27.3% for vitamin C, 3.7% for vitamin E, 17.5% for zinc, and 17% for copper. However, none of the vitamin and element contents reached a possible level of toxicity.
Meaning: Physicians may recommend national brand name vitamins and generic brands in tablet form to their patients with some confidence.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST. Funding/Support: Supported by an unrestricted grant from Research to Prevent Blindness. Financial Disclosures: The authors indicate no conflicts of interest. All authors attest that they meet the current ICMJE criteria for authorship.

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