Multiple Micronutrient Powders

For Point-of-Use Fortification of Foods Consumed by Infants and Children 6-23 Months of Age and Children Aged 2-12 Years

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# Table of Contents

Acronyms and Abbreviations: ............................................................................................................................. 3

General Items ...................................................................................................................................................... 4

1. Summary statement of proposal for inclusion, change or deletion ............................................................. 4

2. Relevant WHO technical department and focal point .................................................................................... 6

3. Name of the organization(s) consulted and/or supporting the application ................................................. 6

4. International Nonproprietary Name (INN), Anatomical Therapeutic Chemical (ATC) code of the medicine and International Classification of Disease (ICD11) .............................................................. 6

5. Dose form(s) and strength(s) proposed for inclusion; age appropriate paediatric dose forms/strengths ... 6

6. Whether listing is requested as an individual medicine or as a representative of a pharmacologic class .. 9

Treatment details, public health relevance and evidence appraisal and synthesis............................................ 10

7. Treatment details (Reference to existing WHO guidelines) ........................................................................ 10

8. Information supporting the public health relevance ...................................................................................... 11


10. Review of harms and toxicity: Summary of evidence .............................................................................. 15

11. Summary of available data on comparative cost and cost-effectiveness of the medicine ...................... 17

Regulatory Information ....................................................................................................................................... 18

12. Regulatory information on MNP .................................................................................................................. 18

13. Availability of pharmacopoeial standards .................................................................................................. 19

References ........................................................................................................................................................... 20

Appendix A: GRADE Tables ............................................................................................................................. 22

Appendix B - USP submission .......................................................................................................................... 25
**Acronyms and Abbreviations:**

CI – 95% Confidence Interval
BNF – British National Formulary
EMLc – Essential Medicines List (for children)
FDA – Food and Drug Administration
GRADE – Grading of Recommendations Assessment, Development and Evaluation
LMICs – Low and Middle-Income Countries
MD – Mean Difference
MHRA – Medicines and Healthcare products Regulatory Agency
MNP – Micronutrient Powder
MSH – Management Sciences for Health
RR – Relative Risk
SRA – Stringent Regulatory Authority
TGA – Therapeutic Goods Administration
UK – United Kingdom
USD – United States Dollar
WHO – World Health Organization
**General Items**

1. **Summary statement of proposal for inclusion, change or deletion**

This application aims to request the inclusion of multiple micronutrient powders (MNP) for infants and young children aged 6-23 months and children aged 2-12 years in the Essential Medicines List for Children (EMLc). The application provides a comprehensive review of the evidence for efficacy and safety of multiple micronutrient powders (MNP) for preventing anemia in infants and children 6 months to 23 months of age and children aged 2-12 years. Recommendations for the use of MNP are supported by the World Health Organization document entitled *WHO guideline: Use of multiple micronutrient powders for point-of-use fortification of foods consumed by infants and young children aged 6-23 months and children aged 2-12 years*, where point-of-use fortification is often referred to as “home fortification” in which various foods prepared in the home can be fortified using MNP.

The evidence synthesized in two Cochrane reviews provide sufficient evidence to show that food fortification at point-of-use with MNP is a feasible and effective public health intervention to reduce anaemia and improve iron status among infants and children 6–23 months of age and children aged 2-12 years.

**Infants and young children 6-23 months of age.** The evidence presented in the first Cochrane review shows that an MNP iron content of 12.5mg was significantly effective at reducing anemia (RR 0.69 CI [0.60, 0.78]), iron deficiency (RR 0.49 CI[0.35, 0.67]), and improving hemoglobin concentrations (MD 5.87 g/L CI[3.25, 8.49 d/L]). MNP zinc content of 5mg or more was also significantly effective at improving anemia (RR0.69 CI[0.60, 0.78]) and iron deficiency (RR 0.51 CI[0.38, 0.67]. In one study, the evidence suggests that inclusion of 5mg of elemental zinc was effective at reducing diarrhea (RR 1.33 CI[1.00, 1.78]).

In general, the use of MNP was well accepted by participants across the studies included in the review. Adherence to the intervention was varied and high adherence was more likely when the product was provided on an intermittent basis.

Based on the body of evidence, it was concluded that the use of MNP at point-of-use fortification is effective to reduce anemia in infants and children 6 – 23 months of age. Furthermore, the intervention is equally efficacious in settings with different prevalence rates of anaemia and malaria endemicity, regardless of the duration of the intervention (2-18 months). In conclusion, an MNP formulation including a dose of 12.5 mg of elemental iron (as ferrous fumarate), 5 mg of zinc and 300 μg of vitamin A is effective and the addition of other vitamins and minerals could be considered within the recommended daily values for this age group.
Children aged 2-12 years. The evidence presented in the second Cochrane review shows that that an MNP iron content of 12.5mg was significantly effective at reducing anemia (RR 0.66 CI [0.49, 0.88]), and improving hemoglobin concentrations (MD 3.37 g/L CI[0.94, 5.80 g/L]) over a duration period between 2-12 months. Also, children were receiving MNP were significantly less likely to have iron deficiency at follow-up (PR 0.35 CI [0.27, 0.47]). Furthermore, children receiving MNP were not at increased risk of diarrhea (3 liquid stools or more per day) at follow-up (RR 0.97 CI [0.53, 1.78])

Based on the body of evidence, it was concluded that the use of iron-containing MNP for point-of-use fortification of foods can help control anemia and iron deficiency in preschool and school-age children (i.e. 2-12 years of age). In conclusion, an MNP formulation including a dose of 12.5 mg of elemental iron (as ferrous fumarate), 5 mg of zinc and 300 μg of vitamin A is effective and the addition of other vitamins and minerals could be considered within the recommended daily values for this age group.

The recommendations for inclusion to the EMLc Section 10.1 - Antianemia Medicines, are as follow:

1. Add Multiple Micronutrient Powder Sachet to the EMLc for the prevention of anemia in infants and children 6 months to 23 months of age.
   a. The composition of the powder is as follow:
      i. Iron (elemental) 12.5mg (preferably as coated ferrous fumarate)
      ii. Zinc (elemental) 5mg
      iii. Vitamin A 300 μg
      iv. Other micronutrients at recommended daily values may be included in the powder.
   b. Regimen: Programme target of 90 sachets/doses over a 6-month period

2. Add Multiple Micronutrient Powder Sachet to the EMLc for the prevention of anemia in children aged 2-12 years.
   a. The composition of the powder is as follow:
      i. Iron (elemental) 12.5mg (preferably as coated ferrous fumarate) for children aged 2-4 years; and 12.5 to 30 mg (preferably as coated ferrous fumarate) for children 5-12 years of age.
      ii. Zinc (elemental) 5mg
      iii. Vitamin A 300 μg
      iv. Other micronutrients at recommended daily values may be included in the powder.
   b. Regimen: Programme target of 90 sachets/doses over a 6-month period
2. Relevant WHO technical department and focal point

Mr. Filiberto Beltran Velazquez, WHO Department of Nutrition for Health and Development.

3. Name of the organization(s) consulted and/or supporting the application

Dr. Stanley Zlotkin, CM, OOnt, MD, PhD, FRCPC. Chief, Global Child Health, The Hospital for Sick Children, Toronto, Canada.

4. International Nonproprietary Name (INN), Anatomical Therapeutic Chemical (ATC) code of the medicine and International Classification of Disease (ICD11)

INN: Not listed. Suggest using USP generic name Vitamins with Minerals Oral Powder (See Appendix B)

ATC: B03AE10, Iron in other combinations, various combinations

ICD 11: 3A00 Iron deficiency anaemia

5. Dose form(s) and strength(s) proposed for inclusion; age appropriate paediatric dose forms/strengths

Table 1 includes a suggested scheme for point-of-use fortification of complementary foods with iron-containing micronutrient powders in infants and young children aged 6–23 months, as adapted from the WHO guideline. (1)

Similarly, Table 2 includes a suggested scheme for point-of-use fortification with iron-containing micronutrient powders in children 2-12 years, as per the WHO guideline. (1)
<table>
<thead>
<tr>
<th>Scheme for fortification</th>
<th>Target group: infants and young children aged 6-23 months</th>
</tr>
</thead>
</table>
| Composition per sachet  | • Iron: 12.5 mg of elemental iron (preferably as coated ferrous fumarate)\(^a\)  
• Vitamin A: 300 µg retinol  
• Zinc: 5 mg elemental zinc  
• With or without other micronutrients to achieve 100% of the RNI\(^b,c\) |
| Regimen                  | Programme target of 90 sachets/doses over a 6-month period |
| Settings                 | Areas where the prevalence of anaemia in children under 2 years or under 5 years is 20% or higher |

\(^a\)12.5 mg of elemental iron equals 37.5 mg of ferrous fumarate or 62.5 mg of ferrous sulfate heptahydrate or equivalent amounts in other iron compounds. In children aged 6–12 months, sodium iron EDTA (NaFeEDTA) is generally not recommended. If NaFeEDTA is selected as a source of iron, the EDTA intake (including other dietary sources) should not exceed 1.9 mg EDTA/kg/day.

\(^b\)Recommended nutrient intake (RNI). Multiple micronutrient powders can be formulated with or without other vitamins and minerals in addition to iron, vitamin A and zinc, to achieve 100% of the RNI, and also taking into consideration the technical and sensory properties.

\(^c\)Where feasible, likely consumption from other sources, including home diet and fortified foods, should be taken into consideration for establishing the composition of the sachet.

<table>
<thead>
<tr>
<th>Scheme for fortification</th>
<th>Target group: 2-12 years of age</th>
</tr>
</thead>
</table>
| Composition per sachet  | • Iron: 12.5 mg of elemental iron (preferable as coated ferrous fumarate) for children aged 2-4 years; and 12.5 to 30 mg elemental iron (preferably as coated ferrous fumarate) for children 5-12 years of age\(^b\)  
• Vitamin A: 300 µg retinol  
• Zinc: 5 mg elemental zinc  
• With or without other micronutrients to achieve 100% of the RNI\(^b,c\) |
| Regimen                  | Programme target of 90 sachets/doses over a 6-month period |
| Settings                 | Areas where the prevalence of anaemia in children under 5 years is 20% or higher |

\(^b\)12.5 mg of elemental iron equals 37.5 mg of ferrous fumarate or 62.5 mg of ferrous sulfate heptahydrate or equivalent amounts in other iron compounds. If sodium iron EDTA (NaFeEDTA) is selected as a source of iron, the dose of elemental iron should be reduced by 3–6 mg due to its higher bioavailability. The appropriate range of NaFeEDTA is an area of research need.

\(^c\)Recommended nutrient intake (RNI). Multiple micronutrient powders can be formulated with or without other vitamin and minerals in addition to iron, vitamin A and zinc to achieve 100% of the RNI, and also taking into consideration the technical and sensory properties.

\(^c\)Where feasible, likely consumption from other sources, including home diet and fortified foods, should be taken into consideration for establishing the composition of the sachet.
In terms of market availability, the following manufacturers were identified in 2016 by UNICEF Supply Division’s *Multiple Micronutrient Powder Supply & Market Outlook* as meeting standards (i.e. Good Manufacturing Practice) and having the capacity to provide suitable, age-appropriate dose forms and strengths of multiple micronutrient powders for administration to infants and children (2):

1. DSM Europe (Switzerland)
2. DSM (Malaysia)- formerly Fortitech
3. Renata (Bangladesh)
4. Piramal (India)
5. DSM (South Africa)

There are several projects in countries targeting infants and young children and school-age children with MNP and it is now a policy within many of these countries. UNICEF (personal communication November 2018) provided a list from their 2015 NutriDash survey of countries that have included MNP, or are in the process of including MNP, in their national list of essential commodities for health/nutrition. The table below provides a list of these countries; however, the dose forms and strengths were not specified. This can be largely explained since MNP are not currently on the EMLc and most LMICs use the model WHO EMLc to build their respective national formularies.
### TABLE 3: Countries self-reporting the inclusion of MNP in the national list of essential commodities for health or nutrition.

<table>
<thead>
<tr>
<th>Country</th>
<th>Is MNP included in the national list of essential commodities for health or nutrition?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>Yes</td>
</tr>
<tr>
<td>Bolivia</td>
<td>Yes</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>Yes</td>
</tr>
<tr>
<td>Burundi</td>
<td>Yes</td>
</tr>
<tr>
<td>China</td>
<td>Yes</td>
</tr>
<tr>
<td>Colombia</td>
<td>Yes</td>
</tr>
<tr>
<td>Cuba</td>
<td>In progress</td>
</tr>
<tr>
<td>Democratic Republic of Congo</td>
<td>Yes</td>
</tr>
<tr>
<td>Djibouti</td>
<td>Yes</td>
</tr>
<tr>
<td>Ecuador</td>
<td>Yes</td>
</tr>
<tr>
<td>Equatorial Guinea</td>
<td>Yes</td>
</tr>
<tr>
<td>Guatemala</td>
<td>Yes</td>
</tr>
<tr>
<td>Korea, Democratic People's Republic of Korea</td>
<td>Yes</td>
</tr>
<tr>
<td>Liberia</td>
<td>In progress</td>
</tr>
<tr>
<td>Malawi</td>
<td>In progress</td>
</tr>
<tr>
<td>Mexico</td>
<td>Yes</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>In progress</td>
</tr>
<tr>
<td>Niger</td>
<td>Yes</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>In progress</td>
</tr>
<tr>
<td>Peru</td>
<td>Yes</td>
</tr>
<tr>
<td>Rwanda</td>
<td>Yes</td>
</tr>
<tr>
<td>Syrian Arab Republic</td>
<td>Yes</td>
</tr>
<tr>
<td>Tanzania, United Republic</td>
<td>Yes</td>
</tr>
<tr>
<td>Timor-Leste</td>
<td>In progress</td>
</tr>
<tr>
<td>Vietnam</td>
<td>In progress</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>In progress</td>
</tr>
</tbody>
</table>

Source: UNICEF NutriDash Survey 2015

6. **Whether listing is requested as an individual medicine or as a representative of a pharmacologic class**

This application is requesting the inclusion of MNP as an individual medicine as part of the core list under Section 10.1 - Antianemia Medicines.
Treatment details, public health relevance and evidence appraisal and synthesis

7. Treatment details (Reference to existing WHO guidelines)

The 2016 WHO guideline, *Use of multiple micronutrient powders for point-of-use fortification of foods consumed by infants and young children 6-23 months and children 2-12 years* (1) is an update of the 2011 WHO guideline, *Use of multiple micronutrient powders for home fortification of foods consumed by infants and young children 6-23 months of age.* (3)

The 2016 WHO guideline makes the following recommendations:

Recommendation 1:

- In populations where anaemia is a public health problem, point-of-use fortification of complementary foods with iron-containing micronutrient powders in infants and young children aged 6–23 months is recommended, to improve iron status and reduce anaemia (strong recommendation, moderate-quality evidence).

Table 1 includes a suggested scheme for point-of-use fortification of complementary foods with iron-containing micronutrient powders in infants and young children aged 6–23 months.

Recommendation 2:

- In populations where anaemia is a public health problem, point-of-use fortification of foods with iron-containing micronutrient powders in children aged 2–12 years is recommended, to improve iron status and reduce anaemia (strong recommendation, moderate-quality evidence).

Table 2 includes a suggested scheme for point-of-use fortification of foods with iron-containing micronutrient powders in children aged 2–12 years.

Furthermore, the 2016 WHO guideline provides several ‘Remarks’ to assist in the implementation of these recommendations including the following with respect to requirements for diagnostic tests, administration requirements and skills level of health care providers:
• The use of multiple micronutrient powders is a preventive strategy for implementation at population level without screening for any condition or disease. Children diagnosed with anaemia should be treated appropriately, according to WHO and national guidelines.¹

• Programmes of point-of-use fortification with micronutrient powders should include a behaviour-change strategy that promotes awareness and correct use of this product, proper and hygienic preparation, feeding of complementary foods for children older than 6 months and a healthy diet for children older than 2 years. Recommended breastfeeding practices, hand washing with soap, prompt attention to fever in malaria settings, and measures to manage diarrhoea should also be included. Further, these programmes should include training for health-care workers or other types of workers to adequately provide nutrition counselling and demonstrate the correct use of multiple micronutrient powders.

• In malaria-endemic areas, the provision of iron in any form, including micronutrient powders for point-of-use fortification, should be implemented in conjunction with measures to prevent, diagnose and treat malaria. Provision of iron through these interventions should not be made to children who do not have access to malaria-prevention strategies (e.g. provision of insecticide-treated bednets and vector-control programmes), prompt diagnosis of malaria illness, and treatment with effective antimalarial drug therapy.

8. Information supporting the public health relevance

Background on Micronutrient Deficiencies. The micronutrients of focus in this application are iron, vitamin A and zinc.

Iron is a vital micronutrient that is used by every cell and organ system in the body. Iron deficiency occurs as a result of depleting iron concentrations in the body which over time lead to decreased hemoglobin concentration in the blood. (4) Iron deficiency is a common childhood condition, resulting from nutritional deficiency; it can be induced by sustained negative iron balance due to inadequate dietary intake, absorption or utilization of iron, increased iron requirements during the growth period, or blood loss due to parasitic infections such as malaria, soil-transmitted helminth infestations and schistosomiasis. (4) With prolonged iron deficiency, the hemoglobin concentrations start to decrease, resulting in anemia. Hemoglobin is responsible

for carrying oxygen from the lungs to the tissue; therefore, during anemia, the blood has a
decreased capacity to carry oxygen through the blood leading to oxygen deficit in the body of the
affected individual. Iron deficiency alone is not the only concern; deficiencies of other
micronutrient deficiencies such as folate, vitamin B12 and vitamin A can also cause or contribute
to anemia. (4) Iron deficiency anemia is diagnosed by obtaining hemoglobin concentration
levels, and with serum ferritin and transferrin levels, two measures can be used to assess iron
status of an individual. A decrease in these values is measured by predefined laboratory tests that
differ by age and sex, which indicate iron deficiency anemia.

Vitamin A is vital for healthy growth and development, cell recognition, vision, and immune
function. Vitamin A deficiency, which can be caused due to poor nutritional status,
malabsorption or excretion as a result of common illnesses, is the leading cause of acquired
blindness in children. Children under 5 years of age and women of childbearing age are at
highest risk of deficiency. (4) Vitamin A deficiency is diagnosed when serum retinol
concentration is less than 0.70 μmol /L.

Zinc deficiency is primarily a result of inadequate dietary intake or absorption. Zinc deficiency is
linked to increased risk of diarrhea, malaria and lower respiratory tract infections such as
pneumonia. (4)

**Public Health Relevance.** There are no global estimates for combined vitamin and mineral
deficiencies in children under 2 years of age; however global estimates for individual
micronutrient deficiencies exist.

The global prevalence of anemia worldwide for preschool children is 43% or an estimated 273
children, of which about 42% is attributable to iron deficiency. (5) Anemia in early childhood
reduces cognitive ability and causes developmental delays and disability. (5) Currently,
epidemiological and experimental data suggest that in order to minimize these risks, prevention
of anemia is preferred over treatment because the physiological impairments due to deficiency
start at an early age and they may be irreversible, even after repletion of iron stores. (6) There are
no direct estimates for prevalence of zinc deficiency; however, it is believed to be as prevalent as
iron deficiency affecting approximately 293 million children under five and is responsible for
13% of lower respiratory tract infections (primarily pneumonia and influenza). (4)

Amongst children under five globally, an estimated 190 million have vitamin A deficiency. The
prevalence of vitamin A deficiency is about 44% amongst children in Africa and about 50% in
children in South-East Asia.(4) Vitamin A deficiency associated with prevalence of night
blindness is around 2% in African children, and about 0.5% in children in parts of South- East
Asia. (4)

Deficiencies of vitamins and minerals such as iron, zinc, vitamin A and others, often occur
simultaneously in children due to factors such as poor nutritional status. (4) The effects of these
deficiencies in neonates can result in serious adverse events including mortality. Furthermore,
the effects of these deficiencies in childhood may result in long-term, life-long irreversible
physical and cognitive problems that lead to negative consequences for health and economic
opportunities. Mineral and vitamin deficiencies particularly in iron, zinc and vitamin A, among
other nutritional risk factors are determined to be responsible for 3.9 million deaths (35% of total
deaths) in children under the age of five annually. These deficiencies are also responsible for 144
million disability-adjusted life years in the same population. (4)

**Current Public Health Interventions.** Currently, the primary interventions to prevent or treat
micronutrient deficiency vary by age. For infants up to 6 months of age, exclusive breastfeeding
in recommended. (1) (5) After which, point-of-use vitamin and mineral fortified complementary
foods are started and nutritional supplements may also be used. (1) (7) However, despite the
proven cost-effectiveness of micronutrient-based interventions (including iron, zinc and vitamin
A supplements) and the evidence of benefits, success of public programs have been limited, due
to issues such as adherence to dosing regimens, side-effects and safety concerns. (3) (8)

As an alternative to the traditional fortification of staple foods methods, a point-of-use
fortification method has been proposed. The idea of this public health intervention is to allow
direct fortification of all types of semi-solid foods for children 6-23 months of age, and up to the
age of 12 years, at the time of consumption at home, in schools or other places. (1) (7) This is
achieved by providing a mixture of iron, zinc and vitamin A (at the minimum) in a single-serving
sachet. The contents of an MNP sachet can be sprinkled over the food before consumption by the
child without altering the taste of the food. (9) (10)

The adoption of point-of-use fortification using MNP has increased significantly over the past
few years, largely replacing iron supplements such as liquid ferrous sulphate drops. The WHO
second *Global Nutrition Policy Review* (GNPR2), undertaken between 2016–2017, reported that
among the 167 countries surveyed, the most common micronutrient supplements provided to
children were MNP. (11) This reflects an increase in 30% uptake since the first GNPR for 2009-
2010. Furthermore, a decrease in iron supplements was observed for children – especially in the
WHO regions of Africa, South-East Asia and the Western Pacific – which probably reflects the
increase in the use of MNPs for children, for which guidelines were issued in 2011 and
subsequently updated in 2016. (11) The 2016 guideline supersedes the 2011 one for infants and
young children aged 6–23 months, and provides new recommendations for children aged 2–12
years.

Following the WHO handbook for guideline development\(^2\), the guideline development group of the 2016 WHO guideline, *Use of multiple micronutrient powders for point-of-use fortification of foods consumed by infants and young children 6-23 months and children 2-12 years*, utilized two systematic reviews (9) (12) following the Cochrane handbook for systematic review of interventions to assess the effects and safety of point-of-use fortification of foods with multiple micronutrient powders. In addition, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology was followed to prepare evidence profiles to determine the quality of evidence based on these up-to-date systematic reviews. (see Appendix A)

The following are summaries of the evidence as highlighted in the WHO guideline for each of the two age groups (1):

**Multiple micronutrient powders in infants and young children aged 6-23 months.** Infants and young children from 6 to 23 months of age who consumed foods fortified at the point-of use with multiple micronutrients powders had a lower risk for the critical outcome of anaemia, with a 26% reduction compared to placebo or no intervention (risk ratio [RR]: 0.74; 95% confidence interval [CI]: 0.66 to 0.83; 10 studies; 2802 participants, high-quality evidence). They also had a lower risk for the critical outcome of iron deficiency, with a 52% reduction (RR: 0.48; 95% CI: 0.36 to 0.62; 5 studies; 796 participants, moderate-quality evidence). Compared to no treatment or placebo, children receiving multiple micronutrient powders had a 5.12 g/L higher haemoglobin concentration at follow-up (mean difference [MD]: 5.12 g/L; 95% CI: 2.70 to 7.54 g/L; 12 studies; 3565 participants, low-quality evidence). With respect to iron status, compared to no treatment or placebo, children receiving multiple micronutrient powders had an average increase in serum ferritin concentration of 16.47 μg/L at follow-up (MD: 16.47 μg/L; 95% CI: 3.03 to 29.91 μg/L; 3 studies; 694 participants, very low-quality evidence). Regarding weight-for-age z-score, the mean difference was minimal (MD: 0.04 in z-score; 95% CI: –0.13 to 0.21; 4 studies; 606 participants, low-quality evidence). None of the trials reported on the outcome of all-cause mortality.

In conclusion, the use of multiple micronutrient powders for point-of-use fortification of foods is an effective intervention to reduce anaemia and iron deficiency in infants and young children aged 6–23 months. The intervention is equally efficacious in settings with different prevalence rates of anaemia and malaria endemicity versus areas with sporadic malarial cases, and regardless of the duration of the intervention (2 to 18 months).

Multiple micronutrient powders in children aged 2-12 years. Children aged 2–12 years receiving iron-containing multiple micronutrient powders for point-of-use fortification of foods were significantly less likely to have anaemia at follow-up than those children receiving no intervention or a placebo (prevalence ratio [PR]: 0.66; 95% CI: 0.49 to 0.88; 10 studies, 2448 participants, moderate-quality evidence). These children also had a 3.37 g/L higher haemoglobin concentration at follow-up (MD: 3.37 g/L; 95% CI: 0.94 to 5.80 g/L; 11 studies; 2746 participants, low-quality evidence). Also, children receiving iron-containing multiple micronutrient powders for point-of-use fortification of foods were significantly less likely to have iron deficiency at follow-up than those children receiving no intervention or a placebo (PR: 0.35; 95% CI: 0.27 to 0.47; 5 studies; 1364 participants, moderate-quality evidence). With respect to ferritin concentrations, children receiving iron-containing multiple micronutrient powders had, on average, 0.42 μg of ferritin more per litre at follow-up than those children receiving no intervention or a placebo (standardized mean difference [SMD]: 0.42 μg/L; 95% CI: –4.36 to 5.19 μg/L; 3 studies; 1066 participants, very low-quality evidence).

In conclusion, the use of iron-containing micronutrient powders for point-of-use fortification of foods can help control anaemia and iron deficiency in preschool and school-age children (i.e. 2–12 years of age).

10. Review of harms and toxicity: Summary of evidence

Data from UNICEF’s 2017 NutriDash report estimate that the total number of children 6–59 months of age worldwide exposed to micronutrient powders was 10,166,753 in 2015 alone. (13) Data on the number of children who received micronutrient powders was available in 54 out of the 65 countries implementing micronutrient powder interventions.

Multiple micronutrient powders in infants and young children aged 6-23 months. With respect to available data on potential adverse effects, the first systematic review mentioned above, included a total of 15 trials with 12,239 infants and young children 6-23 months in low- and middle-income countries in Asia, Africa and the Americas. (9) Data on morbidity, other indicators of vitamin and mineral status and side-effects were scarce due to a lack of standardization; however, none of the trials reported deaths attributable to the intervention and there was difference regarding the patterns of morbidity between children receiving placebo or no intervention and the ones receiving MNP. Also noted is that six of the studies were conducted in settings considered malaria endemic, of which only one study reported results related to malaria and found no difference in the presence of positive malaria smears between the groups (RR 0.24; 95% CI 0.05 to 1.12; 194 children). None of the trials reported on the outcome of all-cause mortality.
**Multiple micronutrient powders in children aged 2-12 years.** With respect to available data on the potential of adverse effects, the second systematic review included a total of 12 trials with 5,720 children aged 2-12 years in low- and middle-income countries in Asia, Africa and the Americas. (12) Regarding all-cause mortality, only one trial reported on this outcome and there were no deaths reported during this trial (MD: 0; 95% CI: –0.03 to 0.03; 1 study; 115 participants, low-quality evidence). Finally, diarrhoea (three liquid stools or more per day) was reported by two trials and children receiving iron-containing multiple micronutrient powders for point-of-use fortification of foods were as likely to have diarrhoea at follow-up as those children receiving no intervention or a placebo (RR: 0.97; 95% CI: 0.53 to 1.78; 2 studies; 366 participants, moderate-quality evidence).

The GRADE summary of findings tables for both systematic reviews can be found in Appendix A. (1)

Lastly, to address concerns with respect to the safety of iron administration, a recent Cochrane review entitled “Oral iron supplements for children in malaria-endemic areas” evaluated the effects and safety of iron supplementation (including MNP), with or without folic acid, in children living in areas with hyperendemic or holoendemic malaria transmission. (14) The review found that overall, iron does not cause an excess of clinical malaria (risk ratio (RR) 0.93, 95% confidence intervals (CI) 0.87 to 1.00; 14 trials, 7168 children, high quality evidence). Iron probably does not cause an excess of clinical malaria in both populations where anaemia is common and those in which anaemia is uncommon. In areas where there are prevention and management services for malaria, iron (with or without folic acid) may reduce clinical malaria (RR 0.91, 95% CI 0.84 to 0.97; seven trials, 5586 participants, low quality evidence), while in areas where such services are unavailable, iron (with or without folic acid) may increase the incidence of malaria, although the lower CIs indicate no difference (RR 1.16, 95% CI 1.02 to 1.31; nine trials, 19,086 participants, low quality evidence). Iron supplementation does not cause an excess of severe malaria (RR 0.90, 95% CI 0.81 to 0.98; 6 trials, 3421 children, high quality evidence). Iron resulted in fewer anaemic children at follow up, and the end average change in haemoglobin from base line was higher with iron.
11. Summary of available data on comparative cost and cost-effectiveness of the medicine

The current listed price of the MNP provided by UNICEF Supply Catalogue website is 0.65 USD per pack (30 sachets), with a cost 0.022 USD per 1g sachet. The price of MNP for customized/country specific layouts is 0.61 USD per pack (30 sachets), with a cost of 0.020 USD per 1g sachet. The composition of this product includes 12 additional micronutrients as presented in the Table 4. (15)

Table 4: Comparison of dosages between UNICEF’s MNP Multiple Micronutrient Powder, single-use 1 gram sachets, pack of 30 sachets and the MNP for the EMLc.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosage of UNICEF’s MNP</th>
<th>Dosage of MNP for the EMLc inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (as dry CWS vitamin A acetate or palmitate beadlets)</td>
<td>400 µg</td>
<td>300 µg</td>
</tr>
<tr>
<td>Iron (as coated Ferrous fumarate, Ferric pyrophosphate, NaFe EDTA* or Ferrous bisglycinate)</td>
<td>10 mg</td>
<td>12.5 mg</td>
</tr>
<tr>
<td>Zinc (as Zinc sulphate, oxide or gluconate)</td>
<td>4.1. mg</td>
<td>5 mg</td>
</tr>
<tr>
<td>Vitamin C (as ascorbic acid or sodium ascorbate)</td>
<td>30 mg</td>
<td>5 µg</td>
</tr>
<tr>
<td>Vitamin D (200IU) (as dry CWS Cholecalciferol)</td>
<td>5 µg</td>
<td>5 mg</td>
</tr>
<tr>
<td>Vitamin E TE (as CWS d or dl-alpha tocopheryl acetate)</td>
<td>0.5 mg</td>
<td>0.5. mg</td>
</tr>
<tr>
<td>Vitamin B1 (as Thiamine mononitrate)</td>
<td>6 mg</td>
<td>0.5. mg</td>
</tr>
<tr>
<td>Vitamin B2 (Riboflavin or riboflavin-5-phosphate)</td>
<td>0.9 µg</td>
<td>0.9 µg</td>
</tr>
<tr>
<td>Vitamin B3 (as Nicotinamide)</td>
<td>90 µg</td>
<td>90 µg</td>
</tr>
<tr>
<td>Vitamin B6 (as Pyridoxine hydrochloride)</td>
<td>0.56 mg</td>
<td>90 µg</td>
</tr>
<tr>
<td>Vitamin B12 (1% or 0.1% Cyanocobalamin on a carrier)</td>
<td>17 µg</td>
<td>90 µg</td>
</tr>
<tr>
<td>Folic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper (as Copper gluconate or sulphate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium (as Sodium selenate or selenite or selenomethionine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine (as Potassium iodide)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other micronutrients may be included</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The price of the MNP provided by the WHO/UNICEF report on sources and prices of selected medicines for children, 2nd edition April 2010 is presented in Table 5 and is based on a survey
done among manufacturers and does not include added costs such as freight, insurance, import duties or taxes and may also vary according to quantities ordered. (16) The average price ranges from 0.017 to 0.030 USD for 1g sachet.

Table 5: Cost of MNP Multiple Micronutrient Powder, single-use 1 gram sachets, pack of 30 sachets

<table>
<thead>
<tr>
<th>Manufacturers (N=5)</th>
<th>Unit</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>25 Perc</th>
<th>(x/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSM Switzerland</td>
<td>1g sachet</td>
<td>0.017</td>
<td>0.030</td>
<td>0.023</td>
<td>0.020</td>
<td>2/2</td>
</tr>
<tr>
<td>Genera Pakistan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lomapharm Germany</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lachifarma Italy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piramal India</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Min: The minimum price listed represents the lowest price among the products in this category Max: The maximum price listed represents the highest price among products in this category Median: The middle price, or in the case of an even number of prices listed, it is the mean of the two middle numbers. 25th percentile: The values point representing the first quartile of quoted prices in ascending order. (x/y) stands for the number of manufacturers (x) per countries (y) reporting prices.

The World Food Programme (WFP) estimates the cost of MNP between 0.015 and 0.035 USD per sachet depending on the quantity of sachets ordered, the composition of the mixture and the site of production. (17)

The World Bank estimates the cost of MNP intervention at 3.60 USD per child 6-23 months of age. The methodology described in Scaling Up Nutrition: What Will it Cost? was applied to calculate the annual cost of scaling up MNP interventions in each of the 68 countries. (8)

A Copenhagen Consensus review found that micronutrient interventions were cost-effective in general (18) and furthermore, it has been estimated that iron-containing MNP recover $37 for every $1 invested due to the positive effects of addressing childhood anaemia among children 6–23 months. (19)

Regulatory Information

12. Regulatory information on MNP

MNP supplements are not reviewed for safety or efficacy and are not approved for sale as medications by the United States Food and Drug Administration (FDA), the Australian Therapeutic Goods Administration (TGA), and the United Kingdom’s Medicines and Healthcare
Products Regulatory Agency (MHRA). Rather, supplements in these jurisdictions are registered as food supplements and are held to good manufacturing practices for purities only. Therefore, no additional specific analysis of regulatory status of iron or folic acid supplements was warranted. However, manufacturers of supplements must be registered entities and certified to adhere to good manufacturing practices. (20)

At the individual country level where MNP are being distributed, MNP have been classified as either pharmaceuticals or food related, with different implications. The regulatory classification of MNP can have implications for how the product is imported, packaged, distributed, and/or promoted. (21) Classification as a pharmaceutical has sometimes exempted MNP from import taxation, particularly in instances where MNP has been included on the national list of essential commodities, which can also help with donor buy-in. (21) The pharmaceutical classification can also help increase demand due to the perceived value of medicines. (22) Classifying MNP as pharmaceuticals may also encourage training of pharmacists and doctors in their appropriate use. (23)

13. **Availability of pharmacopoeial standards**

A submission for MNP to be included within the United States Pharmacopoeia (USP) has been approved under the category *Vitamins with Minerals Oral Powder*. A monograph for *Vitamins and Oral Powder* was balloted and approved in the May-June ballot for USP 42 – NF 37 and will become official on May 1, 2019. All comments received to the monograph proposal published in PF 43(6) [Nov.-Dec. 2017] were reviewed, considered and incorporated as the Expert Committee deemed appropriate. A summary of comments received, and the appropriate Expert Committee's responses will be published in the Revisions and Commentary section of USP.org at the time the official revision is published. A draft copy of the monograph is provided in Appendix B.
References


15. UNICEF. Supply Catalogue. UNICEF. [Online] [Cited: November 28, 2018.] https://supply.unicef.org/unicef_b2c/app/displayApp/(cpgsize=5&layout=7.0-12_1_66_68_115_2&uiarea=2&carea=50D129F16B9F08F2E10000009E710FC1&cpgnum=1)/.do?rf=y#.


### Appendix A: GRADE Tables

#### A. Micronutrient powders for point-of-use fortification of foods versus placebo/no intervention in infants and young children aged 6–23 months

**Micronutrient powders for point-of-use fortification of foods versus placebo/no intervention in infants and young children aged 6–23 months**

<table>
<thead>
<tr>
<th>Patient or population: infants and young children aged 6–23 months</th>
<th>Setting: community settings</th>
<th>Intervention: point-of-use fortification with multiple micronutrient powders</th>
<th>Comparison: placebo/no intervention</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Relative effect (95% CI)</th>
<th>Number of participants (studies)</th>
<th>Quality of the evidence (GRADE) comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia (haemoglobin values lower than 110 g/L)</td>
<td>RR 0.74 (0.66 to 0.83)</td>
<td>2802 (10 RCTs)</td>
<td>HIGH</td>
</tr>
<tr>
<td>Iron deficiency (as defined by trialists)</td>
<td>RR 0.48 (0.39 to 0.58)</td>
<td>796 (5 RCTs)</td>
<td>MODERATE</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>MD 5.12 (2.70 to 7.54)</td>
<td>3565 (12 RCTs)</td>
<td>LOW</td>
</tr>
<tr>
<td>Iron status (ferritin concentrations in μg/L)</td>
<td>MD 16.47 (3.03 to 29.91)</td>
<td>694 (3 RCTs)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Weight-for-age (in z-scores)</td>
<td>MD 0.04 (0.13 to 0.21)</td>
<td>606 (4 RCTs)</td>
<td>LOW</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>Not estimable</td>
<td>0 (0)</td>
<td>None of the trials reported on this outcome</td>
</tr>
</tbody>
</table>

CI: confidence interval; RCT: randomized controlled trial; RR: risk ratio; OR: odds ratio.

* The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

GRADE Working Group grades of evidence:

- **High quality**: we are very confident that the true effect lies close to that of the estimate of the effect.
- **Moderate quality**: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- **Low quality**: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
- **Very-low quality**: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

---

1. One study (29) has serious risk of bias and contributed 23.8% weighting for meta-analysis.
2. Four studies (around 40% weighting) were unclear on randomization, and two studies had high risk of attrition bias.
3. Heterogeneity was high (I² greater than 75%).
4. Two studies were unclear on randomization and had high risk of attrition bias.
5. Sample size was under optimal information size and there was a wide 95% CI.
### Micronutrient powders for point-of-use fortification of foods versus iron supplements in infants and young children aged 6–23 months

**Patient or population:** infants and young children aged 6–23 months  
**Settings:** community settings  
**Intervention:** point-of-use fortification with multiple micronutrient powders  
**Comparison:** placebo/no intervention

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Relative effect (95% CI)</th>
<th>Number of participants (studies)</th>
<th>Quality of the evidence (GRADE comments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia (haemoglobin values lower than 110 g/L)</td>
<td>RR 0.89 (0.58 to 1.39)</td>
<td>145 (1 RCT)</td>
<td>★★★☆☆ LOW</td>
</tr>
<tr>
<td>Iron deficiency (as defined by trialists)</td>
<td>Not estimable</td>
<td>0</td>
<td>None of the trials reported on this outcome</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>MD 2.81 (10.84 to 5.22)</td>
<td>278 (2 RCTs)</td>
<td>★★★☆☆ VERY LOW[1]</td>
</tr>
<tr>
<td>Iron status (ferritin concentrations in µg/L)</td>
<td>Not estimable</td>
<td>0 (0)</td>
<td>None of the trials reported on this outcome</td>
</tr>
<tr>
<td>Weight-for-age (in z-scores)</td>
<td>Not estimable</td>
<td>0 (0)</td>
<td>None of the trials reported on this outcome</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>Not estimable</td>
<td>0 (0)</td>
<td>None of the trials reported on this outcome</td>
</tr>
</tbody>
</table>

CI: confidence interval; RCT: randomized controlled trial; RR: risk ratio; OR: odds ratio.  
* The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).  
GRADE Working Group grades of evidence:  
**High quality:** we are very confident that the true effect lies close to that of the estimate of the effect.  
**Moderate quality:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.  
**Low quality:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.  
**Very-low quality:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.  
[1] Considerable statistical heterogeneity and inconsistency in the results between trials.  
[2] Small sample size with wide 95%CI.
C. Iron-containing micronutrient powders for point-of-use fortification of foods versus placebo/no intervention in children aged 2–12 years

### Patient or population:  
Infants and young children aged 6–23 months

### Settings:  
Community settings

### Intervention:  
Point-of-use fortification with multiple micronutrient powders

### Comparison:  
Placebo/no intervention

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Relative effect (95% CI)</th>
<th>Number of participants (studies)</th>
<th>Quality of the evidence (GRADE) comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia (haemoglobin lower than 110 g/L for children aged 24–59 months and lower than 115 g/L for children aged 5–11.9 years)</td>
<td>RR 0.66 (0.49 to 0.88)</td>
<td>2448 (10 studies)</td>
<td>MODERATE&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>MD 3.37 (0.94 to 5.80)</td>
<td>2746 (11 studies)</td>
<td>low&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron deficiency (as defined by using ferritin concentrations of less than 15 μg/L)</td>
<td>RR 0.35 (0.27 to 0.47)</td>
<td>1364 (5 studies)</td>
<td>MODERATE&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron status (ferritin concentrations in μg/L)</td>
<td>SMD 0.42 (-4.36 to 5.19)</td>
<td>1066 (3 studies)</td>
<td>very low&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>All-cause mortality (number of deaths during the trial)</td>
<td>MD 0 (-0.03 to 0.03)</td>
<td>115 (1 study)</td>
<td>low&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diarrhoea (three liquid stools or more per day)</td>
<td>RR 0.97 (0.53 to 1.78)</td>
<td>366 (2 studies)</td>
<td>MODERATE&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

CI: confidence interval; MD: mean difference; RR: risk ratio; SMD: standardized mean difference.

GRADE Working Group grades of evidence:

- **High quality:** we are very confident that the true effect lies close to that of the estimate of the effect.
- **Moderate quality:** we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- **Low quality:** our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.
- **Very-low quality:** we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

<sup>1</sup> Most of the studies had no blinding. High heterogeneity (72%) with most of the studies showing a positive effect of multiple micronutrient powders. No serious imprecision.

<sup>2</sup> Most of the studies had no blinding. High heterogeneity (93%) with most of the studies showing a positive effect of multiple micronutrient powders. Serious imprecision.

<sup>3</sup> Most of the studies had no blinding. Nil heterogeneity with most of the studies showing a positive effect of multiple micronutrient powders. No serious imprecision.

<sup>4</sup> All the studies had no or unclear blinding. 100% heterogeneity with most inconsistency in the direction of the effect. Serious imprecision.

<sup>5</sup> Only one low-risk trial reported on this outcome. Nil heterogeneity with both studies showing no difference between the intervention and the comparison group. Serious imprecision.

<sup>6</sup> Two low-risk trials reported on this outcome. Nil heterogeneity with both studies showing no difference between the intervention and the comparison group. Serious imprecision.
Appendix B - USP submission
BRIEFING

Vitamins with Minerals Oral Powder. Fortification of foods with powders containing Vitamins with Minerals has been found helpful for increasing the vitamins and minerals intake especially in children 6–23 months of age. This intervention consists of the addition of a mixture of vitamins and minerals in powder form to any semi-solid food. The mixture is provided in single-serving containers, the contents of which are simply sprinkled over the food before consumption. Because there is no existing USP monograph for this dosage form, a new monograph, based on validated methods of analysis, is proposed.

1. The liquid chromatographic procedure for Content of Vitamin A and Vitamin E, for vitamin A as retinyl acetate, is based on analyses performed with the 4.6-mm × 25-cm Phenomenex Synergi Hydro brand of column with L1 packing of 4-µm particle size. The typical retention times for retinyl acetate and tocopheryl acetate are about 6.5 and 12.2 min, respectively.

2. The liquid chromatographic procedure for Content of Vitamin A and Vitamin E, for vitamin A as retinyl palmitate, is based on analyses performed with the 4.6-mm × 25-cm Phenomenex Synergi Hydro brand of column with L1 packing of 4-µm particle size. The typical retention times for tocopheryl acetate and retinyl palmitate are about 5.1 and 13.3 min, respectively.

3. The liquid chromatographic procedure for Content of Vitamin D is based on analyses performed with the 2.1-mm × 10-cm Waters Acquity UPLC BEH Shield brand of column with L1 packing of 1.7-µm particle size. The typical retention time for cholecalciferol is about 11 min.

4. The liquid chromatographic procedure for Content of Vitamins B1, B2, B3, B6, and Folic Acid is based on analyses performed with the 4.6-mm × 25-cm Waters Atlantis T3 brand of column with L1 packing of 5-µm particle size. The typical retention times for ascorbic acid, niacinamide, thiamine, pyridoxine, folic acid, and riboflavin are 5.0, 6.1, 10.7, 20.0, 30.4, and 38.0, respectively.

5. The liquid chromatographic procedure for Content of Vitamin B12 is based on analyses performed with the 2.1-mm × 10-cm Waters Acquity UPLC BEH C18 brand of column with L1 packing of 1.7-µm particle size. The typical retention time for cyanocobalamin is about 4.7 min.

6. The liquid chromatographic procedure for Content of Vitamin C is based on analyses performed with the 4.6-mm × 25-cm Phenomenex Gemini C18 brand of column with L1 packing of 5-µm particle size. The typical retention time for ascorbic acid is about 5.0 min.

Dissolution tests for folic acid, vitamin A, index vitamins, and index minerals are important performance tests for these dosage forms. Interested parties are encouraged to submit proposals for dissolution tests that assure the release of vitamins and minerals from the powder so that these nutrients are available for absorption.

(NBDS: N. Davydo.)
Correspondence Number—C190939

Comment deadline: January 31, 2018
Add the following:

Vitamins with Minerals Oral Powder

**DEFINITION**

Vitamins with Minerals Oral Powder contains one or more of the following oil-soluble vitamins: Vitamin A as retinyl acetate or retinyl palmitate, Vitamin D as cholecalciferol, Vitamin E as *RRR*-alpha-tocopheryl acetate or *all-rac*-alpha-tocopheryl acetate; one or more of the following water-soluble vitamins: Vitamin C as ascorbic acid or sodium ascorbate, Vitamin B1 as thiamine mononitrate, Vitamin B2 as riboflavin, Vitamin B3 as niacin or niacinamide, Vitamin B6 as pyridoxine hydrochloride, Vitamin B12 as cyanocobalamin, and Folic Acid; and one or more minerals: copper, iodine, iron, selenium, and zinc, derived from substances generally recognized as safe and furnishing the elements in ionizable form. [NOTE—NMT 25% of elemental iron from the labeled amount may derive from edetate iron (III) sodium when used as a source of iron.] Vitamins with Minerals Oral Powder contains NLT 90.0% and NMT 150.0% of the labeled amounts of Vitamin A as retinol activity equivalent (C₂₀H₃₀O), Vitamin D as cholecalciferol (C₂₇H₄₄O), and Vitamin E as *RRR*-alpha-tocopherol equivalent (C₂₉H₅₀O₂); NLT 90.0% and NMT 150.0% of the labeled amounts of Vitamin C as ascorbic acid (C₆H₈O₆), Vitamin B1 as thiamine monochloride (C₁₂H₁₇ClN₄O₅S), Vitamin B2 as riboflavin (C₁₇H₂₀N₄O₆), Vitamin B3 as niacin (C₆H₅NO₂) or niacinamide (C₆H₆N₂O), Vitamin B6 as pyridoxine (C₈H₁₁NO₃), Vitamin B12 as cyanocobalamin (C₆₃H₈₈CoN₁₄O₁₄P), and folic acid (C₁₉H₁₉N₇O₆); NLT 90.0% and NMT 125.0% of the labeled amounts of copper (Cu), iron (Fe), and zinc (Zn); and NLT 90.0% and NMT 160.0% of the labeled amounts of iodine (I) and selenium (Se).

**IDENTIFICATION**

- **PROCEDURE:** Proceed as directed in the tests for **Strength**.

**Acceptance criteria:** The retention times of the main peaks of the **Sample solution** correspond to those of the vitamins claimed in their respective **Standard solutions**. The **Sample solution** presents significant emission at the characteristic wavelengths of the elements claimed. When iodine is claimed, the **Sample solution** turns blue upon addition of starch TS in the test for **Content of Iodine**.

**STRENGTH**

- **CONTENT OF VITAMIN A AND VITAMIN E**

  [NOTE—Use low-actinic glassware.]  

**For Oral Powders containing retinyl acetate**

**Mobile phase:** Methanol

**Standard stock solution:** A solution containing the equivalent of 0.15 mg/mL of retinol from USP Retinyl Acetate RS in isopropyl alcohol

**Standard solution:** A solution in methanol of USP Retinyl Acetate RS from **Standard stock solution** and USP Alpha Tocopheryl Acetate RS at concentrations similar to those obtained for the corresponding vitamins in the **Sample solution**.

http://www.usppf.com/pf/pub/data/v436/MON_IPR_436_m11816.xml 11/17/2017
Sample solution: Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a calculated amount of accurately weighed composite into a 200- or 250-mL volumetric flask to obtain a solution with a known nominal concentration of vitamins in the range of 2.5–56 µg/mL of retinol from retinyl acetate and 0.2–2.0 mg/mL of alpha tocopheryl acetate. Add 20 mL of water and immediately swirl the sample to slurry it and prevent clumping. Add about 500 units of leucine amino peptidase from a suitable enzyme preparation from Aspergillus oryzae containing NLT 500 units of LAP/g to the bottom of the flask using a plastic pipette and gently swirl the contents to mix. Place the sample in a water bath set to 60°–65° for 5 min. Swirl the flask 3–4 times during the 5 min to ensure the sample is completely dispersed in the water and that it has not formed clumps or stuck to the walls of the flask. Remove the flask from the water bath, add 80 mL of isopropyl alcohol, and swirl for 1 min to dissolve the vitamins. Place the sample in a sonicator for 5 min, then cool the flask to room temperature. Add 50 mL of methanol and swirl to mix. Dilute with methanol to volume and mix well. Pass a portion of the solution through a 0.45-µm PTFE membrane filter, and discard the first 1 mL of the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC
Detector: UV 325 nm for 11 min, then switch to 280 nm
Column: 4.6-mm × 25-cm; 4-µm packing L1

Temperatures
Sample: 8°. [NOTE—Use a thermostatic autosampler or a cooled bath.]
Column: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: Standard solution

[NOTE—The relative retention times for retinyl acetate and alpha tocopheryl acetate are 1.0 and 2.2, respectively.]

Suitability requirements
Relative standard deviation: NMT 2.0% for each peak

For Oral Powders containing retinyl palmitate

Mobile phase: Methanol

Standard stock solution: A solution containing the equivalent of 0.15 mg/mL of retinol from USP Retinyl Palmitate RS in isopropyl alcohol

Standard solution: A solution in methanol of USP Retinyl Palmitate RS from Standard stock solution and USP Alpha Tocopheryl Acetate RS at concentrations similar to those obtained for the corresponding vitamins in the Sample solution.
Sample solution: Prepare as directed in For Oral Powders containing retinyl acetate, except replace retinyl acetate with retinyl palmitate throughout the text.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC
Detector: UV 280 nm for 10 min, then switch to 325 nm
Column: 4.6-mm × 15-cm; 4-µm packing L1
Temperatures
Sample: 8°.[NOTE—Use a thermostatic autosampler or a cooled bath.]
Column: 30°
Flow rate: 2.0 mL/min
Injection volume: 10 µL

System suitability
Sample: Standard solution

[NOTE—The relative retention times for alpha tocopheryl acetate and retinyl palmitate are 1.0 and 2.6, respectively.]

Suitability requirements
Relative standard deviation: NMT 2.0% for each peak

Analysis
Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of vitamin A, as retinol activity equivalent (C_{20}H_{30}O), in the portion of Oral Powder taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100
\]

\( r_U \) = peak area of the all-trans-retinyl ester from the Sample solution
\( r_S \) = peak area of the all-trans-retinyl ester from the Standard solution
\( C_S \) = concentration of retinol in the Standard solution (µg/mL)
\( C_U \) = nominal concentration of vitamin A, as retinol, in the Sample solution (µg/mL)

Calculate the percentage of the labeled amount of vitamin E as RRR-alpha-tocopherol equivalent (C_{29}H_{50}O_{2}), in the portion of the Oral Powder taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times F \times 100
\]

\( r_U \) = peak area of the alpha-tocopheryl acetate from the Sample solution
\( r_S \) = peak area of the alpha-tocopheryl acetate from the Standard solution
\( C_S \) = concentration of all-rac-alpha-tocopherol from USP Alpha Tocopheryl Acetate RS in the Standard solution (mg/mL)
\( C_U \) = nominal concentration of vitamin E, as RRR-alpha tocopherol equivalent, in the Sample solution (mg/mL)
\( F \)
= conversion factor for the content of alpha tocopherol to \(\text{RRR-\alpha-tocopherol equivalent:}\) 1/2 for products labeled to contain \textit{all-rac}-\(\alpha\)-tocopheryl acetate and 1 for products labeled to contain \textit{RRR-\alpha-tocopheryl acetate}

**Acceptance criteria:** 90.0%–150.0% of the labeled amount of vitamin A, as retinol activity equivalent, and the labeled amount of vitamin E, as \textit{RRR-\alpha-tocopherol equivalent}

**CONTENT OF CHOLECALCIFEROL (VITAMIN D)**

[NOTE—Use amber, low-actinic glassware.]

**For Oral Powders containing less than 10 µg/g of vitamin D**

**Solution A:** Dimethyl sulfoxide in water (10:2)

**Extraction solvent:** 0.02% butylated hydroxytoluene (BHT) in \(n\)-hexane

**Mobile phase:** Methanol and water (84:16)

[NOTE—Wash the column periodically with 100% methanol to eliminate retained substances and avoid carryover interferences.]

**Standard stock solution:** 0.04 mg/mL of cholecalciferol from USP Cholecalciferol RS in methanol

**Standard solution:** Dilute the **Standard stock solution** with methanol to obtain a solution with an accurate vitamin concentration as close as possible to that in the **Sample solution**.

**System suitability solution:** Heat a volume of the **Standard stock solution** at 60° for 1 h to partially isomerize vitamin D to pre-vitamin D.

**Sample solution:** Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a calculated amount of accurately weighed composite (NMT 3 g) into a 50-mL centrifuge tube. Add 15 mL of **Solution A**, insert the stopper, and shake the sample on a horizontal shaker for 60 min. Add 15 mL of **Extraction solvent** into the tube and shake on a horizontal shaker for 30 min. Centrifuge the sample for 10 min at about 1500 rpm. Accurately transfer 10 mL of the upper hexane layer into centrifuge tube. Gently evaporate the hexane to dryness using a stream of nitrogen. [NOTE—Some samples may result in an oil residue after the hexane evaporates.] Redissolve the residue in an accurately pipetted volume of absolute alcohol to obtain a solution with a known nominal concentration in the range of 1.0–8.0 µg/mL of cholecalciferol and mix well. Pass a portion of the solution through a 0.2-µm PTFE membrane filter, and discard the first 1 mL of the filtrate.

**For Oral Powders containing 10 µg/g or more of vitamin D**

**Solution A:** 50 mg/mL of sodium dodecyl sulfate in water

**Mobile phase, Standard stock solution, Standard solution, and System suitability solution:** Prepare as directed in **For Oral Powders containing less than 10 µg/g of vitamin D**.

**Sample solution:** Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a calculated amount of accurately weighed composite (NMT 5 g) into a 50-mL volumetric flask. [NOTE—A larger flask may be used in order to eliminate additional
dilutions. The final content of water in the Sample solution is NMT 20%.) Add 10 mL of Solution A and immediately swirl the flask to mix the contents, and sonicate for 15 min swirling occasionally. Add 5 mL of methyl tert-butyl ether, swirl to mix, add 10 mL of absolute alcohol, and swirl to mix. Continue to add absolute alcohol in 10-ml aliquots, with swirling, almost to volume. Cool to room temperature, and dilute with absolute alcohol to volume to obtain a solution with a known nominal concentration in the range of 1.0–8.0 µg/mL of cholecalciferol. Mix well. [NOTE—Excessive amounts of dissolved solids may be precipitated by placing the flask in a freezer for 15 min, if necessary.] Pass a portion of the solution through a 0.2-µm PTFE membrane filter, and discard the first 1 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC  
**Detector:** 265 nm  
**Column:** 2.1-mm × 10-cm; 1.7-µm packing L1  
**Sample temperature:** 8°  
**Flow rate:** 0.4 mL/min  
**Injection volume:** 1 µL

**System suitability**

**Samples:** Standard solution and System suitability solution

[NOTE—The relative retention times for pre-cholecalciferol and cholecalciferol are 1.0 and 1.1, respectively.]

**Suitability requirements**

**Column efficiency:** NLT 14,000 theoretical plates, Standard solution  
**Resolution:** NLT 3 between pre-cholecalciferol and cholecalciferol, System suitability solution  
**Relative standard deviation:** NMT 2.0%, Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of vitamin D, as cholecalciferol (C_{27}H_{44}O), in the portion of Oral Powder taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100
\]

\[r_U\] = peak area of cholecalciferol from the Sample solution  
\[r_S\] = peak area of cholecalciferol from the Standard solution  
\[C_S\] = concentration of cholecalciferol from USP Cholecalciferol RS in the Standard solution (µg/mL)  
\[C_U\] = nominal concentration of cholecalciferol in the Sample solution (µg/mL)

**Acceptance criteria:** 90.0%–150.0%
• CONTENT OF VITAMINS B1, B2, B3, B6, AND FOLIC ACID

[NOTE—Use low-actinic glassware.]

Solution A: 10 mg/mL of USP Ascorbic Acid RS in water. Prepare fresh at the time of use.
Solution B: Phosphoric acid and water (50:50)
Solution C: 1% sodium bicarbonate in water
Solution D: 0.1 M edetate disodium in water
Mobile phase A: 0.1% trifluoroacetic acid in water
Mobile phase B: 0.1% trifluoroacetic acid in mixture of water and acetonitrile (88:12)
Mobile phase: See Table 1.

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow Rate (mL/min)</th>
<th>Mobile Phase A (%)</th>
<th>Mobile Phase B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>100</td>
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<td>1</td>
<td>0</td>
<td>100</td>
</tr>
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<td>0</td>
<td>100</td>
</tr>
<tr>
<td>40</td>
<td>1.5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>41</td>
<td>1.5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>1.5</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

[NOTE—For preparation of the following Standard stock solutions, dissolve the related Reference Standard in water first. The solution can be swirled or placed on a steam bath for about 5–10 min with frequent swirling to assist in dissolution. When the vitamin has completely dissolved, cool the solution to room temperature, add Solution B, and dilute stepwise with water to final volume. Solution B should be added in an amount resulting in a concentration of 0.5% Solution B in the final volume of stock solution.]

Thiamine standard stock solution: 150 µg/mL of thiamine from USP Thiamine Hydrochloride RS in water containing 0.5% Solution B
Riboflavin standard stock solution: 120 µg/mL of USP Riboflavin RS in water containing 0.5% Solution B
Niacin (or Niacinamide) standard stock solution: 1.5 mg/mL of USP Niacin RS or USP Niacinamide RS in water containing 0.5% Solution B
Pyridoxine standard stock solution: 150 µg/mL of pyridoxine from USP Pyridoxine Hydrochloride RS in water containing 0.5% Solution B
Folic acid standard stock solution: 55 µg/mL of USP Folic Acid RS in Solution C

Standard solution: Accurately transfer calculated volumes of Thiamine standard stock solution, Riboflavin standard stock solution, Niacin standard stock solution (or Niacinamide standard stock solution), Pyridoxine standard stock solution, and Folic acid standard stock solution into a 200-mL volumetric flask to obtain a solution with final vitamin concentrations similar to the nominal concentrations in the Sample solution for the corresponding vitamins. Add 1.0 mL of Solution B and 1.0 mL of Solution A, and dilute with water almost to volume. Add 2–3 drops of polysorbate 80 and add water to final volume. Mix well by completely
inverting the flask at least 20 times. [Note—After inverting the flask 10–12 times, carefully
remove the stopper to release the built-up pressure.]

**Sample solution:** Empty 20 containers and combine and mix their contents to obtain a
homogeneous composite. Weigh the composite and calculate the average fill weight per
container. Transfer a calculated amount of accurately weighed composite into a suitable
volumetric flask to obtain a solution containing known nominal concentrations of vitamins in
the range of 2–130 µg/mL for thiamine, 1.5–110 µg/mL for riboflavin, 10–800 µg/mL for
niacin, 10–500 µg/mL for niacinamide, 2–120 µg/mL for pyridoxine, and 0.5–2.0 µg/mL for
folic acid. Add about half volume of water and an aliquot of Solution D equivalent to 2% the
volume of the flask, swirl to mix thoroughly. Place the flask on a steam bath for 2–3 min,
swirl frequently, and avoid overheating. Cool the flask to room temperature and add an
aliquot of Solution C equivalent to 10% the volume of the flask, mix well, sonicate for 1
min. Cool to room temperature, add an aliquot of Solution A equivalent to 0.5% the volume
of the flask, and an aliquot of Solution B equivalent to 0.3% the volume of the flask, swirl to
mix and dilute almost to volume with water. Add 2–3 drops of polysorbate 80 and bring to
final volume with water. [Note—If more than one dilution is necessary to obtain the
required concentrations of vitamins make the dilutions with water first and add Solution A,
Solution B, and polysorbate 80 to the last dilution.] Mix well by completely inverting the
flask at least 20 times with caution to release the built-up pressure. Pass a portion of the
solution through a suitable filter of 0.45-µm pore size and discard the first milliliter of the
filtrate.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm for 13 min, switch to 280 nm for 33 min, then return to 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** See Table 1.

**Injection volume:** 20 µL

**System suitability**

**Sample:** Standard solution

[Note—The relative retention times for ascorbic acid, niacinamide, thiamine, pyridoxine,
follic acid, and riboflavin are 0.8, 1.0, 1.8, 3.3, 5.0, and 6.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between the ascorbic acid and niacinamide peaks

**Relative standard deviation:** NMT 2.0% for each peak

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of vitamin B1 as thiamine monochloride
(C_{12}H_{17}ClN_{4}OS), vitamin B2 as riboflavin (C_{17}H_{20}N_{4}O_{6}), vitamin B3 as niacin (C_{6}H_{5}NO_{2}) or
niacinamide \((C_6H_6N_2O)\), vitamin B6 as pyridoxine \((C_8H_{11}NO_3)\), and folic acid \((C_{19}H_{19}N_7O_6)\), in the portion of Oral Powder taken:

\[
\text{Result} = \left(\frac{r_U}{r_S}\right) \times \left(\frac{C_S}{C_U}\right) \times 100
\]

- \(r_U\) = peak area of the relevant vitamin from the Sample solution
- \(r_S\) = peak area of the relevant vitamin from the Standard solution
- \(C_S\) = concentration of the relevant vitamin Reference Standard in the Standard solution (µg/mL)
- \(C_U\) = nominal concentration of the relevant vitamin in the Sample solution (µg/mL)

**Acceptance criteria:** 90.0%–150.0% of the labeled amount of each individual vitamin

- **CONTENT OF VITAMIN B12**

  [NOTE—Use low-actinic glassware throughout this procedure. Inject samples within 30 min.]

  **Chelating reagent:** A powdered mixture of ammonium pyrrolidinedithiocarbamate, edetate disodium, and diethylenetriaminepentaacetic acid (1:1:1)

  **Buffer:** Dissolve 470.5 mg of hexanesulfonic acid sodium salt in water, add 1 mL of phosphoric acid, dilute with water to 1000 mL, and mix. Adjust with 50% potassium hydroxide to a pH of 3.5.

  **Mobile phase:** Acetonitrile and Buffer. See Table 2 for gradient.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Acetonitrile (%)</th>
<th>Buffer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>99.0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>99.0</td>
</tr>
<tr>
<td>1.2</td>
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<td>7.0</td>
<td>1.0</td>
<td>99.0</td>
</tr>
<tr>
<td>8.0</td>
<td>1.0</td>
<td>99.0</td>
</tr>
</tbody>
</table>

  **Standard solution:** 0.5 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in water

  **Sample solution:** Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a portion of the composite, nominally equivalent to 4 µg of cyanocobalamin (NMT 4 g of composite), to a flask. Add 25 mg of Chelating reagent and 10 mL of water accurately measured. Record the weight of the flask, sonicate for 5 min, and shake vigorously for 2 min. Add water to the previously recorded weight to compensate for any solvent loss. Immediately pass a portion of the solution through a polysulfone membrane filter of 0.45-µm pore size, and discard the first portion of the filtrate.

  **Chromatographic system**
(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 361 nm

**Column:** 2.1-mm × 10-cm; 1.7-µm packing L1

**Column temperature:** 35°

**Flow rate:** 0.5 mL/min

**Injection volume:** 15 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin B12, as cyanocobalamin (C₆₃H₈₈CoN₁₄O₁₄P), in the portion of Oral Powder taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100
\]

\(r_U\) = peak area of cyanocobalamin from the *Sample solution*

\(r_S\) = peak area of cyanocobalamin from the *Standard solution*

\(C_S\) = concentration of cyanocobalamin from USP Cyanocobalamin RS in the *Standard solution* (µg/mL)

\(C_U\) = nominal concentration of cyanocobalamin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–150.0%

- **CONTENT OF VITAMIN C**

  [NOTE—Protect samples from air, light, and heat.]

**Buffer:** 2.04 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** *Buffer*

**Diluent:** 0.56 g of edetate disodium and 2.04 g of monobasic potassium phosphate per 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

**Standard solution:** 0.25 mg/mL of USP Ascorbic Acid RS in *Diluent*

**Sample solution:** Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a portion of the composite, nominally equivalent to about 25 mg of ascorbic acid, into a 100-mL volumetric flask. Add 60 mL of *Diluent*, shake mechanically for 15 min, dilute with *Diluent* to volume, mix well, and pass through a membrane filter of 0.45-µm pore size, discarding the first 4 mL of the filtrate.

**Chromatographic system**
(See Chromatography (621), System Suitability.)

Mode: LC
Detector: UV 245 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 5 µL

System suitability
Sample: Standard solution
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of vitamin C₇ as ascorbic acid (C₆H₈O₆), in the portion of Oral Powder taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100
\]

- \( r_U \): peak area of the ascorbic acid from the Sample solution
- \( r_S \): peak area of the ascorbic acid from the Standard solution
- \( C_S \): concentration of ascorbic acid from USP Ascorbic Acid RS in the Standard solution (mg/mL)
- \( C_U \): nominal concentration of ascorbic acid in the Sample solution (mg/mL)

Acceptance criteria: 90.0%-150.0%

- CONTENT OF COPPER, IRON, SELENIUM, AND ZINC

Stock aqua regia solution: Prepare a mixture of hydrochloric acid and nitric acid (3:1) by adding the nitric acid to the hydrochloric acid. [NOTE—Periodically vent the solution in an appropriate fume hood.]

Diluent: Prepare a mixture of the Stock aqua regia solution and water (1:9) by adding 1 volume of Stock aqua regia solution to 2 volumes of water. Dilute with additional water to volume, and mix well.

Solution A: 1 mg/mL of yttrium in 5% nitric acid solution
Solution B: 1 mg/mL of scandium in 5% nitric acid solution

System suitability solution: A mixture of Solution A, Solution B, and Diluent (1:1:198)

Standard stock solution 1 [copper (Cu), iron (Fe), and zinc (Zn)]: Use commercially available element standard solutions in 5% nitric acid solution as follows. Pipet the appropriate amount of element standard solution into a volumetric flask, and dilute with 5% nitric acid solution to obtain a solution with final concentrations of about 0.1 mg/mL of copper, 0.25 mg/mL of iron, and 0.25 mg/mL of zinc.

Standard stock solution 2 [selenium (Se)]: Use commercially available element standard solutions in 20% hydrochloric acid solution as follows. Pipet the appropriate amount of
element standard solution into a volumetric flask, and dilute with 20% hydrochloric acid solution to obtain a solution with a final concentration of 0.1 mg/mL of selenium.

**Standard curve solutions:** Prepare a mixture of *Standard stock solution 1* and *Standard stock solution 2*, as required, in *Diluent* to generate a six-point calibration curve to bracket the concentration range of each mineral of interest.

**Sample solution:** Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a calculated amount of accurately weighed composite into a 250-mL volumetric flask. Slowly add 25 mL of the *Stock aqua regia solution* in 5-mL increments, followed by mixing. Bring the solution to a boil on a hot plate. Continue to heat gently for NMT 15 min. Remove from heat, cool, and dilute with water to volume. Pass about 30 mL through a 5-µm pore size nylon syringe filter into a centrifuge tube. If necessary, make any further dilutions using the *Diluent* to concentrations fitting within the range of the *Standard curve solutions* used.

**Instrumental conditions**

*(See Plasma Spectrochemistry (730).)*

**Mode:** Inductively coupled plasma spectrometry, using a spectrophotometer set to measure the emission of each mineral of interest at about the corresponding wavelength.  
[NOTE—The operating conditions may be developed and optimized based on the manufacturer’s recommendation. The wavelengths selected should be demonstrated experimentally to provide sufficient specificity, sensitivity, linearity, accuracy, and precision.]

**System suitability**

**Sample:** *System suitability solution*  

[NOTE—Analyze the *System suitability solution*, and obtain the response as directed in the *Analysis*.]

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard curve solutions* and *Sample solution*

Determine the emission of each mineral of interest in the *Standard curve solutions* and *Sample solution* with an inductively coupled plasma system using the *Diluent* as the blank. Plot the emission of the *Standard curve solutions* versus the concentration, in µg/mL, of the minerals of interest, and draw the straight line best fitting the plotted points. From the graph, determine the concentration (*C*), in µg/mL, for each mineral of interest in the *Sample solution*.

Calculate the percentage of the labeled amount of each mineral in the portion of Oral Powder taken:

\[
\text{Result} = C \times \left( \frac{V}{W} \right) \times F \times \left( \frac{W_{AV}}{L} \right) \times 100
\]
\[ C = \text{concentration of relevant mineral in the Sample solution determined from the standard curve (µg/mL)} \]
\[ V = \text{volume of the Sample solution (mL)} \]
\[ W = \text{weight of the portion of Oral Powder taken (mg)} \]
\[ F = \text{dilution factor for the Sample solution} \]
\[ W_{AV} = \text{average fill weight per container (mg)} \]
\[ L = \text{label claim for each mineral (µg/container)} \]

**Acceptance criteria:** 90.0%–125.0% of the labeled amounts of copper (Cu), iron (Fe), and zinc (Zn); 90.0%–160.0% of the labeled amount of selenium (Se)

- **CONTENT OF IODINE**

  **Bromine water:** To 20 mL of bromine in a glass-stoppered bottle add 100 mL of water. Insert the stopper into the bottle, and shake. Allow to stand for 30 min, and use the supernatant.

  **Sample solution:** Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer accurately weighed composite, nominally equivalent to 1 mg of iodide, to a nickel crucible. Add 5 g of sodium carbonate, 5 mL of 50% sodium hydroxide solution, and 10 mL of alcohol, taking care that the entire specimen is moistened. Heat the crucible on a steam bath to evaporate the alcohol, then dry the crucible at 100° for 30 min to prevent spattering upon subsequent heating. Transfer the crucible with its contents to a furnace heated to 500°, and heat the crucible for 15 min. [NOTE—Heating at 500° is necessary to carbonize any organic matter present; a higher temperature may be used, if necessary, to ensure complete carbonization of all organic matter.]

  Cool the crucible, add 25 mL of water, cover the crucible with a watch glass, and boil gently for 10 min. Filter the solution, and wash the crucible with boiling water, collecting the filtrate and washings in a beaker. Add phosphoric acid until the solution is neutral to methyl orange TS, then add 1 mL excess of phosphoric acid. Add excess of Bromine water, and boil the solution gently until colorless and then for 5 min longer. Add a few crystals of salicylic acid, and cool the solution to 20°.

  **Titrimetric system**

  **Mode:** Direct titration

  **Titrant:** 0.005 N sodium thiosulfate from 0.1 N sodium thiosulfate VS

  **Analysis**

  **Sample:** Sample solution

  Add 1 mL of phosphoric acid and 0.5 g of potassium iodide, and titrate the liberated iodine with Titrant, adding starch TS when the liberated iodine color has nearly disappeared.

  Calculate the percentage of the labeled amount of iodine (I) in the portion of Oral Powder taken:

  \[
  \text{Result} = V \times N_A \times F \times I_{me} \times (W_{AV}/W) \times (100/L)
  \]

  \[ V = \text{volume of sodium thiosulfate consumed (mL)} \]

  \[ N_A = \text{actual normality of the sodium thiosulfate solution used} \]
\[ F = \text{correction factor to convert mg to } \mu\text{g, } 1000 \mu\text{g/mL} \]

\[ I_{me} = \text{milliequivalent of I, } 21.16 \text{ mg/mEq} \]

\[ W_{AV} = \text{average fill weight per container (mg)} \]

\[ W = \text{weight of the portion of Oral Powder taken (mg)} \]

\[ L = \text{label claim for iodine (µg/container)} \]

**Acceptance criteria:** 90.0%–160.0%

**PERFORMANCE TESTS**

- **Weight Variation** (2091)

  **Procedure:** Empty and weigh individually the net contents from 20 containers. Determine the average net content from the sum of the individual net weights. Determine the difference between each individual net content and the average net content.

  **Acceptance criteria:** The requirements are met if (a) NMT 2 of the differences are greater than 10% of the average net content and (b) in no case is the difference greater than 25%.

**CONTAMINANTS**

- **Microbial Enumeration Tests** (2021): The total aerobic microbial count does not exceed \(10^3\) cfu/g, and the total combined molds and yeasts count does not exceed \(\times 10^2\) cfu/g.

- **Absence of Specified Microorganisms** (2022), Test Procedures, Test for Absence of Salmonella Species and Test for Absence of Escherichia coli: Meets the requirements.

**SPECIFIC TESTS**

- **Loss on Drying** (731)

  **Sample:** 2.0 g

  **Analysis:** Dry the Sample at 105° for 2 h.

  **Acceptance criteria:** NMT 6%

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in tight, light- and moisture-resistant packages.

- **Labeling:** The label states the quantity of each vitamin and mineral in terms of metric units per dosage unit and, where necessary, the chemical form in which a vitamin is present, and also states the salt form of the mineral used as the source of each element. Where the product contains vitamin E, the label indicates whether it contains \(RRR\)-alpha-tocopheryl acetate or \(all-rac\)-alpha-tocopheryl acetate.

- **USP Reference Standards** (11)

  USP Alpha Tocopheryl Acetate RS
  USP Ascorbic Acid RS
  USP Cholecalciferol RS
  USP Cyanocobalamin RS
  USP Folic Acid RS
USP Niacin RS
USP Niacinamide RS
USP Pyridoxine Hydrochloride RS
USP Riboflavin RS
USP Thiamine Hydrochloride RS
USP Retinyl Acetate RS
USP Retinyl Palmitate RS

1 A suitable grade is available from Sigma: Protease from Aspergillus oryzae, catalog #P6110.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.