Essential Medicines List Application for Multiple Micronutrient Supplements During Pregnancy

Date: November 27, 2020
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Acronyms & abbreviations

α-TE  Alpha tocopherol equivalent
ATC   Anatomical Therapeutic Chemical Classification System
ATP   Adenosine triphosphate
BMI   Body mass index
CGMP  Current Good Manufacturing Practices
DALY  Disability-adjusted life year
DFE   Dietary folate equivalent
DNA   Deoxyribonucleic acid
EML   Essential Medicines List
GINA  Global Database on the Implementation of Nutrition Action
GMP   Good Manufacturing Practice
ICER  Incremental cost-effectiveness ratio
IFA   Iron and folic acid supplements
INN   International Nonproprietary Name
IPD   Individual patient data
LBW   Low birthweight
LMICs Low- and middle-income countries
mcg   Microgram
mg    Milligram
MMS   Multiple micronutrient supplements
NAD   Nicotinamide adenine dinucleotide
RAE   Retinol activity equivalents
RDA   Recommended dietary allowance
SGA   Small for gestational age
TB    Tuberculosis
UL    Upper intake level
UNICEF United Nations Children’s Fund
UNIMMAP United Nations International Multiple Micronutrient Antenatal Preparation
USP   United States Pharmacopeial Convention
WFP   World Food Programme
WHO   World Health Organization
WRA   Women of reproductive age
General items

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

This application requests the inclusion of daily multiple micronutrient supplements (MMS) for pregnant women in the Essential Medicines List (EML). Micronutrient deficiencies are common in low- and middle-income countries (LMICs) among women of reproductive age (WRA), and MMS can help women meet the increased micronutrient requirements of pregnancy to improve the health and wellbeing of both the mother and infant. MMS is currently recommended for use in pregnant women in three World Health Organization (WHO) guidelines. The guidelines for pregnant women in emergency settings in Preventing and controlling micronutrient deficiencies in populations affected by an emergency and for pregnant women with tuberculosis in Micronutrient supplementation in individuals with active tuberculosis recommend the use of MMS.(1,2) More recently, MMS has been recommended for pregnant women who are in food insecure areas by UNICEF and the World Food Programme (WFP) in their guidance on Protecting Maternal Diets and Nutrition Services and Practices in the Context of COVID-19.(3) In the recent update to the WHO antenatal care recommendations for a positive pregnancy experience, WHO now recommends MMS for pregnant women in the context of rigorous research, based on the recently expanded evidence base of clinical trials.(4)

Two meta-analyses, a Cochrane Review, and an individual patient data (IPD) meta-analysis have compared the effectiveness of MMS, that includes iron and folic acid, with iron and folic acid supplements (IFA) alone in clinical trials. While a range of micronutrient doses was used in the trials, the most commonly used formulation in the trials was the United Nations Multiple Micronutrient Antenatal Preparation (UNIMMAP). These trials and meta-analyses provide ample evidence that MMS during pregnancy provides additional health benefit compared with IFA alone. The Cochrane review found that MMS has a significant effect over IFA in reducing the risk for low birthweight (LBW) (RR 0.88; 95% CI 0.85-0.91) and small for gestational age (SGA) (RR 0.92; 95% CI 0.88-0.97).(5) The IPD meta-analysis shows similar effects on LBW (RR 0.86; 95% CI 0.81-0.92) and SGA 0.94 (0.90-0.98), as well as a significant effect on reducing the risk of preterm birth (RR 0.93; 95% CI 0.97-0.98) with MMS using the random effects model.(6) Sub-group analyses show that infants born to women receiving MMS who are underweight and anaemic have even greater benefits with MMS, as well as female infants who have a 15% reduction in the risk of neonatal and infant mortality. There is no evidence of harm or toxicity from exceeding the upper intake level of any micronutrient, and MMS and IFA are similarly tolerated by pregnant women. Two cost-effectiveness analyses have also demonstrated that MMS is highly cost-effective in all modelled scenarios.(7,8) Thus, this application demonstrates that MMS is a safe and cost-effective intervention for the reduction of adverse pregnancy outcomes.

2. Relevant WHO technical department and focal point

WHO Department of Nutrition for Health and Development, Filiberto Beltran Velazquez, Technical Officer (Regulatory Affairs)

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

The Micronutrient Forum and the New York Academy of Sciences
4. International Nonproprietary Name (INN) and Anatomical Therapeutic Chemical (ATC) code of the medicine and International Classification of Disease (IDC11)

INN does not list a name for MMS. ATC also does not specifically list MMS, but it does have a listing for the individual components (see Table 1). Multivitamins generally are listed in the ATC as A11B.

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

Suggested components and daily dosages (1 tablet/day) are listed in Table 1 and follow the United Nations Multiple Micronutrient Antenatal Preparation (UNIMMAP) formulation as per WHA antenatal care recommendations for a positive pregnancy experience, as well as the range of micronutrients used in the various clinical trials. This preparation is for adults, specifically pregnant women and is not intended for infants or children.

**Table 1. Recommended Food/Dietary/Nutritional Ingredients in the UNIMMAP formulation and the ranges used in MMS clinical trials (9)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Chemical Entity in UNIMMAP *</th>
<th>UNIMMAP Amount</th>
<th>Ranges from Trials**</th>
<th>ATC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Retinol Acetate</td>
<td>800 mcg RAE</td>
<td>800-1600 mcg</td>
<td>A11CA01</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Ascorbic Acid</td>
<td>70 mg</td>
<td>50-500 mcg</td>
<td>A11GA01</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Cholecalciferol</td>
<td>5 mcg (200 IU)</td>
<td>5 - 10 mcg (200-400 IU)</td>
<td>A11CC05</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Alpha Tocopherol Succinate</td>
<td>10 mg α-TE</td>
<td>7.5-30 mg</td>
<td>A11HA03</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>Thiamine Mononitrate</td>
<td>1.4 mg</td>
<td>0.93-2.8 mg</td>
<td>A11DA01</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>Riboflavin</td>
<td>1.4 mg</td>
<td>1.4-2.8 mg</td>
<td>A11HA04</td>
</tr>
<tr>
<td>Vitamin B3</td>
<td>Niacinamide</td>
<td>18 mg NE</td>
<td>1.9-36 mg</td>
<td>A11HA01</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Pyridoxine Hydrochloride</td>
<td>1.9 mg</td>
<td>1.9-25 mg</td>
<td>A11HA02</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>Folic Acid</td>
<td>680 mcg DFE (400 mcg)</td>
<td>215-600 mcg</td>
<td>B03BB01</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Cyanocobalamin</td>
<td>2.6 mcg</td>
<td>1-5.2 mcg</td>
<td>B03BA01</td>
</tr>
<tr>
<td>Iron</td>
<td>Ferrous Fumarate</td>
<td>30 mg</td>
<td>20-60 mg</td>
<td>B03AA02</td>
</tr>
<tr>
<td>Iodine</td>
<td>Potassium Iodide</td>
<td>150 mcg</td>
<td>150-300 mcg</td>
<td>D08AG03</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zinc Oxide</td>
<td>15 mg</td>
<td>12.9-30 mcg</td>
<td>A12CB</td>
</tr>
<tr>
<td>Selenium</td>
<td>Sodium Selenite</td>
<td>65 mcg</td>
<td>65-130 mcg</td>
<td>A12CE02</td>
</tr>
<tr>
<td>Copper</td>
<td>Cupric Oxide</td>
<td>2 mg</td>
<td>1.2-4 mg</td>
<td>V03AB20***</td>
</tr>
</tbody>
</table>

*α-TE = alpha tocopherol equivalent; mg = milligram; mcg = microgram; RAE = retinol activity equivalents; DFE = dietary folate equivalent

* These chemical entities may be replaced by other chemical entities if they demonstrate equal or better performance (e.g., stability).

** Based on studies included in the IPD meta-analysis, excluding the study targeted at women with HIV. Many of the high values come from Kaestel, 2005 that was testing approximately 2 RDA for most micronutrients. Not all trials included B6, zinc, copper, selenium, iodine; some trials included additional micronutrients not included here.

***Copper sulfate
Based on the NutriDash Survey from the United Nations Children’s Fund (UNICEF), 18 low- and middle-income countries (LMICs) have either already implemented policies or are interested in implementing MMS policies and 38 LMICs have procured MMS for pregnant women. According to the Global Database on the Implementation of Nutrition Action (GINA), five countries have national policies that specifically include multiple micronutrient supplementation (Cambodia, Cameroon, Madagascar, Mongolia and Mozambique) and five additional countries have national policies (Gambia, Kenya, Morocco, Nepal, and Nicaragua) that reference an unspecified micronutrient supplementation.(10) Currently 68 LMICs have multivitamins on their national Essential Medicines Lists (EMLs) though not all adhere to the UNIMMAP formulation (Appendix A).(11)

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

Intended as an individual medicine for either Section 22- Medicines for Reproductive Health and Perinatal Care under the subsection for Other Medicines Administered to the Mother (22.5) or Section 27- Vitamins and Minerals.
Treatment details, public health relevance, and evidence appraisal and synthesis

7. Treatment details (requirements for diagnosis, treatment, and monitoring)

Two guidelines put forward by the WHO recommend MMS for pregnant and lactating women in emergency settings and for pregnant women with active tuberculosis (TB), where the risk of micronutrient deficiencies is especially high. In 2007, the WHO, UNICEF, and World Food Programme (WFP) issued a statement in *Preventing and controlling micronutrient deficiencies in populations affected by an emergency* recommending MMS for pregnant and lactating women. (1) The recommendation states that MMS should be given in addition to iron and folic acid supplements (IFA) in settings where IFA supplementation is available, even in the presence of fortified rations, and should continue for the duration of the emergency. In the case of active TB, the 2013 WHO guideline, *Micronutrient supplementation in individuals with active tuberculosis*, also recommend MMS for pregnant and lactating women. (2) In addition, MMS is recommended by UNICEF in its guidance on maternal nutrition during COVID-19 to support to ensure adequate micronutrient intake in populations with a high prevalence of micronutrient deficiencies or where food distribution is disrupted. (12)

The 2020 update to the *WHO antenatal care recommendations for a positive pregnancy experience* recommends MMS in the context of rigorous research. (4) Specifically, the guidelines encourage research to better estimate gestational age in order to more accurately assess the effect of MMS on pregnancy outcomes, including SGA. They also recommend implementation research where MMS is being considered to assess the acceptability, feasibility, sustainability, equity and cost-effectiveness of MMS compared with IFA alone.

MMS can be used to prevent micronutrient deficiencies, which are common in LMICs, and to help meet the increased micronutrient requirements during pregnancy. The iron, folic acid, and vitamin A contained in the supplement can help prevent maternal anaemia. MMS can also reduce the risks of LBW, preterm birth, SGA and female infant mortality, as discussed in more detail in section 9. The infants of women who are underweight or anaemic also have even greater benefits with MMS.

MMS should begin as early in pregnancy as possible, as folic acid is especially beneficial in reducing the risk of neural tube defects that can occur in the first week of pregnancy. While it is generally not feasible to begin supplementation at conception, an individual patient data (IPD) meta-analysis of randomized clinical trials compared trials that started MMS before 20 weeks’ gestation with those beginning after 20 weeks and found that both conferred benefits on pregnancy outcomes. Specifically, trials that began MMS after 20 weeks of gestation had a greater reduction in the risk of small for gestational age compared to initiating supplementation before 20 weeks (RR 0.91, 95% CI 0.86-0.96; *p*-value heterogeneity 0.004). On the other hand, starting supplementation before 20 weeks had a greater reductions in the risk of preterm birth (RR 0.89, 95% CI 0.85-0.93; *p*-value for heterogeneity 0.03). (6) Regardless of when supplementation began (before or after 20 weeks), MMS conferred a similar reduction on LBW.

It is advised that MMS should be taken throughout pregnancy with at least 180 tablets and one tablet per day. (13) This should be provided in conjunction with routine antenatal care and with counselling on eating a healthy and balanced diet. (14) To improve adherence to supplementation, women should be
provided with information on the importance of MMS during pregnancy, how to take the tablet, and how to reduce the risk of side effects (e.g. take the tablet with a meal to avoid an upset stomach or just before going to sleep).

8. Information supporting the public health relevance

The role of micronutrients in pregnancy
Combining multiple micronutrients into a single supplement provides several benefits for pregnant women, as several micronutrient deficiencies are addressed at the same time. Since there are some synergistic interactions between micronutrients (e.g. iron absorption is increased in the presence of vitamins A and C), which may result in a stronger effect from multiple supplementation as compared to single micronutrient supplementation.(5) While multiple micronutrient supplementation is recommended, it is useful to highlight evidence around the individual micronutrients included in antenatal MMS, their role during pregnancy, and the consequences of deficiency.

Vitamin A is necessary for embryonic growth and development, visual health, and immune function.(15) During pregnancy, it contributes to cellular differentiation (related to embryonic development and immunity, induced by retinoic acid) and has a positive effect on iron metabolism and haemoglobin production by enhancing non-haem iron absorption. Vitamin A deficiency during pregnancy is associated with increased maternal mortality and increased infant mortality rates within the first year. Vitamin A deficiency in pregnancy is also associated with an increased risk of preterm delivery, maternal anaemia, preeclampsia, and slower infant growth and development.(16,17)

Vitamin C contributes to antioxidant function as a reducing agent by protecting against free-radical-induced oxidative damage and the regeneration of other antioxidants within the body (e.g. vitamin E).(18) It also supports the synthesis of L-carnitine, some neurotransmitters, and collagen (a protein that gives structure to bones, cartilage, muscle, and blood vessels). During pregnancy, it also supports immune function by stimulating the production and function of leukocytes) and increases the bioavailability of non-haem iron by enhancing its intestinal absorption. Finally, vitamin C provides a positive effect on iron metabolism and haemoglobin production. Deficiencies of vitamin C during pregnancy has been linked to scurvy and impaired synthesis of collagen.

Vitamin D supports the absorption of calcium, which is essential for foetal bone development and growth.(15) Since calcium demands increase in the third trimester, vitamin D levels become more important for maternal health, foetal skeletal growth, and optimal maternal and foetal outcomes. Vitamin D deficiency is common in pregnant women (18-84% depending on the country of residence) and can result in preeclampsia, LBW, neonatal hypocalcaemia, poor postnatal growth, and bone fragility.(19,20) Vitamin D deficiency has also been linked to increased risks of autoimmune diseases.(21)

Folic acid, alongside iron supplementation, prevents anaemia in pregnancy. Folate plays an important role in foetal brain and spinal cord development as well as the heart and circulatory system. Early in pregnancy, folic acid supplementation can reduce the risk of neural-tube defects like spina bifida by up to 70%. (22) A 2015 Cochrane review found that supplementation of iron and folic acid supplementation was associated with a reduced risk of anaemia and iron deficiency for pregnant women.(23)
Vitamin B1, also known as thiamine, is essential for key reactions in energy metabolism (growth, development, and function of cells). In its active form, thiamine diphosphate, is an essential cofactor for several enzymes involved in glucose, amino acid, and lipid metabolism. During pregnancy, vitamin B1 contributes to foetal growth and development and the production of adenosine triphosphate (ATP) from glucose in the brain. Deficiencies of vitamin B1 during pregnancy have been associated with widespread metabolic disturbances affecting both the foetus and the placenta.(24)

Vitamin B2, also known as riboflavin, is required for foetal bone development and growth, muscles, and nerves—and also reduces the risk of preeclampsia, cardiac outflow tract defects, and preterm growth.(17) No additional roles in pregnancy have been identified.

Vitamin B3, also known as niacin, has an important role in prenatal health; the body requires B3 to produce the metabolic cofactor nicotinamide adenine dinucleotide (NAD), which is an essential part of cell development. Disruptions to NAD production interfere with cellular function and can lead to birth defects. Deficiencies of vitamin B3 during pregnancy have been linked to pellagra, which clinically presents as dermatitis, diarrhoea, and dementia.

Vitamin B6 is required for a number of metabolic processes during pregnancy, including nervous system development and functioning.(25) In non-randomized studies, it has been associated with some benefits during pregnancy, including higher Apgar scores, higher birthweights, and reduced incidence of preeclampsia and pre-term birth.(25) A deficiency of vitamin B6 during pregnancy can negatively influence foetal nervous system development.(26)

Vitamin B12 deficiencies in early pregnancy can increase the risk of foetal neural tube defects and may lead to preterm delivery or LBW, ultimately increasing the risk of developmental and long-term adverse health outcomes.(27) When combined with folic acid, B12 supplementation can reduce the risk of spina bifida and other central nervous system defects.(27) Deficiencies of vitamin B12 during pregnancy can result in slowed deoxyribonucleic acid (DNA) synthesis and its consequences, such as neural tube defects, and elevated homocysteine and megaloblastic anaemia.

Zinc supplementation during pregnancy has been shown to have a modest impact on reducing preterm births and may also affect infant growth.(28) Studies of the impact of zinc supplementation among women with low incomes found a 14% relative reduction in preterm birth for zinc compared to a placebo, which has relevance for MMS supplementation in areas with high perinatal mortality.(28)

Selenium supplementation has been associated with a reduction in postpartum thyroiditis.(29) Copper is an essential micronutrient for the proper growth and development of bone, connective tissue, brain, heart, and organ tissue.

Iodine requirements significantly increase during pregnancy, and supplementation is often necessary to ensure proper regulation of thyroid hormones for both the mother and the baby and to regulate the development of the brain and nervous system. Studies have demonstrated that iodine deficiencies during pregnancy can result in infants with stunted growth and is harmful to normal mental development.(15,30)
Prevalence of micronutrient deficiencies in pregnant women

Sufficient intakes of micronutrients are required during pregnancy to support maternal health and normal foetal development. (18) Globally, many pregnant women do not meet these requirements, which has negative consequences for their own health as well as for the health, growth, and development of their infants. Insufficient nutrient intakes before and during pregnancy, combined with increased metabolic demands during pregnancy result in severe nutritional deficiencies, particularly in low- and middle-income countries (LMICs) where many women enter pregnancy already malnourished.

Maternal anaemia is the most common micronutrient deficiency, affecting 40% of pregnant women globally. (31) Southeast Asia (49%), Africa (46%), and the Eastern Mediterranean (41%) have the highest prevalence followed by Western Pacific (33%), the Americas (26%), and Europe (27%). (31) While anaemia is not always due to iron deficiency, a 2013 analysis suggests that 19.2% of pregnant women in low- and middle-income countries had iron deficiency anaemia. (32)

A literature review of micronutrient deficiencies found that vitamin D, iodine, and zinc deficiencies were widespread in WRA. On average in LMICs, 63.2% of WRA were vitamin D deficient, 41.4% were zinc deficient, 31.2% were anemic, and 22.7% were folate deficient, and 15.9% were vitamin A deficient, using WHO cutoff criteria for each indicator. More specific regional estimates of micronutrient deficiencies are shown in Figure 1.

Figure 1. Regional estimates of micronutrient deficiencies and anaemia as reported in women of reproductive age. Black circles are not representative (<3 countries). Data calculated from 52 national and regional surveys, published between 2013 and July 2017. Missing bars means no data were found for that micronutrient in the specific region. (33)

Prevalence of adverse pregnancy outcomes

Adverse pregnancy outcomes, including LBW, SGA, preterm birth, and peri-natal mortality, are relatively common in LMICs, and are associated with micronutrient deficiencies. Table 2 outlines the available
data on the prevalence of adverse pregnancy outcomes. Overall, 14.6% of all live births in LMICs in 2015 were LBW (<2,500 g) with South Asia carrying the largest burden (26.4%).(34) SGA, preterm, and both preterm and SGA infants have an increased mortality risk.(35) On the basis of secondary analyses of data from the 2012 Child Health Epidemiology Reference Group in LMICs, an estimated 23.3 million infants, or 19.3% of all live births, were SGA; and an estimated 606,500 neonatal deaths (21.9% of all neonatal deaths) were attributable to SGA. SGA was defined as infants weighing <10th percentile of birth weight-for-gestational age and sex according to the multiethnic, INTERGROWTH-21st birth weight standard.(36) South Asia also had the highest prevalence of SGA (34% of all live births).

A 2018 review—assessing data from National Registries, Reproductive Health Surveys and published studies—estimated that 14.84 million babies, or 10.6% of live births, were born preterm worldwide in 2014. More than 80% of preterm babies were born in Asia (7.8 million, or 10.4% of live births) and sub-Saharan Africa (4.2 million, or 12.0% of live births).(37) Global estimates for stillbirths and neonatal mortality are 18.4 per 1000 total births and 18.6 per 1000 live births, respectively, with regionally higher prevalence in sub-Saharan Africa and South Asia.(38,39)

**Table 2. Prevalence of adverse birth outcomes** (13)

<table>
<thead>
<tr>
<th></th>
<th>Sub-Saharan Africa</th>
<th>Asia</th>
<th>South East Asia</th>
<th>South Asia</th>
<th>World</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm births (2014) (%)</td>
<td>12.0</td>
<td>10.4</td>
<td>N/R</td>
<td>N/R</td>
<td>10.6</td>
</tr>
<tr>
<td>SGA (2012) (%)</td>
<td>16.5</td>
<td>10.4</td>
<td>21.6</td>
<td>34.2</td>
<td>19.3</td>
</tr>
<tr>
<td>LBW (2015) (%)</td>
<td>13.7*</td>
<td>17.3</td>
<td>12.3</td>
<td>26.4</td>
<td>14.6</td>
</tr>
<tr>
<td>Stillbirths (per 1000 total births)</td>
<td>28.7</td>
<td>N/R</td>
<td>12.2</td>
<td>25.5</td>
<td>18.4</td>
</tr>
<tr>
<td>Neonatal mortality (per 1000 live births)</td>
<td>27.7</td>
<td>N/R</td>
<td>13.5</td>
<td>27.6</td>
<td>18.6</td>
</tr>
</tbody>
</table>

*a Estimated mean preterm birth rate defined as all live births before 37 weeks of completed weeks of gestation, whether singleton, twin, or higher order multiples, divided by all live births in the population(37)

*b SGA; Small for Gestational Age, defined as birth weight less than 10th centile for a specific completed gestational age by sex, using the Intergrowth standard, SGA rate defined as all term and preterm SGA, divided by all livebirths in the population(36)

*c Low Birthweight defined as the number of live births weighing less than 2500 g divided by all live births in the population(34)

*d Stillbirth rates estimates are based on the late fetal death definition: 1000 g or more with an assumed equivalent of 28 weeks’ gestation or more or 35 cm or more. 2015 estimates per 1000 total births(40)

*e Neonatal mortality defined as deaths in the first 28 days of life per 1000 live births. 2016 estimates(39)

*A of Africa

N/R, not reported

**Current public health interventions and current use**

MMS is commonly used by pregnant women in high-income countries (with access to a high-quality diet) to meet the increased micronutrient demands in pregnancy and is widely available globally. Several countries have either recently adapted or are currently updating their national policies to provide MMS for pregnant women. The policies are primarily aimed at reducing micronutrient deficiencies among pregnant women as well as reducing the risk of adverse pregnancy outcomes.

**9. Review of benefits: summary of evidence of comparative effectiveness**

To date, 21 clinical trials have compared the use of MMS with IFA in pregnant women in LMICs, and ten of these trials have used the UNIMMAP formulation. Two meta-analyses have been carried out to
compare MMS versus IFA from trial data, including a 2019 Cochrane Review that included 19 trials and a 2017 individual patient data (IPD) meta-analysis of 17 trials.\(^{(5,6)}\) Fifteen of these trials were included in both the Cochrane Review and the IPD meta-analysis. Appendix B provides information on each trial, including its location and number of participants.

The analyses in the Cochrane Review demonstrated that overall MMS resulted in a 12% reduction in LBW (RR: 0.88; 95% CI: 0.85–0.91) and a 8% reduction in SGA births (RR: 0.92; 95% CI: 0.88–0.97), compared with IFA supplementation, with high and moderate quality evidence (based on GRADE criteria)\(^{(41)}\), respectively.\(^{(5)}\) There were no significant differences identified for other maternal or pregnancy outcomes assessed, including preterm birth, stillbirth, maternal anaemia in the third trimester, miscarriage, maternal mortality, perinatal mortality, neonatal mortality, or risk of delivery by caesarean section when MMS was compared with supplementation with iron or without folic acid. A summary of these analyses is presented in Table 3, alongside the results from the IPD meta-analysis for comparison, which is discussed in more detail below.\(^{(5,6)}\) However, it should be noted that the IPD was based on voluntary participation of the trial’s investigators and was primarily aimed at conducting subgroup analyses, while the Cochrane Review was focused on the overall effects of all available trials.

**Table 3. Overall effects of MMS on pregnancy outcomes in comparison with iron, with or without folic acid in low- and middle-income countries based on the Cochrane Review and an IPD meta-analysis (RR, 95% CI)**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Cochrane Review (19 RCTs) Relative Risks</th>
<th>IPD Meta-analysis (17 RCTs) Relative Risks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Random effects</td>
<td>Random effects</td>
</tr>
<tr>
<td>SGA (&lt;10(^{th}) percentile)</td>
<td>0.92 (0.88-0.97)(^{a})</td>
<td>0.94 (0.90-0.98)(^{b})</td>
</tr>
<tr>
<td>LBW (&lt;2500g)</td>
<td>0.88 (0.85-0.91)</td>
<td>0.86 (0.81-0.92)</td>
</tr>
<tr>
<td>VLBW (&lt;2000g)</td>
<td>Not Reported</td>
<td>Not Reported</td>
</tr>
<tr>
<td>Preterm Birth (&lt;37 weeks)</td>
<td>0.96 (0.90-1.01)</td>
<td>0.93 (0.97-0.98)</td>
</tr>
<tr>
<td>Very Preterm Birth (&lt;34 weeks)</td>
<td>Not Reported</td>
<td>Not Reported</td>
</tr>
<tr>
<td>LGA (&gt;90(^{th}) percentile Oken)</td>
<td>Not Reported</td>
<td>1.04 (0.92-1.18)</td>
</tr>
<tr>
<td>LGA (&gt;90(^{th}) percentile INTERGROWTH)</td>
<td>Not Reported</td>
<td>Not Reported</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>0.95 (0.86-1.04)</td>
<td>0.97 (0.85-1.11)</td>
</tr>
<tr>
<td>Neonatal Mortality (≤28 days)</td>
<td>1.00 (0.89-1.12)</td>
<td>0.99 (0.89-1.09)</td>
</tr>
<tr>
<td>Infant Mortality (≤365 days)</td>
<td>Not Reported</td>
<td>0.97 (0.88-1.06)</td>
</tr>
</tbody>
</table>

\(^{a}\)SGA defined by authors of trials.

\(^{b}\)SGA defined by the INTERGROWTH-21 standard.

**RCTs,** randomized controlled trials; **SGA,** small for gestational age; **LBW,** low birthweight; **VLBW,** very low birthweight; **LGA,** large for gestational age.
To evaluate possible effect modifications, the Cochrane Review performed several population-level subgroup analyses, including analyses that stratify, on the basis of the study-specific averages of maternal body mass index (BMI), maternal height, timing of supplementation, and the iron dose. Among the 10 trials in which the average maternal BMI was at least 20 kg/m$^2$, there was evidence of a lower incidence of SGA among those who received MMS compared with IFA, while there was no evidence of a difference among the three trials where the mean BMI was less than 20 kg/m$^2$ ($P$ value for subgroup differences = 0.001). Similarly, among the six trials in which the average maternal height was at least 154.9 cm, MMS was associated with a reduction in SGA, while no effect was apparent in the eight trials in which the average maternal height was less than 154.9 cm ($P$ value for subgroup differences <0.0001). Thus, while the review suggests that MMS reduces the risk of SGA, this effect was only observed in populations with better nutritional status, as defined by a height of at least 154.9 cm or a BMI of at least 20 kg/m$^2$. However, among trials in which the average maternal BMI was less than 20 kg/m$^2$, those receiving MMS had a lower rate of preterm birth, whereas no difference for preterm birth was observed among trials in which the average BMI was greater than or equal to 20 kg/m$^2$ ($P$ value for subgroup differences <0.0001). The authors found no significant subgroup differences by the dose of iron with regard to preterm birth, SGA, or perinatal mortality, on the basis of the 15 studies included in this subgroup analysis.(42)

The IPD meta-analysis found that MMS reduces the risk of stillbirth (on the basis of fixed effects analysis), very LBW (VLBW), LBW, early preterm birth, preterm birth, and SGA (by INTERGROWTH-21 standards and Oken reference) when compared with IFA. These are additional benefits to those reported in the Cochrane Review, i.e., a reduction of LBW and SGA associated with MMS. Table 3 contains a summary of these results in comparison to the results from the Cochrane Review. In addition, Smith et al. also found an increased risk of being born large for gestational age (LGA) (by Intergrowth standards but not by Oken reference) associated with the use of MMS, which was not examined in the Cochrane Review. While this may raise concerns about increased risk of obstructed labour/asphyxiation, the authors noted that MMS was not associated with increased risk of stillbirth or mortality at any time point, including among women with short stature (less than 150 cm), who are more likely to be at risk of obstructed labour.(6)

Twenty-six subgroup analyses were conducted with numerous outcomes in the IPD meta-analysis to identify individual characteristics that may modify the effect of MMS as compared with IFA alone. Importantly, these subgroup analyses were done based on individual patient data, rather than the trial population means used in the Cochrane Review. These analyses revealed the evidence of larger benefits of MMS among women who were anaemic (defined as haemoglobin <110 g/L for pregnant women), were underweight (BMI <18.5 kg/m$^2$), started supplementation before 20 weeks of gestation, had higher supplement adherence (>95%), were carrying a female foetus, and had a skilled birth attendant. Specifically, the subgroup analyses found:

- The beneficial effects of MMS compared with IFA were greater among anaemic pregnant women than non-anaemic pregnant women for:
  - LBW (19% reduction versus 9%, respectively; $P$ value for heterogeneity 0.049),
  - SGA (8% reduction versus 1% reduction, respectively; $P$ value for heterogeneity 0.03), and
  - 6-month infant mortality (29% reduction versus 7% reduction, respectively; $P$ value for heterogeneity 0.04).
• The effects of MMS compared with IFA were greater among underweight pregnant women than non-underweight women in reducing the risk of preterm birth (16% reduction versus 6% reduction, respectively; P value for heterogeneity 0.01).

• The effects of maternal MMS compared with IFA were greater among female infants than male infants, for:
  - neonatal mortality (15% reduction versus 6% increase, respectively; P value for heterogeneity 0.007),
  - 6-month mortality (15% reduction versus 2% reduction, respectively; P value 0.06), and
  - infant mortality (13% reduction versus 5% increase, respectively; P value for heterogeneity 0.04).

• The beneficial effects of MMS compared with IFA were greater among women who started supplementation before 20 weeks of gestation than those who started after 20 weeks in reducing the risk of preterm birth (11% reduction versus no change after 20 weeks, respectively; P value for heterogeneity 0.03).

• The effects of MMS compared with IFA were greater among women who started supplementation after 20 weeks of gestation than those who started before 20 weeks in reducing the risk of stillbirth (19% reduction versus 3% reduction, respectively; p-value for heterogeneity 0.05).

• The effects of MMS compared with IFA appeared greater when adherence was greater than or equal to 95% versus less than 95% for:
  - neonatal mortality (12% reduction versus 5% increase, respectively; p-value for heterogeneity 0.05) and
  - infant mortality (15% reduction versus 6% increase, respectively; p-value for heterogeneity 0.02).

• MMS did not have any significant effect on risk of stillbirth, neonatal, 6-month, or infant mortality in any of the 26 subgroups analysed compared with IFA.
Box 1 presents a summary of beneficial effects of MMS (vs IFA) in the subgroup analysis carried out in the IPD meta-analysis.

**Box 1. Summary of Subgroup Analysis from the IPD meta-analysis**

**MMS reduces the risk of stillbirth**
- by 8% in the overall population of pregnant women
- by 21% in the group of anaemic pregnant women

**MMS reduces the risk of mortality among 6-month infants**
- by 29% in the group of anaemic pregnant women
- by 15% in female infants

**MMS reduces the risk of low birth weight (<2500g)**
- by 12% in the overall population of pregnant women
- by 19% in the group of anaemic pregnant women

**MMS reduces the risk of preterm (<37 weeks) birth**
- by 8% in the overall population of pregnant women
- by 16% in the group of underweight women

**MMS reduces the risk of being born small-for-gestational age**
- by 3% in the overall population of pregnant women
- by 8% in the group of anaemic pregnant women

10. Review of harms and toxicity: summary of evidence of safety

**Estimation of total patient exposure to date**
Currently, there are three main distributors of MMS: Kirk Humanitarian, UNICEF, and Vitamin Angels. Although there is limited coverage data the three organizations have distributed over eight million regimes of MMS over the past three years across at least 80 countries (Table 4).

**Table 4. Women receiving at least 100 doses of MMS during 2017, 2018, and 2019**

<table>
<thead>
<tr>
<th>Organization</th>
<th>2017</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirk Humanitarian*</td>
<td>1,160,107</td>
<td>597,435</td>
<td>2,104,952</td>
</tr>
<tr>
<td>UNICEF**</td>
<td>2,090,000</td>
<td>2,090,000</td>
<td>2,090,000</td>
</tr>
<tr>
<td>Vitamin Angels</td>
<td>447,649</td>
<td>2,024,357</td>
<td>2,150,782</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,697,756</td>
<td>4,711,792</td>
<td>6,345,734</td>
</tr>
</tbody>
</table>

* Kirk Humanitarian does not distribute product directly, rather they donate production to partner organizations and governments. See Appendix D for a list of countries. This data represents Kirk Humanitarian donated product to organizations other than Vitamin Angels, to avoid double counting.

**UNICEF’s average annual distribution between 2017-2019. Distribution includes 100 count bottles and 1000 count bottles.**

**Description of the adverse effects/reactions and estimates of their frequency**
The 2019 Cochrane Review found no harms of MMS for mortality outcomes (stillbirths, perinatal, and neonatal mortality). (5) This conclusion was further supported by two trials that were statistically powered to analyse the effect on early infant mortality. A large trial in Bangladesh did not find an
increase in neonatal or early infant mortality risk in the MMS group versus the control (iron and folic acid). (43) In a post-hoc analysis, however, the trial investigators reported a higher neonatal mortality among male infants due to birth asphyxia but the Cochrane Review concluded that these findings should be interpreted with caution due to potential misclassification. (5)

A recent analysis examined the risk of exceeding the upper intake level (UL), as set by the National Academy of Medicine, of any micronutrient in the UNIMMAP formulation if it is consumed with a nutritionally adequate diet. In this case, most of the micronutrient intakes remain well below the UL and only iron, folic acid, and niacin would meet or slightly exceed the UL. (44)

For niacin, the UL is based on the side effect of flushing and only occurs with the synthetic form nicotinic acid, which is not used in dietary supplements. UNIMMAP contains 18 mg of nicotinamide (not nicotinic acid) so this does not contribute to the UL. If it were present in the form of nicotinic acid, it would still be well below the 35 mg/day UL. (44)

For folic acid, there are no known side effects for reaching the UL. Rather, the UL is set based on the risk of masking the diagnosis of pernicious anaemia, which can occur with high folate intake in the presence of vitamin B12 deficiency. However, MMS contains vitamin B12, which mitigates this risk. (44)

The National Academy of Medicine’s UL for iron is 45mg/day and is based on gastrointestinal side effects, which are most commonly reported when a supplement is consumed on an empty stomach and would be a concern for both MMS and IFA. WHO recommends pregnant women receive between 30-60 mg of iron per day, which is met by the UNIMMAP formulation. Importantly, the ULs are set for the healthy population and do not apply to the treatment of iron deficiency anaemia in which case the daily iron intake may need to exceed the UL. (44)

Summary of the available data
The GRADE summary of the findings table from the 2019 Cochrane Review can be found in Appendix C.

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

The current listed price of the MMS supplements provided by the UNICEF Supply Catalogue website is 1.75 USD per 100 supplements (MMS: Product No. S1580101). (45) To provide 6-month coverage per individual, the unit cost adjusted to 180 supplements is 3.15 USD (Table 5). Depending on the packaging and other variables, price can vary dramatically, but based on information from global manufactures and UNICEF, the median price for a 180-count bottle is 2.50 USD for a purchase at scale. All prices include packaging (Table 5).

Table 5. Cost of MMS according to manufacturers & UNICEF

<table>
<thead>
<tr>
<th>Unit</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 count</td>
<td>2.20 USD</td>
<td>3.15 USD</td>
<td>2.50 USD</td>
</tr>
</tbody>
</table>

The composition of this product includes 15 micronutrients as presented in Table 1. (4)
The cost of the supplements is important to understand alongside the program implementation costs, including national-level administration, training, nutrition education programs, and supervision. The calculation methodology to determine the program costs have been detailed in Johns et al; the estimated programmatic roll-out cost is 0.42 USD per patient (USD 2016).(46)

Several recent studies have demonstrated the cost-effectiveness of MMS compared to IFA. A 2019 analysis modelled the cost-effectiveness of the two interventions in three South Asian countries (Pakistan, India, and Bangladesh) using eight health outcomes reported in the two meta-analyses described above (Cochrane Review and the IPD meta-analysis).(7) The researchers found that MMS would avert 4,391, 5,769, and 8,578 more disability-adjusted life years (DALYs) than IFA per 100,000 pregnancies in Pakistan, India, and Bangladesh, respectively (62.6%, 76.8%, and 82.6% certainty). The incremental cost-effectiveness ratio (ICER) of transitioning IFA to MMS was 41.54, 31.62, and 21.26 USD (USD 2016) per DALY averted, respectively. Concluding that MMS was cost-effective and resulted in positive health outcomes for both infants and pregnant women, this research was supportive of a transition from IFA to MMS in Pakistan, India, and Bangladesh. This modelling was subsequently done for 29 additional countries, which found MMS was highly cost-effective in all modelled scenarios.(47)

Another 2019 modelling analysis evaluated the cost-effectiveness of transitioning from IFA to MMS; this study focused on Bangladesh and Burkina Faso.(48) Transitioning to multiple micronutrient supplementation could avert more than 15,000 deaths and 30,000 cases of preterm birth annually in Bangladesh (as compared to iron and folic acid supplementation) and more than 5,000 deaths and 5,000 cases of preterm births in Burkina Faso. The cost per death averted was 175-185 USD in Bangladesh and 112-125 USD in Burkina Faso. Finally, the cost per DALY averted was 3-15 USD, influenced by the country and subgroup effects.
Regulatory information

12. Summary of regulatory status and market availability of the medicine

Antenatal micronutrient supplements, with similar formulations to UNIMMAP, providing roughly 1 recommended dietary allowance (RDA) per day of approximately 15 micronutrients, are widely available in pharmacies and other marketplaces globally. A market assessment—conducted by Sight and Life—of 32 LMICs found that every country had either locally manufactured or imported maternal MMS products containing at least ten micronutrients. (49,50) While none of the MMS products matched the UNIMMAP formulation, the wide scale availability of multiple micronutrient formulations indicates that there is global manufacturing capacity that could meet the global need for a UNIMMAP-MMS product. If manufacturers complied with the requirements of the *Expert Consensus UNIMMAP-MMS Product Specification* (See Appendix F), the product quality would be similar to that of a drug product.

As with most nutrition supplements, there is no global consensus on the regulatory status of the MMS product for pregnant women. At individual country-level it can be considered either as a dietary supplement and regulated as a food, or as a therapeutic product that is regulated as a drug. In some countries, including the US, India, Japan, and the European Union, MMS is regulated as a dietary supplement, while in other countries, such as Australia, Bangladesh, New Zealand, and Mexico, MMS is regulated as a drug. The regulatory classification of MMS can have implications on how the product is manufactured, imported, packaged, distributed, and/or promoted. To help establish a product that conforms to internationally recognized Good Manufacturing Practice (GMP) requirements and pharmacopeial standards, the *Expert Consensus UNIMMAP-MMS Product Specification* was created by an expert panel hosted jointly by the New York Academy of Sciences and the Micronutrient Forum. Manufacturers of supplements must be registered entities and certified to adhere to GMP requirements. For GMP, these include the requirements of United States Food and Drug Administration, Health Canada, the Australian Therapeutics Goods Administration or the WHO for the manufacture of nutritional products. For Pharmacopeias, these include quality standard for nutritional supplements as set by the United States Pharmacopeia, the European Pharmacopeia, British Pharmacopeia, Japanese Pharmacopeia or the International Pharmacopeia.

Currently, there are four companies manufacturing a UNIMMAP formulated product for global distribution, Contract Pharmacal Corporation, DSM Nutritional Products, Lekapharm, and Lomapharm. Additionally, there are companies manufacturing an MMS product for local distribution, such as Beximco and Renata in Bangladesh, and the Renata product will be conformant with the *Expert Consensus UNIMMAP – MMS Product Specification*. Nevertheless, manufacturers producing an MMS product that conforms to internationally recognized GMP requirements and pharmacopeial standards should be able to produce a basic, affordable, and efficacious UNIMMAP-formulated nutritional supplement.

The United States Pharmacopeial Convention (USP) has approved an official USP product monograph for "Oil- and Water-Soluble Vitamins with Minerals Tablets" which includes UNIMMAP-MMS and is a public standard containing tests, test procedures, and acceptance criteria for the identity, strength, and purity (with limits on contaminants) for a nutritional supplement (Appendix E). The use of USP standards in conjunction with Current Good Manufacturing Practices (CGMPs) helps to ensure the quality and consistency of nutritional products. The UNIMMAP-MMS Product Specification provides information on the manufacturing of UNIMMAP formulation and includes the requirement that manufacturers follow United States Food and Drug Administration’s Current Good Manufacturing Practice in Manufacturing, Packing, Labelling, or Holding Operations for Dietary Supplements (21 CFR Part 111) and the USP–NF general chapter <2750> Manufacturing Practices for Dietary Supplements.
References


### Appendix A. Countries with MMS included in their national Essential Medicines List

<table>
<thead>
<tr>
<th>Afghanistan</th>
<th>El Salvador</th>
<th>Mozambique</th>
</tr>
</thead>
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<td>Angola</td>
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<td>Niue</td>
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<td>Botswana</td>
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<td>Papua New Guinea</td>
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<td>Guyana</td>
<td>Paraguay</td>
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<td>Cameroon</td>
<td>India</td>
<td>Saint Lucia</td>
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<tr>
<td>Central African Republic</td>
<td>Iran (Islamic Republic of)</td>
<td>Senegal</td>
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<td>Chile</td>
<td>Iraq</td>
<td>Solomon Islands</td>
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<td>Jamaica</td>
<td>Thailand</td>
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<td>Jordan</td>
<td>Timor-Leste</td>
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<td>Kenya</td>
<td>Togo</td>
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<td>Kiribati</td>
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<td>Cuba</td>
<td>Lebanon</td>
<td>Tunisia</td>
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<td>Democratic People’s Republic of Korea</td>
<td>Liberia</td>
<td>Tuvalu</td>
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<td>Democratic Republic of Congo</td>
<td>Madagascar</td>
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### Appendix B. Clinical trial information

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<tr>
<th>Study, as identified in Cochrane review</th>
<th>Location</th>
<th>Years of Study</th>
<th>Participants (n)</th>
<th>In IPD meta-analysis?</th>
<th>In 2019 Cochrane Review?</th>
<th>UNIMMAP formulation?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashorn, 2015</td>
<td>Mangochi District, Malawi</td>
<td>2011-2015</td>
<td>929</td>
<td>Yes</td>
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<td>Bhutta, 2009a</td>
<td>Bilal colony, Karachi, Kot Diji, Sindh, Pakistan</td>
<td>2002-2004</td>
<td>2378</td>
<td>Yes</td>
<td>Yes</td>
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<td>Biggs, 2010 (Hanieh 2013)</td>
<td>Ha Nam, Vietnam</td>
<td>2010-2012</td>
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<td>Christian, 2003</td>
<td>Sarlahi, Nepal</td>
<td>1998-2001</td>
<td>4926</td>
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<td>Fawzi, 2007</td>
<td>Dar es Salaam, Tanzania</td>
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<td>Yes</td>
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<td>Friis, 2004</td>
<td>Harare, Zimbabwe</td>
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<td>Kaestel, 2005</td>
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<td>2100</td>
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<td>Liu, 2013</td>
<td>Hebei Province, China</td>
<td>2006-2009</td>
<td>18775</td>
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<td>Moore, 2009 (Johnson, 2017)</td>
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<td>2009-2013</td>
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<td>Osrin, 2005</td>
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<td>1200</td>
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<td>Ramakrishnan, 2003</td>
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<td>Hounde health district, Burkina Faso</td>
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<td>SUMMIT, 2008 (Shankar, 2008)</td>
<td>Lombok island, Indonesia</td>
<td>2001-2004</td>
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<td>Sunawang, 2009</td>
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<td>2000-2003</td>
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<td>Tofail 2008 (Persson, 2012)</td>
<td>Matlab, Bangladesh</td>
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<td>West, 2014</td>
<td>Gaibandha and Rangpur, Bangladesh</td>
<td>2007-2012</td>
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<td>Zagre, 2007</td>
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<td>2002-2006</td>
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### Appendix C. GRADE assessment from 2019 Cochrane Review

#### SUMMARY OF FINDINGS FOR THE MAIN COMPARISON

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Anticipated absolute effects* (95% CI)</th>
<th>Relative effect (95% CI)</th>
<th>∼ of participants (trials)</th>
<th>Quality of the evidence (GRADE)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm births</td>
<td>Study population</td>
<td>RR 0.95 (0.80 to 1.01)</td>
<td>91,425 (18 RCTs)</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>197 per 1000</td>
<td>186 per 1000 (179 to 199)</td>
<td></td>
<td></td>
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<tr>
<td>Small-for-gestational age</td>
<td>Study population</td>
<td>RR 0.92 (0.88 to 0.97)</td>
<td>57,348 (17 RCTs)</td>
<td>Moderate</td>
<td></td>
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<tr>
<td></td>
<td>337 per 1000</td>
<td>310 per 1000 (299 to 327)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birthweight</td>
<td>Study population</td>
<td>RR 0.88 (0.85 to 0.91)</td>
<td>68,801 (18 RCTs)</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td></td>
<td>212 per 1000</td>
<td>187 per 1000 (181 to 193)</td>
<td></td>
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<tr>
<td>Perinatal mortality</td>
<td>Study population</td>
<td>RR 1.00 (0.90 to 1.11)</td>
<td>63,922 (15 RCTs)</td>
<td>High</td>
<td>Raw event and participant data were unavailable for Ramakrishnan 2003 and West 2014, so have not been included in No of participants column.</td>
</tr>
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<tr>
<td></td>
<td>39 per 1000</td>
<td>39 per 1000 (35 to 43)</td>
<td></td>
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<tr>
<td>Stillbirths</td>
<td>Study population</td>
<td>RR 0.95 (0.86 to 1.04)</td>
<td>97,927 (17 RCTs)</td>
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<td>28 per 1000 (26 to 31)</td>
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<td>Neonatal mortality</td>
<td>Study population</td>
<td>RR 1.00 (0.89 to 1.12)</td>
<td>80,964 (14 RCTs)</td>
<td>High</td>
<td>Raw event and participant data were unavailable for Bhutta 2009a and Fawzi 2007 so have not been included in No of participants column.</td>
</tr>
<tr>
<td></td>
<td>29 per 1000</td>
<td>29 per 1000 (26 to 33)</td>
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</tbody>
</table>

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

CI: confidence interval; RR: risk ratio

**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 the effect.

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*Strong evidence of funnel plot asymmetry, indicating possible publication bias.

Source: 2019 *Cochrane Review* (5)
Appendix D. Countries that have directly or through partners received MMS procured by Kirk Humanitarian

<table>
<thead>
<tr>
<th>Afghanistan</th>
<th>Guyana</th>
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<tr>
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<td>Guinea</td>
<td>Palestine</td>
<td>Zimbabwe</td>
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Appendix E. United States Pharmacopeial Convention Product Monograph

The United States Pharmacopeial Convention (USP) has approved an official USP product monograph for "Oil- and Water-Soluble Vitamins with Minerals Tablets" which includes UNIMMAP-MMS and is a public standard containing tests, test procedures, and acceptance criteria for the identity, strength, and purity (with limits on contaminants) for a nutritional supplement. The full product Monograph is available upon request courtesy of USP.

Appendix F. Expert Consensus UNIMMAP-MMS Product Specification

Abstract: The multiple micronutrient supplement (MMS) based on the United Nations International Multiple Micronutrient Antenatal Preparation (UNIMMAP) formula provides women and their offspring with a healthy start to life in an efficacious, safe, and cost-effective way. To date, however, no precise and transparent specifications exist to support the manufacturing and distribution of UNIMMAP–MMS globally. To palliate for this need, the MMS Technical Advisory Group at the New York Academy of Sciences and the Micronutrient Forum convened a technical consultation to develop an open access UNIMMAP–MMS Product Specification for the manufacturing of this product. The specifications offered in this paper cover: ingredients, excipients, and processing aids used in the manufacturing of UNIMMAP–MMS; stability studies recommended under different testing conditions and climatic zones; packaging considerations; manufacturing standards, including pharmacopeia standards, manufacturing practices, certificates of analysis, change control, and quality agreement; finished product specifications, including tablet characterization and purity, potency assay; analytical test methods; and storage and transportation requirements.

Full text can be found at www.nyas.org/mmsprodspec and is attached below in the pdf version of this application.
Oil- and Water-Soluble Vitamins with Minerals Tablets

DEFINITION

Change to read:

Oil- and Water-Soluble Vitamins with Minerals Tablets contain one or more of the following oil-soluble vitamins: Vitamin A as retinyl acetate or retinyl palmitate, vitamin D as Ergocalciferol (vitamin D₂) or Cholecalciferol (vitamin D₃), Vitamin E as RRR- or all-rac-alpha-tocopherol, RRR- or all-rac-alpha-tocopheryl acetate, or RRR- or all-rac-alpha-tocopheryl acid succinate, Phytomenadione (vitamin K₁), and Beta Carotene; and one or more of the following water-soluble vitamins: Ascorbic Acid or its equivalent as Calcium Ascorbate or Sodium Ascorbate, Biotin, Cyanocobalamin, Folic Acid, Niacin or Niacinamide, pantothenic acid (as Calcium Pantothenate or Racemic Calcium Pantothenate), Pyridoxine Hydrochloride, Riboflavin, and Thiamine Hydrochloride or Thiamine Mononitrate; and one or more minerals derived from substances generally recognized as safe, furnishing one or more of the following elements in ionizable form: boron, calcium, chromium, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, tin, vanadium, and zinc. Tablets contain NLT 90.0% and NMT 165.0% of the labeled amount of vitamin A as retinyl acetate (C₂₂H₂₄O₂), vitamin D as cholecalciferol (C₂₇H₂₈O) or ergocalciferol (C₂₇H₄₈O₂), vitamin E as 2R-alpha-tocopherol (USP 1-May-2020) (C₂₆H₄₀O₅), phytomenadione (vitamin K₁), and Beta Carotene; and one or more of the following water-soluble vitamins: Ascorbic Acid or its equivalent as Calcium Ascorbate or Sodium Ascorbate, Biotin, Cyanocobalamin, Folic Acid, Niacin or Niacinamide, pantothenic acid (as Calcium Pantothenate or Racemic Calcium Pantothenate), Pyridoxine Hydrochloride, Riboflavin, and Thiamine Hydrochloride or Thiamine Mononitrate; and one or more minerals derived from substances generally recognized as safe, furnishing one or more of the following elements in ionizable form: boron, calcium, chromium, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, tin, vanadium, and zinc. Tablets contain NLT 90.0% and NMT 150.0% of the labeled amount of vitamin A as retinyl acetate (C₂₂H₂₄O₂), vitamin D as cholecalciferol (C₂₇H₂₈O) or ergocalciferol (C₂₇H₄₈O₂), vitamin E as 2R-alpha-tocopherol (USP 1-May-2020) (C₂₆H₄₀O₅), phytomenadione (vitamin K₁), and Beta Carotene; and one or more of the following water-soluble vitamins: Ascorbic Acid or its equivalent as Calcium Ascorbate or Sodium Ascorbate, Biotin, Cyanocobalamin, Folic Acid, Niacin or Niacinamide, pantothenic acid (as Calcium Pantothenate or Racemic Calcium Pantothenate), Pyridoxine Hydrochloride, Riboflavin, and Thiamine Hydrochloride or Thiamine Mononitrate; and one or more minerals derived from substances generally recognized as safe, furnishing one or more of the following elements in ionizable form: boron, calcium, chromium, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, tin, vanadium, and zinc. Tablets contain NLT 90.0% and NMT 160.0% of the labeled amount of boron (B), chromium (Cr), fluoride (F), iodine (I), molybdenum (Mo), nickel (Ni), selenium (Se), tin (Sn), and vanadium (V). They may contain other added substances that are generally recognized as safe, in amounts that are unobjectionable.

IDENTIFICATION

Add the following:

• A. Characteristic absorption of each mineral as obtained in the tests in Strength (USP 1-May-2020)

Add the following:

• B. The retention times of the vitamin peaks of the Sample solutions correspond to those of the corresponding vitamin peaks of the Standard solutions as obtained in the tests for Strength (USP 1-May-2020)

STRENGTH

[Note—Where more than one assay method is given for an individual ingredient, the requirements may be met by following any one of the specified methods, with the method used being stated in the labeling only if Method 1 is not used.]

Change to read:

• VITAMIN A, Method 1
  • Proceed as directed in Vitamin A Assay (571), Assay, Chromatographic Methods, Procedure 1, except for the Sample stock solution and Sample solution (USP 1-May-2020)

Sample stock solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 5 Tablets, to a container having a polytetrafluoroethylene screw-cap. Add 10 mL of dimethyl sulfoxide and 15 mL of n-hexane, and shake for 45 min on a wrist-action shaker in a water bath maintained at 60°. [Note—Set up the wrist-action shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a 100-mL volumetric flask. Add 15 mL of n-hexane to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the 100-mL volumetric flask. Repeat this extraction with 3 additional 15-mL portions of n-hexane. Dilute the extracts in the volumetric flask with n-hexane to volume.

Sample solution: Dilute a 10-mL volume of the Sample stock solution with n-hexane to obtain a solution with a concentration of 15 µg/mL of vitamin A as retinol (C₂₂H₂₄O₂).

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin A as retinol (C₂₂H₂₄O₂)

Change to read:

• VITAMIN A, Method 2
  • Proceed as directed in Vitamin A Assay (571), Assay, Chromatographic Methods, Procedure 2 (USP 1-May-2020)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin A as retinol (C₂₂H₂₄O₂)

Change to read:

• VITAMIN A, Method 3
  • Proceed as directed in Vitamin A Assay (571), Assay, Chromatographic Methods, Procedure 3 (USP 1-May-2020)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin A as retinol (C₂₂H₂₄O₂)

Change to read:

• VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL), Method 1
  • Proceed as directed in Vitamin D Assay (581), Assay, Chromatographic Methods, Procedure 1, except for the Sample solution (USP 1-May-2020)

Sample solution: Prepare as directed for the Sample stock solution in Vitamin A, Method 1. Transfer NLT 20 mL of this solution to a suitable container, and if necessary, evaporate under vacuum at room temperature to obtain a concentration of 2 µg/mL of cholecalciferol or ergocalciferol.

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol (C₂₇H₃₄O) or ergocalciferol (C₂₇H₄₆O)
Change to read:

**VITAMIN D (CHOLECALCIFEROL or ERGOCALCIFEROL)**, Method 2

▲Proceed as directed in Vitamin D Assay (581), Assay, Chromatographic Methods, Procedure 2. ▲(USP 1-May-2020)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol (C_{27}H_{44}O) or ergocalciferol (C_{28}H_{44}O)

Change to read:

**VITAMIN D (CHOLECALCIFEROL or ERGOCALCIFEROL)**, Method 3

▲Proceed as directed in Vitamin D Assay (581), Assay, Chromatographic Methods, Procedure 3, except for the Analysis. ▲(USP 1-May-2020)

Analysis

Samples: Standard solution and Sample solution

Measure the peak areas for vitamin D.

Calculate the percentage of the labeled amount of cholecalciferol (C_{27}H_{44}O) or ergocalciferol (C_{28}H_{44}O) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{r_s}{r_u} \right) \times \left( \frac{C_s}{C_u} \right) \times 100
\]

\(r_s\) = peak area of cholecalciferol or ergocalciferol from the Standard solution

\(r_u\) = peak area of cholecalciferol or ergocalciferol from the Sample solution

\(C_s\) = concentration of USP Cholecalciferol RS or USP Ergocalciferol RS in the Standard solution (µg/mL)

\(C_u\) = nominal concentration of cholecalciferol or ergocalciferol in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol (C_{27}H_{44}O) or ergocalciferol (C_{28}H_{44}O)

Change to read:

**VITAMIN E, Method 1**

▲Proceed as directed in Vitamin E Assay (551), Assay, Procedure 1, except for the Sample solution and Analysis. ▲(USP 1-May-2020)

Sample solution: Prepare as directed for the Sample stock solution in Vitamin A, Method 1. Transfer NLT 20 mL of this solution to a suitable container, and evaporate under vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to volume to obtain a concentration of 2 mg/mL of alpha-tocopherol, alpha-tocopheryl acetate, or alpha-tocopheryl acid succinate.

▲(USP 1-May-2020)

Analysis

Samples: Standard solution and Sample solution

Measure the peak areas.

Calculate the percentage of the labeled amount of alpha-tocopherol (C_{29}H_{50}O), alpha-tocopheryl acetate (C_{31}H_{52}O_3), or alpha-tocopheryl acid succinate (C_{33}H_{54}O_3) ▲as 2R-alpha-tocopherol (C_{29}H_{50}O) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{r_s}{r_u} \right) \times \left( \frac{C_s}{C_u} \right) \times F_s \times 100
\]

\(r_s\) = peak area of the relevant vitamin E form from the Sample solution

\(r_u\) = peak area of the relevant vitamin E form from the Sample solution

\(C_s\) = concentration of all-rac-alpha-tocopherol from either USP Alpha Tocopherol RS or USP Alpha Tocopheryl Acid Succinate RS in the Standard solution (mg/mL)

\(C_u\) = nominal concentration of the corresponding form of vitamin E ▲as 2R-alpha-tocopherol ▲(USP 1-May-2020)

\(F_s\) = conversion factor for the content of all-rac-alpha-tocopherol to 2R-alpha-tocopherol, 1/2 (for products labeled to contain all-rac vitamin E sources) and 1 (for products labeled to contain RRR vitamin E sources) ▲(USP 1-May-2020)

Acceptance criteria: 90.0%–165.0% of the labeled amount of alpha-tocopherol (C_{29}H_{50}O), alpha-tocopheryl acetate (C_{31}H_{52}O_3), or alpha-tocopheryl acid succinate (C_{33}H_{54}O_3) ▲as 2R-alpha-tocopherol ▲(USP 1-May-2020)

Change to read:

**VITAMIN E, Method 2**

▲Proceed as directed in Vitamin E Assay (551), Assay, Procedure 2, except for the Analysis. ▲(USP 1-May-2020)

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of alpha-tocopherol (C_{29}H_{50}O), alpha-tocopheryl acetate (C_{31}H_{52}O_3), or alpha-tocopheryl acid succinate (C_{33}H_{54}O_3) as 2R-alpha-tocopherol (C_{29}H_{50}O) in the portion of Tablets taken as follows:

\[
\text{Result} = \left( \frac{r_s}{r_u} \right) \times \left( \frac{C_s}{C_u} \right) \times F \times 100
\]

\(r_s\) = peak area of the relevant vitamin E form from the Sample solution

\(r_u\) = peak area of the relevant vitamin E form from the Sample solution

\(C_s\) = concentration of all-rac-alpha-tocopherol from either USP Alpha Tocopherol RS or USP Alpha Tocopheryl Acetate RS or RRR-alpha-tocopherol from USP Alpha Tocopheryl Acid Succinate RS in the Standard solution (mg/mL)

\(C_u\) = nominal concentration of the corresponding form of vitamin E as 2R-alpha-tocopherol in the Sample solution (mg/mL)

\(F\) = conversion factor for the content of all-rac-alpha-tocopherol to 2R-alpha-tocopherol, 1/2 (for products labeled to contain all-rac vitamin E sources) and 1 (for products labeled to contain RRR vitamin E sources)

Acceptance criteria: 90.0%–165.0% of the labeled amount of alpha-tocopherol (C_{29}H_{50}O), alpha-tocopheryl acetate (C_{31}H_{52}O_3), or alpha-tocopheryl acid succinate (C_{33}H_{54}O_3) as 2R-alpha-tocopherol ▲(USP 1-May-2020)

Change to read:

**VITAMIN E, Method 3**

▲Proceed as directed in Vitamin E Assay (551), Assay, Procedure 3, except for the Analysis.

Analysis

Samples: Standard solution and Sample solution
Chromatographic system

System suitability

Standard stock solution: Methanol and water (19:1)

System suitability solution: 0.65 mg/mL of USP Alpha Tocopherol RS or USP Alpha Tocopherol Acetate RS or RRR-alpha-tocopherol from USP Alpha Tocopherol Acid Succinate RS in the Standard solution (mg/mL)

Sample stock solution: Prepared as directed for the Sample solution

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin E as 2R-alpha-tocopherol in the Sample solution (mg/mL)

Flow rate: 2 mL/min

Detector: LC

Suitability requirements

Resolution: NLT 5 between alpha-tocopherol acetate and phytonadione, System suitability solution

Relative standard deviation: NM<sup>T</sup> 3.0%, Standard solution

Analysis

Samples: Standard solution and Sample solution

Measure the peak areas. Calculate the percentage of the labeled amount of phytonadione (C<sub>3</sub>H<sub>5</sub>O<sub>2</sub>) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{r_b}{r_t} \times \frac{C_t}{C_b} \right) \times 100
\]

where:
- \( r_b \) = peak area of phytonadione from the Sample solution
- \( r_t \) = peak area of phytonadione from the Standard solution
- \( C_t \) = concentration of USP Phytonadione RS in the Standard solution (µg/mL)
- \( C_b \) = nominal concentration of phytonadione in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–165.0% of the labeled amount of phytonadione (C<sub>3</sub>H<sub>5</sub>O<sub>2</sub>) in the Sample solution (mg/mL)

Phytonadione, Method 1

[Note—Use low-acetic glassware throughout this procedure.]

Mobile phase: Methanol and water (19:1)

Standard stock solution: 200 µg/mL of USP Phytonadione RS in methanol. Dissolve with the aid of sonication if necessary.

System suitability solution: 0.65 mg/mL of USP Alpha Tocopherol Acetate RS and 20 µg/mL of USP Phytonadione RS from the Standard stock solution diluted with methanol. [Note—Dissolve USP Tocopherol Acetate RS in a portion of methanol, add the Standard stock solution, and then dilute with methanol to volume.]

Standard solution: 20 µg/mL of USP Phytonadione RS from the Standard stock solution diluted with methanol

Sample solution: Prepared as directed for the Sample stock solution in Vitamin A, Method 1. Transfer NLT 20 mL of the solution to a suitable container, and, if necessary, evaporate under vacuum at room temperature to dryness. Transfer the residue to a suitable volumetric flask with the aid of methanol, and dilute with methanol to volume to obtain a concentration of 20 µg/mL of phytonadione.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 8-mm × 10-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

Samples: System suitability solution and Standard solution

[Note—The relative retention times for alpha-tocopherol acetate and phytonadione are 0.68 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 5 between alpha-tocopherol acetate and phytonadione, System suitability solution

Relative standard deviation: NM<sup>T</sup> 3.0%, Standard solution

Phytonadione, Method 2

[Note—Use low-acetic glassware throughout this procedure.]

Solvent: Methanol and isopropyl alcohol (19:1)

Mobile phase: Mix 800 mL of methanol, 200 mL of methylene chloride, 0.1 mL of glacial acetic acid, 1.36 g of zinc chloride, and 0.41 g of sodium acetate.

Internal standard solution: 5 µg/mL of menaquinone 4 (vitamin K<sub>3</sub>) in the Solvent. [Note—A concentrated stock solution of menaquinone 4 (100 µg/mL) can be stored for 2 months in a refrigerator.]

Standard stock solution: 5 µg/mL of USP Phytonadione RS, prepared by dissolving in methylene chloride with the aid of sonication and diluting with Solvent to final volume

Standard solution: Transfer 1.0 mL of the Standard stock solution and 1.0 mL of the Internal standard solution to a suitable flask, and dilute with Solvent to 5 mL. Pass through a membrane filter of 0.45-µm or finer pore size.

Sample solution: Finely powder NLT 20 Tablets. To a centrifuge tube fitted with a cap transfer an amount of powder not exceeding 800 mg and equivalent to an amount of phytonadione not exceeding 50 µg. Add 4 mL of water. Insert the stopper, and mix using a vortex mixer until the sample is dispersed. Place the tube in a water bath at 60° for 5 min. Remove from the bath, and again shake or mix using a vortex mixer for 1 min while the preparation is still hot. Add 8 mL of alcohol, and swirl the contents to mix. Place the tube in a water bath at 60° for 5 min. Remove from the bath, and again shake or mix using a vortex mixer for 5 min while the preparation is still hot. Cool to room temperature. Add a volume of the Internal standard solution, equivalent to 1.0 mL per each 5 µg of the expected amount of phytonadione in the aliquot taken. Add 20.0 mL of petroleum ether, and cap the tube tightly. Shake or mix using a vortex mixer for 15 min to thoroughly mix the contents. Centrifuge to separate the 2 layers. Transfer a volume of the top layer of petroleum ether, equivalent to 5–50 µg of the nominal amount of phytonadione, to an appropriate flask. Place the flask in a water bath at 35°–45°, and evaporate the solvent under a stream of nitrogen until an oily residue is left. Dissolve the residue in a volume of the Solvent to obtain a concentration of 1 µg/mL of phytonadione.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: Fluorometric detector

Excitation: 320 nm

Emission: 420 nm


@2020 USPC
Column: 4.6-mm × 25-cm; 5-µm, end-capped packing L1, and a postcolumn reactor constituted with a 4.6-mm × 3-cm PEEK column tightly packed with zinc powder. [Note—Prepare the postcolumn reactor daily, or if necessary, to meet the System suitability requirements.]

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: Standard solution

Solution A: 0.1% (v/v) trifluoroacetic acid in methanol and water (90:10, v/v)

Suitability requirements

Column efficiency: NLT 2500 theoretical plates for the phytonadione peak

Tailing factor: NMT 1.5 for the phytonadione peak

Relative standard deviation: NMT 3.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of phytonadione (C₁₁H₁₄O₂) in the portion of Tablets taken:

\[ \text{Result} = \left( \frac{R_2}{R_1} \right) \times \left( \frac{C_s}{C_u} \right) \times 100 \]

R₂ = peak response ratio of phytonadione to the internal standard from the Sample solution

R₁ = peak response ratio of phytonadione to the internal standard from the Standard solution

Cₛ = concentration of USP Phytonadione RS in the Standard solution (µg/mL)

Cᵤ = nominal concentration of phytonadione in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–165.0% of labeled amount of phytonadione (C₁₁H₁₄O₂)

Add the following:

**Vitamin A, Vitamin D (Cholecalciferol or Ergocalciferol), and Vitamin E, Method 4; Phytonadione, Method 3**

**Vitamin A** (retinyl acetate or retinyl palmitate), vitamin D (cholecalciferol or ergocalciferol), or vitamin E (alpha-tocopherol, alpha-tocopheryl acetate, or alpha-tocopheryl acid succinate) are specified in the following procedure, use the chemical form present in the formulation and the relevant Reference Standard. Use low-acetic glassware.

Diluent: Methanol and water (90:10, v/v)

Solution A: 0.1% (v/v) trifluoroacetic acid in Diluent

Solution B: 0.1% (v/v) trifluoroacetic acid in methanol

Mobile phase: See Table 1.

<table>
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<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
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<tr>
<td>30</td>
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</tbody>
</table>

Vitamin A standard stock solution: A solution containing the equivalent of 0.2 mg/mL of retinol from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in isopropyl alcohol. Dissolve with the aid of sonication if necessary.

Vitamin D standard stock solution: 0.05 mg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in isopropyl alcohol.

Vitamin E standard stock solution: 3.0 mg/mL of USP Alpha Tocopheryl RS, USP Alpha Tocopheryl Acetate RS, or USP Alpha Tocopheryl Acid Succinate RS in isopropyl alcohol. Dissolve with the aid of sonication if necessary.

Phytonadione standard stock solution: 0.04 mg/mL of USP Phytonadione RS in isopropyl alcohol. Dissolve with the aid of sonication if necessary.

Sample solution 1: Finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder into a 50-mL volumetric flask to obtain a solution containing known nominal concentrations in the ranges of 0.05–0.1 mg/mL of vitamin A and 0.5–0.15 mg/mL of vitamin E. Add 10 mL of dimethyl sulfoxide and sonicate for 30 min with vigorous intermittent shaking. Cool to room temperature, dilute with isopropyl alcohol to volume, and mix well. Pass a portion of the solution through a nylon filter of 0.45-µm pore size, and discard the first milliliter of the filtrate.

Sample solution 2: Transfer an accurately weighed portion of the powder from NLT 20 finely powdered Tablets into a 50-mL volumetric flask to obtain a solution containing known nominal concentrations in the ranges of 1.0–3.5 µg/mL of vitamin D and 1.0–3.5 µg/mL of phytonadione. Add 5 mL of water and sonicate for 10 min with intermittent shaking. Add 10 mL of dimethyl sulfoxide and sonicate for 30 min with vigorous intermittent shaking. Cool to room temperature, dilute with isopropyl alcohol to volume, and mix well. Pass a portion of the solution through a nylon filter of 0.45-µm pore size, and discard the first milliliter of the filtrate.

Standard solution: Transfer calculated volumes of Vitamin A standard stock solution, Vitamin D standard stock solution, Vitamin E standard stock solution, and Phytonadione standard stock solution into a suitable volumetric flask, and dilute with isopropyl alcohol to volume to obtain a solution with final vitamin concentrations similar to those obtained for the corresponding vitamins in Sample solution 1 and Sample solution 2.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detectors

For Sample solution 1 containing retinyl acetate: UV 325 nm for 7 min, then switch to 265 nm

For Sample solution 1 containing retinyl palmitate: UV 265 nm for 18 min, then switch to 325 nm

For Sample solution 2: UV 265 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Temperatures

Sample: 4°

Column: 35°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: Sample solution 1, Sample solution 2, and Standard solution

Relative standard deviation: NMT 2.0% for each individual peak, Standard solution

Peak purity of vitamin D and vitamin A (as retinyl palmitate): Monitor the chromatograms of Sample solution 1 and Sample solution 2 at 450 nm. The area of any peak at the retention time of retinyl palmitate from Sample solution 1 and any peak at the retention time of vitamin D (as cholecalciferol or ergocalciferol) from Sample solution 2 should be NMT 5% of the corresponding peak areas detected at 325 nm and
Potassium hydroxide solution: Dissolve 58.8 g potassium hydroxide in 50 mL of water.

Iodine solution: 0.01 mg/mL of iodine in cyclohexane.

[Note—Prepare this solution fresh daily.]

Sample solution: Weigh NLT 20 Tablets. Grind the Tablets to a fine powder, and transfer a quantity of the powder, equivalent to 2 mg of beta carotene, to a 500-mL saponification flask. Add 100 mL of alcohol, 6 mL of Potassium hydroxide solution, and a magnetic stirring bar. Attach an air condenser to the flask, and heat under reflux for 45 min with constant stirring. Cool to room temperature. Add 170 mL of solvent hexane, and stir for 30 min. Quantitatively transfer the contents of the flask to a 500-mL separatory funnel with portions of solvent hexane. Allow the layers to separate for 5–10 min, and transfer the upper organic layer to a 500-mL volumetric flask. Transfer the lower aqueous layer into the saponification flask. Add 170 mL of solvent hexane, and stir for an additional 20 min. Quantitatively transfer the contents of the saponification flask to the separatory funnel with the aid of portions of solvent hexane. Allow the layers to separate for 10 min. Drain the lower aqueous layer, and discard. Transfer the organic layer to the volumetric flask containing the previously collected organic layer. Rinse the separatory funnel with small portions of solvent hexane, and transfer the washings to the volumetric flask. Dilute the hexane extracts with solvent hexane to volume. Add 3 g of anhydrous sodium sulfate, shake, and allow to settle. Quantitatively transfer a volume of this solution, equivalent to 100 µg of beta carotene, to a 50-mL volumetric flask. Evaporate under a stream of nitrogen to dryness, and immediately add cyclohexane. Add 2 mL of Iodine solution, and heat for 15 min in a water bath maintained at 65°. Cool rapidly, and dilute with cyclohexane to volume.

Instrumental conditions
(See Ultraviolet-Visible Spectroscopy (857).)

Mode: Vis
Analytical wavelength: 452 nm
Blank: Cyclohexane

Analysis
Sample: Sample solution
Determine the absorbance against the Blank.

Calculate the percentage of the labeled amount of beta carotene (C\text{\textsubscript{40}}H\text{\textsubscript{56}}) in the portion of Tablets taken:

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

\(A_U\) = absorbance of the Sample solution
\(F\) = absorptivity of beta carotene at 452 nm, 223
\(C_U\) = nominal concentration of beta carotene in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–165.0% of the labeled amount of beta carotene (C\text{\textsubscript{40}}H\text{\textsubscript{56}})

Change to read:

• **ASCORBIC ACID, CALCIUM ASCORBATE, and SODIUM ASCORBATE**

(See Vitamin C Assay (580).)

[Note—For labeling purposes, consider (580), Method I—Titrimetric Method as Method 1; (580), Method II—Chromatographic Method, Procedure 1 as Method 2; and (580), Method II—Chromatographic Method, Procedure 2 as Method 3.]

Acceptance criteria: 90.0%–150.0% of the labeled amount of ascorbic acid (C\text{\textsubscript{6}}H\text{\textsubscript{8}}O\text{\textsubscript{6}}), or its salts as calcium ascorbate (C\text{\textsubscript{12}}H\text{\textsubscript{14}}CaO\text{\textsubscript{6}} \cdot 2H\text{\textsubscript{2}}O) or sodium ascorbate (C\text{\textsubscript{6}}H\text{\textsubscript{7}}NaO\text{\textsubscript{6}})
• **Biotin, Method 1**
  [Note—Use low-actinic glassware throughout this procedure.]
  
  Mobile phase: Mix 85 mL of acetonitrile, 1 g of sodium perchlorate, 1 mL of phosphoric acid, and dilute with water to 1000 mL.
  
  **Standard stock solution:** 0.333 mg/mL of USP Biotin RS in dimethyl sulfoxide
  
  **Standard solution:** 5 µg/mL of USP Biotin RS prepared by diluting the Standard stock solution in water
  
  **Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 1 mg of biotin, to a 200-mL volumetric flask. Add 3 mL of dimethyl sulfoxide, and swirl to wet the contents. Place the flask in a water bath at 60°–70° for 5 min. Sonicate for 5 min, dilute with water to volume, and filter.
  
  **Chromatographic system**
  (See Chromatography (621), System Suitability.)
  
  **Mode:** LC
  **Detector:** UV 200 nm
  **Column:** 4.6-mm × 15-cm; 3-µm packing L7
  **Flow rate:** 1.2 mL/min
  **Injection volume:** 100 µL
  
  **System suitability**
  **Sample:** Standard solution
  **Suitability requirements**
  **Relative standard deviation:** NMT 3.0%
  
  **Analysis**
  **Samples:** Standard solution and Sample solution
  Measure the peak areas of biotin. Calculate the percentage of the labeled amount of biotin (C₁₉H₁₈N₂O₅S) in the portion of Tablets taken:
  
  \[
  \text{Result} = \left( \frac{r_0}{r_s} \right) \times \left( \frac{C_s}{C_0} \right) \times 100
  \]
  
  \[r_0\] = peak area of biotin from the Sample solution
  \[r_s\] = peak area of biotin from the Standard solution
  \[C_s\] = concentration of USP Biotin RS in the Standard solution (µg/mL)
  \[C_0\] = nominal concentration of biotin in the Sample solution (µg/mL)
  
  **Acceptance criteria:** 90.0%–150.0% of the labeled amount of biotin (C₁₉H₁₈N₂O₅S)
  
  **Change to read:**
  
  • **Biotin, Method 2**
  [Note—Use low-actinic glassware throughout this procedure.]
  
  Dehydrated mixtures yielding formulations similar to the media described herein may be used provided that, when constituted as directed, they have growth-promoting properties equal to or superior to those obtained with the media prepared as described herein.
  
  **Standard stock solution:** 50 µg/mL of USP Biotin RS in 50% alcohol
  
  **Standard solution:** 0.1 ng/mL of USP Biotin RS in water, prepared by dilution of the Standard stock solution with water on the day of the assay
  
  **Sample solution:** Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 100 µg of biotin, to a 200-mL volumetric flask. Add 3 mL of 50% alcohol, and swirl to wet the contents. Heat the flask in a water bath at 60°–70° for 5 min. Sonicate for 5 min, dilute with 50% alcohol to volume, and filter. Dilute a volume of the filtrate quantitatively, and stepwise if necessary, with water to obtain a solution with a concentration of 0.1 ng/mL.
  
  **Acid-hydrolyzed casein solution:** Mix 100 g of vitamin-free casein with 500 mL of 6 N hydrochloric acid, and reflux the mixture for 8–12 h. Remove the hydrochloric acid from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in water, adjust the solution with 1 N sodium hydroxide to a pH of 3.5 ± 0.1, and dilute with water to make 1000 mL. Add 20 g of activated charcoal, stir for 1 h, and filter. Repeat the treatment with activated charcoal. Store under toluene in a cool place at a temperature not below 10°. Filter the solution if a precipitate forms during storage.
  
  **Cystine–tryptophan solution:** Suspend 4.0 g of l-cystine in a solution of 1.0 g of L-tryptophan (or 2.0 g of D,L-tryptophan) in 700–800 mL of water. Heat to 70°–80°, and add dilute hydrochloric acid (1 in 2) dropwise, with stirring, until the solids are dissolved. Cool, and add water to make 1000 mL. Store under toluene in a cool place at a temperature NLT 10°.
  
  **Adenine–guanine–uracil solution:** Dissolve 200 mg each of adenine sulfate, guanine hydrochloride, and uracil, with the aid of heat, in 10 mL of 4 N hydrochloric acid. Cool, and add water to make 200 mL. Store under toluene in a refrigerator.
  
  **Polysorbate 80 solution:** 100 mg/mL of polysorbate 80 in alcohol
  
  **Calcium pantothenate solution:** 10 µg/mL of calcium pantothenate in 50% alcohol. Store in a refrigerator.
  
  **Riboflavin–thiamine hydrochloride solution:** 20 µg/mL of riboflavin and 10 µg/mL of thiamine hydrochloride in 0.02 N acetic acid. Store under toluene, protected from light, in a refrigerator.
  
  **p-Aminobenzoic acid–niacin–pyridoxine hydrochloride solution:** 10 µg/mL of p-aminobenzoic acid, 50 µg/mL of niacin, and 40 µg/mL of pyridoxine hydrochloride in a mixture of neutralized alcohol and water (1:3). Store in a refrigerator.
  
  **Salt solution A:** 25 g of monobasic potassium phosphate and 25 g of dibasic potassium phosphate in water to make 500 mL. Add 5 drops of hydrochloric acid. Store under toluene.
  
  **Salt solution B:** 10 g of magnesium sulfate, 0.5 g of sodium chloride, 0.5 g of ferrous sulfate, and 0.5 g of manganese sulfate in water to make 500 mL. Add 5 drops of hydrochloric acid, and mix. Store under toluene.
  
  **Basal medium stock solution:** Dissolve the anhydrous dextrose and anhydrous sodium acetate in the solutions previously mixed according to Table 2 and adjust with 1 N sodium hydroxide to a pH of 6.8. Dilute with water to 250 mL.
  
  **Table 2**
  "(USP 1-May-2020)
  
<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-hydrolyzed casein solution</td>
<td>25 mL</td>
</tr>
<tr>
<td>Cystine–tryptophan solution</td>
<td>25 mL</td>
</tr>
<tr>
<td>Polysorbate 80 solution</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Dextrose, anhydrous</td>
<td>10 g</td>
</tr>
<tr>
<td>Sodium acetate, anhydrous</td>
<td>5 g</td>
</tr>
<tr>
<td>Adenine–guanine–uracil solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Calcium pantothenate solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Riboflavin–thiamine hydrochloride solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>p-Aminobenzoic acid–niacin–pyridoxine hydrochloride solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Salt solution A</td>
<td>5 mL</td>
</tr>
<tr>
<td>Salt solution B</td>
<td>5 mL</td>
</tr>
</tbody>
</table>

Stock culture of *Lactobacillus plantarum*: Dissolve 2.0 g of yeast extract in 100 mL of water. Add 500 mg of anhydrous dextrose, 500 mg of anhydrous sodium acetate, and 1.5 g of agar, and heat the mixture on a steam bath, with stirring, until the agar dissolves. Add 10-mL portions of the hot solution to test tubes, close or cover the tubes, sterilize in an autoclave at 121° for 15 min, and allow the tubes to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of *Lactobacillus plantarum*,\(^1\) incubating for 16–24 h at a temperature between 30° and 37° held constant to within ±0.5°. Store in a refrigerator. Prepare a fresh stab of the stock culture every week, and do not use for *Inoculum* if the culture is more than 1 week old.

**Culture medium:** To each of a series of test tubes containing 5.0 mL of *Basal medium stock solution* add 5.0 mL of water containing 0.5 ng of biotin. Plug the tubes with cotton, sterilize in an autoclave at 121° for 15 min, and cool.

**Inoculum:** \([\text{Note—A frozen suspension of } Lactobacillus plantarum \text{ may be used as the stock culture, provided it yields an inoculum comparable to a fresh culture.}]\) Transfer cells from the *Stock culture of Lactobacillus plantarum* to a sterile tube containing 10 mL of *Culture medium*. Incubate this culture for 16–24 h at a temperature between 30° and 37° held constant to within ±0.5°. The cell suspension is the *Inoculum*.

**Analysis**

**Samples:** Standard solution and Sample solution

To similar separate test tubes add, in duplicate, 1.0 and/or 1.5, 2.0, 3.0, 4.0, and 5.0 mL of the *Standard solution*. To each tube and to 4 similar but empty tubes add 5.0 mL of *Basal medium stock solution* and sufficient water to make 10 mL.

To similar test tubes add, in duplicate, volumes of the *Sample solution* corresponding to 3 or more of the levels specified for the *Standard solution*, including the levels of 2.0, 3.0, and 4.0 mL. To each tube add 5.0 mL of the *Basal medium stock solution* and sufficient water to make 10 mL. Place one complete set of Standard and sample tubes together in one tube rack and the duplicate set in a second rack or section of a rack, preferably in random order. Cover the tubes of both series to prevent contamination, and sterilize in an autoclave at 121° for 5 min. Cool. Add 1 drop of *Inoculum* to each tube, except 2 of the 4 tubes containing no *Standard solution* (the uninoculated blanks). Incubate the tubes at a temperature between 30° and 37° held constant to within ±0.5° until, following 16–24 h of incubation, there has been no substantial increase in turbidity in the tubes containing the highest level of Standard during a 2-h period.

Determine the transmittance of the tubes in the following manner. Mix the contents of each tube, and transfer to a spectrophotometer cell. Place the cell in a spectrophotometer that has been set at a specific wavelength of 540–660 nm, and read the transmittance while a steady state is reached. This steady state is observed a few seconds after agitation when the galvanometer reading remains constant for 30 or more. Allow approximately the same time interval for the reading on each tube.

With the transmittance set at 1.00 for the uninoculated blank, read the transmittance of the inoculated blank. With the transmittance set at 1.00 for the inoculated blank, read the transmittance for each of the remaining tubes. If there is evidence of contamination with a foreign microorganism, disregard the result of the assay.

\(<1\text{ ATCC No. 8014 is suitable. This strain was formerly known as } Lactobacillus arabinosus 17-5.\>

**Calculation:** Prepare a standard concentration-response curve as follows. For each level of the Standard, calculate the response from the sum of the duplicate values of the transmittance (\(\Sigma_y\)) as the difference, \(y = 2.00 - \Sigma_y\). Plot this response on the ordinate of cross-section paper against the logarithm of the milliliter of the *Standard solution* per tube on the abscissa, using for the ordinate either an arithmetic or a logarithmic scale, whichever gives the better approximation to a straight line. Draw the straight line or smooth curve that best fits the plotted points.

Calculate the response, \(y = 2.00 - \Sigma_y\) adding together the two transmittances (\(\Sigma_y\)) for each level of the *Sample solution*. Read from the standard curve the logarithm of the volume of the *Standard solution* corresponding to each of those values of \(y\) that falls within the range of lowest and highest points plotted for the Standard. Subtract from each logarithm so obtained the logarithm of the volume, in mL of the *Sample solution* to obtain the difference, \(X\), for each dosage level. Average the values of \(X\) for each of 3 or more dosage levels to obtain \(\bar{X}\), which equals the log-relative potency, \(M\), of the *Sample solution*.

Determine the quantity, in \(\mu\)g, of biotin (\(C_{16}H_{24}N_{2}O_{5}\)) in the portion of Tablets taken:

\[
antilog M = \frac{\text{antilog} (M' + \log R)}{N} \times 100
\]

Calculate the percentage of the labeled amount of biotin (\(C_{16}H_{24}N_{2}O_{5}\)) in the portion of the Tablets taken:

\[
\text{Result } = \frac{\text{[(antilog } M)/N]}{\times 100}
\]

**Replication:** Repeat the entire determination at least once, using separately prepared *Sample solutions*. If the difference between the two log-potencies \(M\) is NMT 0.08, their mean, \(\bar{M}\), is the assayed log-potency of the test material (see *Design and Analysis of Biological Assays* (111), *The Confidence Interval and Limits of Potency*). If the two determinations differ by more than 0.08, conduct one or more additional determinations. From the mean of two or more values of \(M\) that do not differ by more than 0.15, compute the mean potency of the preparation under assay.

**Acceptance criteria:** 90.0%–150.0% of the labeled amount of biotin (\(C_{16}H_{24}N_{2}O_{5}\)).

- **BIOTIN, Method 3**
  \([\text{Note—Use low-actinic glassware throughout this procedure.}]\)

**Solution A:** Transfer 800 mL of water and 100 mL of triethylamine to a 1000-mL volumetric flask. Add 80 mL of 85% phosphoric acid, and dilute with water to volume.

**Mobile phase:** Transfer 80 mL of acetonitrile and 10 mL of *Solution A* to a 1000-mL volumetric flask. Dilute with water to volume.

**Standard solution:** 0.6 \(\mu\)g/mL of USP Biotin RS in water. [\text{Note—A portion of the *Standard solution* will be used to determine the percent recovery of biotin from the Solid-phase extraction procedure.}]

**Sample solution:** Finely powder NLT 20 Tablets. Transfer an amount of powdered Tablets to a volumetric flask to obtain a nominal concentration of 0.6 \(\mu\)g/mL of biotin. Add water up to 80% of the flask capacity, and sonicate for 30–40 min, with occasional mixing, to dissolve. Dilute with water to volume, and filter. Adjust the pH of the solution.
with either dilute acetic acid or 0.1 N sodium hydroxide to a pH of 6.0–7.0.

Solid-phase extraction: [Note—Condition the extraction column specified in this procedure in the following manner. Wash the column with a 2-mL portion of methanol. Equilibrate with a 2-mL portion of water.] Separately pipet 5.0 mL each of the Sample solution and Standard solution into freshly conditioned solid-phase extraction columns consisting of a mixed-mode packing with a sorbent-mass of 60 mg. [Note—The mixed-mode packing consists of anion-exchange and reversed-phase sorbents. The reverse-phase component is a polymer of copolymer N-vinylpyrrolidone and divinylbenzene. The anion exchange moiety is a trialkylamino group.2] Wash the column with 10 mL of 30% (v/v) methanol in water. Apply an appropriate volume (4.9 mL) of 30% (v/v) methanol in 0.1 N hydrochloric acid to the column. Collect the eluate in a 5-mL volumetric flask, containing 100 µL of 40% (v/v) sodium acetate in water, and dilute with 30% (v/v) methanol in 0.1 N hydrochloric acid to volume.

Chromatographic system
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: UV 200 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 100 µL

System suitability
Samples: Standard solution and portion of the Standard solution that has undergone Solid-phase extraction
Suitability requirements
Tailing factor: NMT 1.5, Standard solution
Relative standard deviation: NMT 2.0%, Standard solution and Standard solution that has undergone Solid-phase extraction
Recovery: 95%–100%, Standard solution that has undergone Solid-phase extraction

Analysis
Samples: Standard solution and the Sample solution that have both undergone Solid-phase extraction
Measure the peak areas of biotin. Calculate the percentage of the labeled amount of biotin (C₁₀H₁₈N₄O₅S) in the portion of Tablets taken:

Result = (rₒ/ᵣₛ) × (Cₛ/Cₒ) × 100

rₒ = peak area from the Sample solution
rₛ = peak area from the Standard solution
Cₛ = concentration of USP Biotin RS in the Standard solution (µg/mL)
Cₒ = nominal concentration of biotin in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of biotin (C₁₀H₁₈N₄O₅S)

• CYANOCOBALAMIN, Method 1
[Note—Use low-actic glassware throughout this procedure.]
Mobile phase: Methanol and water (7:13)
Standard stock solution: 10 µg/mL of USP Cyanocobalamin (Crystalline) RS in water. [Note—Store in a dark place, and discard after 1 week.]
Standard solution: 1 µg/mL of USP Cyanocobalamin (Crystalline) RS from Standard stock solution diluted with water

Sample solution: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 100 µg of cyanocobalamin, to a 250-mL flask. Quantitatively add 100.0 mL of water, and carefully extract for 2 min. Filter 10 mL of the extract, and use the filtrate.

Chromatographic system
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: 550 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 0.5 mL/min
Injection volume: 200 µL

System suitability
Sample: Standard solution
Suitability requirements
Relative standard deviation: NMT 3.0%

Analysis
Samples: Standard solution and Sample solution
Measure the peak areas of cyanocobalamin.
Calculate the percentage of the labeled amount of cyanocobalamin (C₆₃H₆₈CoN₁₃O₁₄P) in the portion of Tablets taken:

Result = (rₒ/ᵣₛ) × (Cₛ/Cₒ) × 100

rₒ = peak area from the Sample solution
rₛ = peak area from the Standard solution
Cₛ = concentration of USP Cyanocobalamin (Crystalline) RS in the Standard solution (µg/mL)
Cₒ = nominal concentration of cyanocobalamin in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of cyanocobalamin (C₆₃H₆₈CoN₁₃O₁₄P)

Change to read:

• CYANOCOBALAMIN, Method 2
[Note—Use low-actic glassware throughout this procedure.]
Standard stock solution: 1.0 µg/mL of USP Cyanocobalamin (Crystalline) RS in 25% alcohol. Store in a refrigerator.

Standard solution: Dilute a suitable volume of the Standard stock solution with water to a measured volume such that after the incubation period as described in the Analysis, the difference in transmittance between the inoculated blank and the 5.0-mL level of the Standard solution is NLT that which corresponds to a difference of 1.25 mg in dried cell weight. This concentration usually falls between 0.01 and 0.04 mg/mL of the Standard solution. Prepare this solution fresh for each assay.

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powdered Tablets, equivalent to 1.0 µg of cyanocobalamin, to an appropriate vessel containing, for each gram of powdered Tablets taken, 25 mL of an aqueous extracting solution prepared just before use to contain, in each 100 mL, 1.9 g of dibasic sodium phosphate, 1.1 g of anhydrous citric acid, and 1.0 g of sodium metabisulfite. Autoclave the mixture at 121°C for 10 min. Allow any undissolved particles of the extract to settle, and filter or centrifuge if necessary. Dilute an aliquot of the clear solution with water to obtain a final solution containing vitamin B₁₂ activity equivalent to the nominal activity of the Standard solution.

Acid-hydrolyzed casein solution: Prepare as directed in Biotin, Method 2.
Asparagine solution: Dissolve 2.0 g of L-asparagine in water to make 200 mL. Store under toluene in a refrigerator.

2 A suitable cartridge is the Waters Oasis MAX Vac RC, particle size 30 µm, part #186000371.
Adenine–guanine–uracil solution: Prepare as directed in *Biotin, Method 2*.

Xanthine solution: Suspend 0.20 g of xanthine in 30–40 mL of water, heat to 70°C, add 6.0 mL of 6 N ammonium hydroxide, and stir until the solid is dissolved. Cool, and dilute with water to make 200 mL. Store under toluene in a refrigerator.

Salt solution A: Dissolve 10 g of monobasic potassium phosphate and 10 g of dibasic potassium phosphate in water to make 200 mL, and add 2 drops of hydrochloric acid. Store this solution under toluene.

Salt solution B: Dissolve 4.0 g of magnesium sulfate, 0.20 g of sodium chloride, 0.20 g of ferrous sulfate, and 0.20 g of manganese sulfate in water to make 200 mL. Add 2 drops of hydrochloric acid. Store this solution under toluene.

Polysorbate 80 solution: Dissolve 20 g of polysorbate 80 in alcohol to make 200 mL. Store in a refrigerator.

Vitamin solution A: Dissolve 10 mg of riboflavin, 10 mg of thiamine hydrochloride, 100 μg of biotin, and 20 mg of niacin in 0.02 N acetic acid to make 400 mL. Store under toluene, protected from light, in a refrigerator.

Vitamin solution B: Dissolve 20 mg of p-aminobenzoic acid, 10 mg of calcium pantothenate, 40 mg of pyridoxine hydrochloride, 40 mg of pyridoxal hydrochloride, 8 mg of pyridoxamine dihydrochloride, and 2 mg of folic acid in a mixture of water and neutralized alcohol (3:1) to make 400 mL. Store, protected from light, in a refrigerator.

**Basal medium stock solution:** Prepare the medium according to the following formula and directions. A dehydrated mixture containing the same ingredients may be used provided that, when constituted as directed in the labeling, it yields a medium equivalent to that obtained from the formula given herein. Dissolve 0.75 g of yeast extract, 0.75 g of dried peptone, 1.0 g of anhydrous dextrose, and 0.20 g of monobasic potassium phosphate in 60–70 mL of water. Add 10 mL of *Tomato juice preparation* and 1 mL of *Polysorbate 80 solution*. Adjust with 1 N sodium hydroxide to a pH of 6.8, and add water to make 100 mL. Place 10-mL portions of the solution in test tubes, and plug with cotton. Store the tubes and contents in an autoclave at 121°C for 15 min. Cool as rapidly as possible to avoid color formation resulting from overheating the medium.

**Suspension medium:** Dilute a measured volume of the *Basal medium stock solution* with an equal volume of water. Place 10-mL portions of the diluted medium in test tubes. Sterilize, and cool as directed for *Culture medium*.

**Stock culture of Lactobacillus leichmannii:** To 100 mL of *Culture medium* add 1.0–1.5 g of agar, and heat the mixture on a steam bath, with stirring, until the agar dissolves. Place 10-mL portions of the hot solution in test tubes, cover the tubes, sterilize at 121°C for 15 min in an autoclave, and allow the tubes to cool in an upright position. Inoculate 3 or more of the tubes by stab transfer of a pure culture of *Lactobacillus leichmannii*. [Note—Before using a fresh culture in this assay, make NLT 10 successive transfers of the culture in a 2-week period.] Incubate for 16–24 h at a temperature between 30°C and 40°C held constant to within ±0.5°C. Store in a refrigerator.

Prepare fresh stab cultures at least 3 times each week, and do not use them for preparing the *Inoculum* if more than 4 days old. The activity of the microorganism can be increased by daily or twice-daily transfer of the stab culture, to the point where definite turbidity in the liquid *Inoculum* can be observed 2–4 h after inoculation. A slow-growing culture seldom gives a suitable response curve and may lead to erratic results.

**Inoculum:** [Note—A frozen suspension of *Lactobacillus leichmannii* may be used as the stock culture, provided it yields an *Inoculum* comparable to a fresh culture.] Transfer cells from the *Stock culture of Lactobacillus leichmannii* to 2 sterile tubes containing 10 mL each of the *Culture medium*. Incubate these cultures for 16–24 h at a temperature between 30°C and 40°C held constant to within ±0.5°C. Under aseptic conditions centrifuge the cultures, and decant the supernatant. Suspend the cells from the culture in 5 mL of sterile *Suspension medium*, and combine. Use sterile *Suspension medium*, adjust the volume so that a 1-in-20 dilution in saline *TS* produces 70% transmittance when read on a suitable spectrophotometer that has been set at a wavelength of 530 nm, equipped with a 10-mm cell, and read against saline *TS* set at 100% transmittance. Prepare a 1-in-400 dilution of the adjusted suspension using sterile *Basal medium stock solution*. [Note—This dilution may be altered, when necessary, to obtain the desired test response.] The cell suspension is the *Inoculum*.

**Calibration of spectrophotometer:** Check the wavelength of the spectrophotometer periodically using a standard wavelength cell or other suitable device. Before reading any tests, calibrate the spectrophotometer for 0% and 100% transmittance, using water, and with the wavelength set at 530 nm.

### Table 3

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Cystine</td>
<td>0.1 g</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.05 g</td>
</tr>
<tr>
<td>1 N hydrochloric acid</td>
<td>10 mL</td>
</tr>
<tr>
<td>Adenine–guanine–uracil solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Xanthine solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Vitamin solution A</td>
<td>10 mL</td>
</tr>
<tr>
<td>Vitamin solution B</td>
<td>10 mL</td>
</tr>
<tr>
<td>Salt solution A</td>
<td>5 mL</td>
</tr>
<tr>
<td>Salt solution B</td>
<td>5 mL</td>
</tr>
<tr>
<td>Asparagine solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Acid-hydrolyzed casein solution</td>
<td>25 mL</td>
</tr>
<tr>
<td>Dextrose, anhydrous</td>
<td>10 g</td>
</tr>
<tr>
<td>Sodium acetate, anhydrous</td>
<td>5 g</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1 g</td>
</tr>
<tr>
<td>Polysorbate 80 solution</td>
<td>5 mL</td>
</tr>
</tbody>
</table>

**Tomato juice preparation:** Centrifuge commercially canned tomato juice so that most of the pulp is removed. Suspend 5 g/L of analytical filter aid in the supernatant, and pass with the aid of reduced pressure through a layer of the filter aid. Repeat if necessary until a clear, straw-colored filtrate is obtained. Store under toluene in a refrigerator.

**Culture medium:** [Note—A dehydrated mixture containing the same ingredients may be used provided that, when constituted as directed in the labeling, it yields a medium equivalent to that obtained from the formula given herein.] Dissolve 0.75 g of yeast extract, 0.75 g of dried peptone, 1.0 g of anhydrous dextrose, and 0.20 g of monobasic potassium phosphate in 60–70 mL of water. Add 10 mL of *Tomato juice preparation* and 1 mL of *Polysorbate 80 solution*. Adjust with 1 N sodium hydroxide to a pH of 6.8, and add water to make 100 mL. Place 10-mL portions of the solution in test tubes, and plug with cotton. Sterilize the tubes and contents in an autoclave at 121°C for 15 min. Cool as rapidly as possible to avoid color formation resulting from overheating the medium.

**Suspension medium:** Dilute a measured volume of the *Basal medium stock solution* with an equal volume of water. Place 10-mL portions of the diluted medium in test tubes. Sterilize, and cool as directed for *Culture medium*.

**Stock culture of Lactobacillus leichmannii:** To 100 mL of *Culture medium* add 1.0–1.5 g of agar, and heat the mixture on a steam bath, with stirring, until the agar dissolves. Place 10-mL portions of the hot solution in test tubes, cover the tubes, sterilize at 121°C for 15 min in an autoclave, and allow the tubes to cool in an upright position. Inoculate 3 or more of the tubes by stab transfer of a pure culture of *Lactobacillus leichmannii*. [Note—Before using a fresh culture in this assay, make NLT 10 successive transfers of the culture in a 2-week period.] Incubate for 16–24 h at a temperature between 30°C and 40°C held constant to within ±0.5°C. Store in a refrigerator.

Prepare fresh stab cultures at least 3 times each week, and do not use them for preparing the *Inoculum* if more than 4 days old. The activity of the microorganism can be increased by daily or twice-daily transfer of the stab culture, to the point where definite turbidity in the liquid *Inoculum* can be observed 2–4 h after inoculation. A slow-growing culture seldom gives a suitable response curve and may lead to erratic results.

**Inoculum:** [Note—A frozen suspension of *Lactobacillus leichmannii* may be used as the stock culture, provided it yields an *Inoculum* comparable to a fresh culture.] Transfer cells from the *Stock culture of Lactobacillus leichmannii* to 2 sterile tubes containing 10 mL each of the *Culture medium*. Incubate these cultures for 16–24 h at a temperature between 30°C and 40°C held constant to within ±0.5°C. Under aseptic conditions centrifuge the cultures, and decant the supernatant. Suspend the cells from the culture in 5 mL of sterile *Suspension medium*, and combine. Use sterile *Suspension medium*, adjust the volume so that a 1-in-20 dilution in saline *TS* produces 70% transmittance when read on a suitable spectrophotometer that has been set at a wavelength of 530 nm, equipped with a 10-mm cell, and read against saline *TS* set at 100% transmittance. Prepare a 1-in-400 dilution of the adjusted suspension using sterile *Basal medium stock solution*. [Note—This dilution may be altered, when necessary, to obtain the desired test response.] The cell suspension is the *Inoculum*.

**Calibration of spectrophotometer:** Check the wavelength of the spectrophotometer periodically using a standard wavelength cell or other suitable device. Before reading any tests, calibrate the spectrophotometer for 0% and 100% transmittance, using water, and with the wavelength set at 530 nm.

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3 Pure cultures of *Lactobacillus leichmannii* (listed as *Lactobacillus delbruekii*) may be obtained as #7830 from ATCC, 10801 University Blvd., Manassas, VA 20110-2209 ([www.atcc.org](http://www.atcc.org)).
Analysis

Samples: Standard solution and Sample solution

Because of the high sensitivity of the test, it is necessary to maintain amounts of vitamin B₁₂ activity and to traces of many contaminating agents, cleanse meticulously by suitable means, followed preferably by heating at 121°C for 2 h using hard-glass 20-mm × 150-mm test tubes and other necessary glassware.

To separate test tubes add, in duplicate, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mL of the Standard solution. To each of these tubes and 4 similar but empty tubes add 5.0 mL of the Basal medium stock solution and sufficient water to make 10 mL.

To similar separate test tubes add, in duplicate, 1.0, 1.5, 2.0, 3.0, and 4.0 mL of the Sample solution. To each tube add 5.0 mL of the Basal medium stock solution and sufficient water to make 10 mL. Place one complete set of Standard and sample tubes together in one tube rack and the duplicate set in a second rack or section of a rack, preferably in random order.

Cover the tubes to prevent bacterial contamination, and sterilize in an autoclave at 121°C for 5 min, arranging to reach this temperature in NMT 10 min by preheating the autoclave if necessary. Cool as rapidly as possible to avoid color formation resulting from overheating the medium. Take precautions to maintain uniformity of sterilizing and cooling conditions throughout the assay, because packing the tubes too closely in the autoclave or overloading it may cause variation in the heating rate.

Aseptically add 0.5 mL of Inoculum to each tube so prepared, except 2 of the 4 containing no Standard solution (the uninoculated blanks). Incubate the tubes at a temperature between 30° and 40°, held constant to within ±0.5°C, for 16–24 h.

Terminate growth by heating to a temperature NLT 80°C for 5 min. Cool to room temperature. After agitating its contents, read the transmittance at 530 nm when a steady state is reached. This steady state is observed a few seconds after agitation when the reading remains constant for 30 s or more. Allow approximately the same time interval for the reading on each tube.

With the transmittance set at 100% for the uninoculated blank, read the transmittance of the inoculated blank. If the difference is greater than 5% or if there is evidence of contamination with a foreign microorganism, disregard the results of the assay.

With the transmittance set at 100% for the uninoculated blank, read the transmittance of each of the remaining tubes. Disregard the results of the assay if the slope of the standard curve indicates a problem with sensitivity.

Calculation: Prepare a standard concentration-response curve by the following procedure. Test for and replace any aberrant individual transmittances. For each level of the Standard, calculate the response from the sum of the duplicate values of the transmittances (Σ̂) as the difference, y = 2.00 − Σ̂. Plot this response on the ordinate of cross-section paper against the logarithm of the milliliter of the Standard solution per tube on the abscissa, using for the ordinate either an arithmetic or a logarithmic scale, whichever gives the better approximation to a straight line. Draw the straight line or smooth curve that best fits the plotted points.

Calculate the response, y = 2.00 − Σ̂, adding together the two transmittances (Σ̂) for each level of the Sample solution. Read from the standard curve the logarithm of the volume of the Standard solution corresponding to each of those values of y that falls within the range of the lowest and highest points plotted for the Standard. Subtract from each logarithm so obtained the logarithm of the volume, in mL, of the Sample solution to obtain the difference, X, for each dosage level. Average the values of X for each of 3 or more dosage levels to obtain X, which equals the log-relative potency, Mᵣ, of the Sample solution.

Determine the quantity, in µg, of cyanocobalamin (C₆₃H₉₈CoN₁₂O₁₉P) in the portion of Tablets taken:

\[ \text{antilog } M = \text{antilog } (M' + \log R) \]

\[ R = \text{amount of cyanocobalamin assumed to be present in the portion of Tablets taken (µg)} \]

Calculate the percentage of the labeled amount of cyanocobalamin (C₆₃H₉₈CoN₁₂O₁₉P) in the portion of the Tablets taken:

\[ \text{Result} = \left( \frac{\text{antilog } M}{N} \right) \times 100 \]

Change to read:

• FOLIC ACID, Method 1
  • Proceed as directed in Folic Acid Assay (411), Assay, Procedure 1 (USP 1-May-2020)
  • Acceptance criteria: 90.0%–150.0% of the labeled amount of folic acid (C₇₁H₁₇N₁₂O₆)

Change to read:

• FOLIC ACID, Method 2
  • Proceed as directed in Folic Acid Assay (411), Assay, Procedure 2 (USP 1-May-2020)
  • Acceptance criteria: 90.0%–150.0% of the labeled amount of folic acid (C₇₁H₁₇N₁₂O₆)

• CALCIUM PANTOTHENATE, Method 1
  • Mobile phase: Phosphoric acid and water (1:1000)
  • Standard solution: Add 50 mL of water and 7.1 g of dibasic sodium phosphate, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 6.7.
  • Standard solution: 0.6 mg/mL of USP Calcium Pantothenate RS in the Internal standard solution
  • Sample solution: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 15 mg of calcium pantothenate, to a centrifuge tube. Add 25.0 mL of the Internal standard solution, and shake vigorously for 10 min. Centrifuge, filter, and use the clear filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.5 mL/min
Injection volume: 10 µL

System suitability

Sample: Standard solution

[NOTE—The relative retention times for calcium pantothenate and p-hydroxybenzoic acid are about 0.5 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: Standard solution and Sample solution

Measure the peak areas of calcium pantothenate and the internal standard.

Calculate the percentage of the labeled amount of calcium pantothenate (C₁₈H₂₇CaN₂O₁₀) in the portion of Tablets taken:

Result = \( R_R \times C_C/C_C \times 100 \)

Where:

\( R_R \) = peak area ratio of calcium pantothenate to p-hydroxybenzoic acid from the Sample solution

\( R_S \) = peak area ratio of calcium pantothenate to p-hydroxybenzoic acid from the Standard solution

\( C_C \) = concentration of USP Calcium Pantothenate RS in the Standard solution (mg/mL)

\( C_U \) = nominal concentration of calcium pantothenate in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of calcium pantothenate (C₁₈H₂₇CaN₂O₁₀)

Change to read:

• **CALCIUM PANTOTHENATE, Method 2**
  
  **Standard stock solution:** Dissolve 50 mg of USP Calcium Pantothenate RS in 500 mL of water in a 1000-mL volumetric flask. Add 10 mL of 0.2 N acetic acid and 100 mL of sodium acetate solution (1 in 60), and dilute with water to volume to obtain a concentration of 50 µg/mL of USP Calcium Pantothenate RS. Store under toluene in a refrigerator.

  **Standard solution:** On the day of the assay, dilute a volume of the Standard stock solution with water to obtain a concentration of 0.01–0.04 µg/mL of calcium pantothenate, the exact concentration being such that the responses obtained as directed in the Analysis, using 2.0 and 5.0 µL of the Standard solution, are within the linear portion of the log-concentration-response curve.

  **Sample solution:** Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 50 mg of calcium pantothenate, to a 1000-mL volumetric flask containing 500 mL of water. Add 10 mL of 0.2 N acetic acid and 100 mL of sodium acetate solution (1 in 60), dilute with water to volume, and filter. Dilute a volume of this solution to obtain a solution having approximately the same concentration as that of the Standard solution.

  **Acid-hydrolyzed casein solution:** Mix 100 g of vitamin-free casein with 500 mL of 6 N hydrochloric acid, and reflux the mixture for 8–12 h. Remove the hydrochloric acid from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in water, adjust the solution with 1 N sodium hydroxide to a pH of 3.5 ± 0.1, and dilute with water to make 1000 mL. Add 20 g of activated charcoal, stir for 1 h, and filter. Repeat the treatment with activated charcoal. Store under toluene in a cool place at a temperature not below 10°C. Filter the solution if a precipitate forms during storage.

  **Cystine–tryptophan solution:** Suspend 4.0 g of L-cystine in a solution of 1.0 g of L-tryptophan (or 2.0 g of DL-tryptophan) in 700–800 mL of water, heat to 70°C–80°C, and add dilute hydrochloric acid (1 in 2) dropwise, with stirring, until the solids are dissolved. Cool, and dilute with water to make 1000 mL. Store under toluene in a cool place at a temperature not below 10°C.

  **Adenine–guanine–uracil solution:** Dissolve 200 mg each of adenine sulfate, guanine hydrochloride, and uracil, with the aid of heat, in 10 mL of 4 N hydrochloric acid. Cool, and dilute with water to make 200 mL. Store under toluene in a refrigerator.

  **Polysorbate 80 solution:** 100 mg/mL of polysorbate 80 in alcohol.

  **Riboflavin–thiamine hydrochloride–biotin solution:** 20 µg/mL of riboflavin, 10 µg/mL of thiamine hydrochloride, and 0.04 µg/mL of biotin in 0.02 N acetic acid. Store under toluene, protected from light, in a refrigerator.

  **p-Aminobenzoic acid–niacin–pyridoxine hydrochloride solution:** 10 µg/mL of p-aminobenzoic acid, 50 µg/mL of niacin, and 40 µg/mL of pyridoxine hydrochloride in a mixture of neutralized alcohol and water (1:3). Store in a refrigerator.

  **Salt solution A:** Dissolve 25 g of monobasic potassium phosphate and 25 g of dibasic potassium phosphate in water to make 500 mL. Add 5 drops of hydrochloric acid. Store under toluene.

  **Salt solution B:** Dissolve 10 g of magnesium sulfate, 0.5 g of sodium chloride, 0.5 g of ferrous sulfate, and 0.5 g of manganese sulfate in water to make 500 mL. Add 5 drops of hydrochloric acid. Store under toluene.

**Basal medium stock solution:** Dissolve the anhydrous dextrose and anhydrous sodium acetate in the solutions previously mixed according to • Table 4 (USP 1-May-2020) and adjust with 1 N sodium hydroxide to a pH of 6.8. Dilute with water to 250 mL.

**Table 4** (USP 1-May-2020)

<table>
<thead>
<tr>
<th>Solution Type</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-hydrolyzed casein solution</td>
<td>25 mL</td>
</tr>
<tr>
<td>Cystine–tryptophan solution</td>
<td>25 mL</td>
</tr>
<tr>
<td>Polysorbate 80 solution</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>Dextrose, anhydrous</td>
<td>10 g</td>
</tr>
<tr>
<td>Sodium acetate, anhydrous</td>
<td>5 g</td>
</tr>
<tr>
<td>Adenine–guanine–uracil solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Riboflavin–thiamine hydrochloride–biotin solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>p-Aminobenzoic acid–niacin–pyridoxine hydrochloride solution</td>
<td>5 mL</td>
</tr>
<tr>
<td>Salt solution A</td>
<td>5 mL</td>
</tr>
<tr>
<td>Salt solution B</td>
<td>5 mL</td>
</tr>
</tbody>
</table>

**Stock culture of Lactobacillus plantarum:** Dissolve 2.0 g of yeast extract in 100 mL of water. Add 500 mg of anhydrous dextrose, 500 mg of anhydrous sodium acetate, and 1.5 g of agar, and heat the mixture on a steam bath, with stirring, until the agar dissolves. Add 10–15 mL portions of the hot solution to the test tubes, close or cover the tubes, sterilize in an autoclave at 121°C for 15 min, and allow the tubes to cool in an upright position. Prepare stab cultures in 3 or more of the tubes using a pure culture of Lactobacillus plantarum, incubating for 16–24 h at a temperature between 30°C and 37°C. Store in a refrigerator. Prepare a fresh stab of the stock culture every week, and do not use for the Inoculum if the culture is more than 1 week old.

**Culture medium:** To each of a series of test tubes containing 5.0 mL of Basal medium stock solution add 5.0 mL of water.
containing 0.2 µg of calcium pantothenate. Plug the tubes with cotton, sterilize in an autoclave at 121° for 15 min, and cool.

Inoculum: [NOTE—A frozen suspension of Lactobacillus plantarum may be used as the stock culture, provided it yields an Inoculum comparable to a fresh culture.] Transfer cells from the Stock culture of Lactobacillus plantarum to a sterile tube containing 10 mL of the Culture medium. Incubate this culture for 16–24 h at a temperature between 30° and 37° held constant to within ±0.5°. The cell suspension is the Inoculum.

Analysis

Samples: Standard solution and Sample solution

To similar separate test tubes add, in duplicate, volumes of Standard solution corresponding to 3 or more of the levels specified for the Standard solution, including the levels of 2.0, 3.0, and 4.0 mL. To each tube add 5.0 mL of the Basal medium stock solution and sufficient water to make 10 mL.

To similar separate test tubes add, in duplicate, volumes of the Sample solution corresponding to 3 or more of the levels specified for the Standard solution, including the levels of 2.0, 3.0, and 4.0 mL. To each tube add 5.0 mL of the Basal medium stock solution and sufficient water to make 10 mL. Place one complete set of Standard and sample tubes together in one tube rack and the duplicate set in a second rack or section of a rack, preferably in random order.

Cover the tubes of both series to prevent contamination, and sterilize in an autoclave at 121° for 5 min. Cool, and add 1 drop of Inoculum to each tube, except 2 of the 4 tubes containing no Standard solution (the uninoculated blanks). Incubate the tubes at a temperature between 30° and 37°, held constant to within ±0.5° until, following 16–24 h of incubation, until there has been no substantial increase in turbidity in the tubes containing the highest level of Standard during a 2-h period.

Determine the transmittance of the tubes in the following manner. Mix the contents of each tube, and transfer to an optical container if necessary. Read the transmittance between 540 and 660 nm when a steady state is reached. This steady state is observed a few seconds after agitation when the galvanometer reading remains constant for 30 s or more. Allow approximately the same time interval for the reading on each tube.

With the transmittance set at 1.00 for the uninoculated blank, read the transmittance of the inoculated blank. With the transmittance set at 1.00 for the inoculated blank, read the transmittance for each of the remaining tubes. If there is evidence of contamination with a foreign microorganism, disregard the result of the assay.

Calculation: Prepare a standard concentration-response curve as follows. For each level of the Standard calculate the response from the sum of the duplicate values of the transmittance (Σy) as the difference, y = 2.00 – Σy, Plot this response on the ordinate of cross-section paper against the logarithm of the milliliter of the Standard solution per tube on the abscissa, using for the ordinate either an arithmetic or a logarithmic scale, whichever gives the better approximation to a straight line. Draw the straight line or smooth curve that best fits the plotted points.

Calculate the response, y = 2.00 – Σy, adding together the two transmittances (Σy) for each level of the Sample solution. Read from the standard curve the logarithm of the volume of the Standard solution corresponding to each of those values of y that falls within the range of the lowest and highest points plotted for the Standard.

Subtract from each logarithm so obtained the logarithm of the volume, in mL, of the Sample solution to obtain the difference, X, for each dosage level. Average the values of X for each of 3 or more dosage levels to obtain X̄, which equals the log-relative potency, M′, of the Sample solution. Determine the quantity, in mg, of calcium pantothenate (C_{18}H_{32}CaN_{2}O_{12}) in the portion of Tablets taken:

\[
\text{antilog } M = \text{antilog} (M' + \log R)
\]

\[
R = \frac{\text{amount of calcium pantothenate assumed to be present in the portion of the Tablets taken (mg)}}{\text{nominal amount of calcium pantothenate in the portion of the Tablets taken (mg)}}
\]

\[
N = \frac{\text{[(antilog } M)\times N]}{100}
\]

Replication: Repeat the entire determination at least once, using separately prepared Sample solutions. If the difference between the two log-potencies M is NMT 0.08, their mean, M̄, is the assayed log-potency of the test material (see Design and Analysis of Biological Assays (111), The Confidence Interval and Limits of Potency). If the two determinations differ by more than 0.08, conduct one or more additional determinations. From the mean of two more values of M that do not differ by more than 0.15, compute the mean potency of the preparation under assay.

Acceptance criteria: 90.0%–150.0% of the labeled amount of calcium pantothenate (C_{18}H_{32}CaN_{2}O_{12}).

- **CALCIUM PANTOTHENATE, Method 3**

Buffer solution: Dissolve 10.0 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Methanol and Buffer solution (1:9)

Standard stock solution: 0.25 mg/mL of USP Calcium Pantothenate RS in water. Prepare fresh every 4 weeks. Store in a refrigerator.

Standard solution: 40 µg/mL of USP Calcium Pantothenate RS from the Standard stock solution diluted with water

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 10 mg of calcium pantothenate, to a 250-mL volumetric flask. Add 10 mL of methanol, and swirl the flask to disperse. Dilute with water to volume, mix, and filter.

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

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm × 30-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 25 µL

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: Standard solution and Sample solution

Measure the peak areas of calcium pantothenate.

Calculate the percentage of calcium pantothenate (C_{18}H_{32}CaN_{2}O_{12}) in the portion of Tablets taken:

\[
\text{Result} = \left(\frac{r_f}{r_i}\right) \times \left(\frac{C_f}{C_i}\right) \times 100
\]
Acetonitrile, glacial acetic acid, and water
A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium

Chromatographic system
Column: 4.6-mm × 25-cm; packing L1
Injection volume: 10 µL
Detector: LC
Mode: LC

Acceptance criteria: 90.0%–150.0% of the labeled amount of calcium pantothenate

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

Change to read:

• Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine, Method 1
[NOTE—Use low-actinic glassware throughout this procedure.]
Diluent: Acetonitrile, glacial acetic acid, and water (5:1:94)
Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL
Standard solution: [NOTE—Use USP Niacin RS in place of USP Niacinamide RS for formulations containing niacin.]
Transfer 80 mg of USP Niacinamide RS, 20 mg of USP Pyridoxine Hydrochloride RS, 20 mg of USP Riboflavin RS, and 20 mg of USP Thiamine Hydrochloride RS to a 200-mL volumetric flask, and add 180 mL of Diluent. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer, until all the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with Diluent to volume.
Sample solution: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 10 mg of niacinamide and 2.5 mg each of pyridoxine hydrochloride, riboflavin, and thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of Diluent, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Change to read:

• Niacin, Method 2
[Proceed as directed in Niacin or Niacinamide Assay (441), Assay, Chromatographic Methods, Procedure Z (USP 1-May-2020)]
Acceptance criteria: 90.0%–150.0% of the labeled amount of niacin (C_{6}H_{5}NO_{2})

Change to read:

• Niacinamide, Method 2
[Proceed as directed in Niacin or Niacinamide Assay (441), Assay, Chromatographic Methods, Procedure Z (USP 1-May-2020)]
Acceptance criteria: 90.0%–150.0% of the labeled amount of niacinamide (C_{6}H_{7}NaN_{2}O_{3})

• Pyridoxine Hydrochloride, Method 2
[NOTE—Use low-actinic glassware throughout this procedure.]
Extraction solvent, Mobile phase, and Sample solution: Prepare as directed for Niacin, Method 2.
Standard stock solution: 0.1 mg/mL of USP Pyridoxine Hydrochloride RS in the Extraction solvent
Standard solution: Transfer 5.0 mL of Standard stock solution to a 25-mL volumetric flask, and dilute with the Extraction solvent to volume.
Chromatographic system
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: Standard solution
Suitability requirements
Relative standard deviation: NMT 3.0%
Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of the labeled amount of vitamin B_{1}, as thiamine ion (C_{2}H_{5}N_{2}OS+), vitamin B_{2} as riboflavin (C_{7}H_{9}NO_{4}), vitamin B_{3} as niacin (C_{6}H_{4}NO_{2}) or niacinamide (C_{6}H_{7}NO_{3}), vitamin B_{6} as pyridoxine (C_{6}H_{11}NO_{3}) (ERR 1-May-2020) in the portion of Tablets taken:

\[ \text{Result} = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_s}{C_u} \right) \times 100 \]

where:
- \( r_u \) = peak area of the relevant vitamin from the corresponding Sample solution
- \( r_s \) = peak area of the relevant vitamin from the corresponding Standard solution
- \( C_s \) = concentration of the relevant vitamin in the corresponding Standard solution (µg/mL)
- \( C_u \) = nominal concentration of the relevant vitamin in the Sample solution (mg/mL)

Example:

\[ \text{Result} = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_s}{C_u} \right) \times 100 \]

Result = (r_u/r_s) × (C_s/C_u) × 100

Example:

\[ \text{Result} = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_s}{C_u} \right) \times 100 \]

Result = (r_u/r_s) × (C_s/C_u) × 100

Example:

\[ \text{Result} = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_s}{C_u} \right) \times 100 \]

Result = (r_u/r_s) × (C_s/C_u) × 100
Acceptance criteria: 90.0%–150.0% of the labeled amount of pyridoxine hydrochloride (C₆H₅N₂O₂·HCl)

Change to read:

- **Riboflavin, Method 2**
  ▲ Proceed as directed in Riboflavin Assay (481), Assay, Chromatographic Methods, Procedure 2.▲ (USP 1-May-2020)
  Acceptance criteria: 90.0%–150.0% of the labeled amount of riboflavin (C₁₇H₂₀N₄O₅) ▲ (USP 1-May-2020)

Change to read:

- **Thiamine, Method 2**
  ▲ Proceed as directed in Thiamine Assay (531), Assay, Chromatographic Methods, Procedure 2.▲ (USP 1-May-2020)
  Acceptance criteria: 90.0%–150.0% of the labeled amount of thiamine ▲ as thiamine ion (C₁₇H₂₀N₄OS+) ▲ (USP 1-May-2020)

Change to read:

- **Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine**
  [Note—Use low-actinic glassware throughout this procedure.] Reagent: 25 mg/mL of edetate disodium in water
  Mobile phase: Transfer 0.4 mL of triethylamine, 150 mL of 1.5 mg/mL of USP Niacin RS or USP Niacinamide RS, 0.24 mg/mL of USP Pyridoxine Hydrochloride RS, 0.08 mg/mL of USP Riboflavin RS, and 0.24 mg/mL of USP Thiamine Hydrochloride RS in the Reagent, with heating if necessary
  Standard stock solution: 1.5 mg/mL of USP Niacin RS or USP Niacinamide RS, 0.24 mg/mL of USP Pyridoxine Hydrochloride RS, 0.08 mg/mL of USP Riboflavin RS, and 0.24 mg/mL of USP Thiamine Hydrochloride RS in the Reagent, with heating if necessary
  Standard solution: Transfer 5.0 mL of the Standard stock solution to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°C, and cool. Filter, discarding the first few milliliters of the filtrate.
  Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 7.5 mg of niacin or niacinamide, 1.2 mg of pyridoxine hydrochloride, 0.4 mg of riboflavin, and 1.2 mg of thiamine hydrochloride to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°C, and cool. Filter, discarding the first few milliliters of the filtrate.

Chromatographic system
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; packing L7
Column temperature: 50°C
Flow rate: 2.0 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 B₆ as thiamine ion (C₁₇H₂₀N₄OS+), vitamin B₂ as riboflavin (C₁₇H₂₀N₄O₅), vitamin B₃ as niacin (C₆H₅N₂O₂·HCl) or niacinamide (C₆H₄N₂O₄), vitamin B₁₂ as pyridoxine (C₁₇H₂₀N₄O₅) ▲ (E1 May-2020) in the portion of Tablets:

\[ \text{Result} = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_s}{C_u} \right) \times 100 \]

\( r_u \) = peak area of the relevant vitamin from the corresponding Sample solution
\( r_s \) = peak area of the relevant vitamin from the corresponding Standard solution
\( C_s \) = concentration of the relevant vitamin Reference Standard in the corresponding Standard solution (µg/mL)
\( C_u \) = nominal concentration of the relevant vitamin in the corresponding Sample solution (µg/mL) ▲ (USP 1-May-2020)

Acceptance criteria: 90.0%–150.0% of the labeled amount of each individual vitamin ▲ (USP 1-May-2020)

Add the following:

- **Folic Acid Method 3: Ascorbic Acid, Niacin or Niacinamide, Pyridoxine Hydrochloride, Calcium Pantothenate, Riboflavin, and Thiamine, Method 4**
  [Note—Use low-actinic glassware.] Solution A: 0.56 g of edetate disodium and 2.04 g of potassium phosphate monobasic per 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.
  Solution B: Acetonitrile, acetic acid, glacial, and water (5:1:94)
  Solution C: Aqueous solution containing 2 mL of ammonium hydroxide, 1 g of sodium perchlorate per 100 mL
  Solution D: Aqueous solution containing 2 mL of ammonium hydroxide, 1 g of sodium perchlorate and 2 g of sodium ascorbate per 100 mL
  Mobile phase A: 0.02% trifluoroacetic acid in water
  Mobile phase B: Acetonitrile
  Mobile phase: See Table 5.

<table>
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<th>Time (min)</th>
<th>Mobile Phase A (%)</th>
<th>Mobile Phase B (%)</th>
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<tr>
<td>25.0</td>
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</tbody>
</table>

Ascorbic acid standard stock solution: 1.0 mg/mL of ascorbic acid from USP Ascorbic Acid RS in Solution A
Thiamine standard stock solution: 0.2 mg/mL of thiamine from USP Thiamine Hydrochloride RS in Solution B. The solution can be placed on a steam bath for about 5–10 min with frequent swirling to assist in dissolution.
Riboflavin standard stock solution: 0.2 mg/mL of USP Riboflavin RS in Solution B. Heat the solution to 65°–70º for 10 min to assist in dissolution.
Niacin or niacinamide standard stock solution: 1.0 mg/mL of USP Niacin RS or USP Niacinamide RS in Solution A
Pyridoxine standard stock solution: 0.2 mg/mL of pyridoxine from USP Pyridoxine Hydrochloride RS in Solution C

Folic acid standard stock solution: 0.2 mg/mL of folic acid from USP Folic Acid RS in Solution C

Calcium pantothenate standard stock solution: 1.0 mg/mL of calcium pantothenate from USP Calcium Pantothenate RS in Mobile phase A

System suitability solution: Transfer 0.5 mL of Ascorbic Acid standard stock solution, 2.5 mL of Thiamine standard stock solution, and 0.5 mL of Niacin or niacinamide standard stock solution into 10-mL volumetric flask and dilute to volume with Mobile phase A.

Sample solution 1: Weigh and finely powder NLT 20 Tablets. Transfer a calculated amount of accurately weighed powder into a 100-mL volumetric flask to obtain a solution containing known nominal concentrations of thiamine, riboflavin, and calcium pantothenate in the ranges of 5–30 µg/mL for thiamine, 10–60 µg/mL for riboflavin, and 25–150 µg/mL for calcium pantothenate. Add Solution B to about half the volume and mix on a vortex mixer for 5 min. Heat the flask at 65°–70° for 5 min, swirling frequently and sonicate additionally for 5 min. Cool to room temperature, dilute with Mobile phase A to volume, and mix well. Pass a portion of the solution through a suitable filter of 0.45-µm pore size and discard the first milliliter of the filtrate.

Sample solution 2: Transfer a portion of finely powdered Tablets into a 100-mL volumetric flask to obtain a solution containing known nominal concentrations of ascorbic acid and niacin or niacinamide in the ranges of 50–300 µg/mL for ascorbic acid and 12–65 µg/mL for niacin or niacinamide, add Solution A to about half the volume and mix on a vortex mixer for 5 min, sonicate for 10 min with occasional shaking. Cool to room temperature, dilute with Mobile phase A to volume, mix well. Pass a portion of the solution through a suitable filter of 0.45-µm pore size and discard the first milliliter of the filtrate.

Sample solution 3: Transfer a portion of finely powdered Tablets into a 100-mL volumetric flask to obtain a solution containing known nominal concentrations of pyridoxine hydrochloride and folic acid in the ranges of 5–30 µg/mL for pyridoxine hydrochloride and 2–12 µg/mL for folic acid, add Solution D to about half the volume, heat on a water bath at 40° for 5 min and sonicate for 10 min with occasional shaking. Cool to room temperature, dilute with Solution D to volume, and mix well. Pass a portion of the solution through a suitable filter of 0.45-µm pore size and discard the first milliliter of the filtrate.

Standard solution 1: Accurately transfer calculated volumes of Thiamine standard stock solution, Riboflavin standard stock solution, and Calcium pantothenate standard stock solution into a suitable volumetric flask to obtain a solution with final vitamin concentrations similar to the nominal concentrations in the Sample solution 1 for the corresponding vitamins, dilute with Mobile phase A to volume, mix well.

Standard solution 2: Accurately transfer calculated volumes of Ascorbic acid standard stock solution and Niacin or niacinamide standard stock solution, into a suitable volumetric flask to obtain a solution with final vitamin concentrations similar to the nominal concentrations in the Sample solution 2 for the corresponding vitamins, dilute with Mobile phase A to volume, mix well.

Standard solution 3: Accurately transfer calculated volumes of Pyridoxine standard stock solution and Folic acid standard stock solution into a suitable volumetric flask to obtain a solution with final vitamin concentrations similar to the nominal concentrations in Sample solution 3 for the corresponding vitamins, dilute with Solution C to volume, and mix well.

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

Mode: LC

Detectors
For calcium pantothenate: UV 210 nm
For thiamine, ascorbic acid, niacin or niacinamide, and riboflavin: UV 254 nm
For pyridoxine hydrochloride and folic acid: UV 280 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25°
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Samples: System suitability solution, Standard solution 1, Standard solution 2 and Standard solution 3

Suitability requirements
Resolution: NLT 3.5 between the thiamine and ascorbic acid peaks and NLT 4.0 between the ascorbic acid and niacin or niacinamide peaks, System suitability solution

Relative standard deviation: NMT 2.0% for each individual peak, Standard solution 1, Standard solution 2, and Standard solution 3

Analysis
Samples: Sample solutions 1–3 and Standard solutions 1–3

Calculate the percentage of the labeled amount of vitamin B1 as thiamine ion (C6H5N2O5H+, vitamin B2 as riboflavin (C12H6N2O5), vitamin B3 as niacin (C6H4NO) or niacinamide (C6H4NO2), vitamin B6 as pyridoxine (C12H14N2O5), calcium pantothenate (C15H25CaN2O10), ascorbic acid (C6H8O6), and folic acid (C19H19N7O6), in the portion of Tablets taken:

Result = (r0/r1) × (C/C0) × 100

r0 = peak area of the relevant vitamin from the corresponding Sample solution
r1 = peak area of the relevant vitamin from the corresponding Standard solution
C = concentration of the relevant vitamin Reference Standard in the corresponding Standard solution (µg/mL)
C0 = nominal concentration of the relevant vitamin in the corresponding Sample solution (µg/mL)

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

[NOTE—In the following assays commercially available atomic absorption standard solutions for the minerals, where applicable, may be used where preparation of a Standard stock solution is described. Use deionized water where water is specified. Where atomic absorption spectrophotometry is specified in the assay, the Standard solutions and the Sample solution may be diluted quantitatively with the solvent specified, if necessary, to yield solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

• CALCIUM, Method 1

Lanthanum chloride solution: 267 mg/mL of lanthanum chloride heptahydrate in 0.125 N hydrochloric acid

Calcium standard solution: 400 µg/mL of calcium. Dissolve 1.001 g of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, in 25 mL of 1 N hydrochloric acid. Boil to expel carbon dioxide, and dilute with water to 1000 mL.


15/24
Standard stock solution: 100 µg/mL of calcium from the
Calcium standard solution diluted with 0.125 N hydrochloric acid

Standard solutions: Into separate 100-mL volumetric flasks pipet 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the Standard stock solution. To each flask add 1.0 mL of the Lanthanum chloride solution, and dilute with water to volume to obtain concentrations of 1.0, 1.5, 2.0, 2.5, and 3.0 µg/mL of calcium.

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 5 Tablets, to a porcelain crucible. Heat the crucible in a muffle furnace maintained at 550° for 6–12 h, and cool. Add 60 mL of hydrochloric acid, and boil gently on a hot plate or steam bath for 30 min, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask. Rinse the crucible with small portions of 6 N hydrochloric acid, and add the rinsings to the flask. Dilute with water to volume, and filter, discarding the first 5 mL of the filtrate. Dilute this solution quantitatively, with 0.125 N hydrochloric acid, to obtain a concentration of 2 µg/mL of calcium, adding 1 mL of the Lanthanum chloride solution per 100 mL of the final volume.

Instrumental conditions
(See Atomic Absorption Spectroscopy (852).)
Mode: Atomic absorption spectroscopy
Analytical wavelength: Calcium emission line at 422.7 nm
Lamp: Calcium hollow-cathode
Flame: Air–acetylene
Blank: 0.125 N hydrochloric acid

Analysis
Samples: Standard solutions and Sample solution
Determine the absorbances of the solutions against the Blank. Plot the absorbances of the Standard solutions versus the concentration, in µg/mL, of chromium, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of chromium in the Sample solution.
Calculate the percentage of the labeled amount of chromium (Cr) in the portion of Tablets taken:

\[
\text{Result} = (C/C_r) \times 100
\]

C = measured concentration of chromium in the Sample solution (µg/mL)
C_r = nominal concentration of chromium in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–160.0% of the labeled amount of chromium (Cr)

• COPPER, Method 1
Copper standard solution: Dissolve 1.00 g of copper foil in a minimum volume of a 50% solution of nitric acid, and dilute with a 1% solution of nitric acid to 1000 mL. This solution contains 1000 µg/mL of copper.

Standard stock solution: 100 µg/mL of copper from the Copper standard solution diluted with 0.125 N hydrochloric acid

Standard solutions: To separate 200-mL volumetric flasks transfer 1.0, 2.0, 4.0, 6.0, and 8.0 mL of the Standard stock solution. Dilute with water to volume to obtain concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 µg/mL of copper.

Sample solution: Prepare as directed for Calcium, Method 1, except obtain a concentration of 2 µg/mL of copper and omit the use of the Lanthanum chloride solution.

Instrumental conditions
(See Atomic Absorption Spectroscopy (852).)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Copper emission line at 324.7 nm
Lamp: Copper hollow-cathode
Flame: Air–acetylene
Blank: 0.125 N hydrochloric acid

Analysis
Samples: Standard solutions and Sample solution
Determine the absorbances of the solutions against the Blank. Plot the absorbances of the Standard solutions versus the concentration, in µg/mL, of copper, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of copper in the Sample solution.
Calculate the percentage of the labeled amount of copper (Cu) in the portion of Tablets taken:

\[
\text{Result} = (C/C_u) \times 100
\]

C = concentration of copper in the Sample solution from the graph (µg/mL)
Fluoride, in 15 mL of water, and shake vigorously. Rinse the sides of the flask with 15 mL of water, and allow to stand for 10 min. Dilute with water to 85 mL, adjust with 1 N sodium hydroxide to a pH of 10.4 ± 0.1, and dilute with water to 100 mL. Prepare as directed for the Standard solution, beginning with “Filter, discarding the first 15 mL of the filtrate”.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC
Detector: Conductivity
Columns
Guard: 4.6-mm × 3-cm; packing L17
Analytical: 7.8-mm × 30-cm; packing L17
Flow rate: 0.5 mL/min
Injection volume: 100 µL
System suitability
Sample: Standard solution
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution
Measure the peak areas of fluoride. Calculate the percentage of the labeled amount of fluoride (F) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{C}{C_0} \right) \times 100
\]

- \(C\) = measured concentration of fluoride in the Sample solution (µg/mL)
- \(C_0\) = nominal concentration of fluoride in the Sample solution (µg/mL)
- \(r_f\) = peak area from the Sample solution
- \(r_s\) = peak area from the Standard solution
- \(C_s\) = concentration of fluoride in the Standard solution (µg/mL)
- \(C_0\) = nominal concentration of fluoride in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–160.0% of the labeled amount of fluoride (F)
• **IODIDE**

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.

**Analysis:** Transfer an amount of finely powdered Tablets, equivalent to 3 mg of iodide, to a nickel crucible. Add 5 g of sodium carbonate, 5 mL of 50% (w/v) 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 watchglass, 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, 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°. Add 1 mL of phosphoric acid and 0.5 g of potassium iodide, and titrate the liberated iodine with 0.005 N sodium thiosulfate VS, 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 Tablets taken:

\[
\text{Result} = \frac{V \times N_A \times F \times I_{eq} \times (A/w)}{W} \times 100/L
\]

- \( V \) = volume of sodium thiosulfate consumed (mL)
- \( N_A \) = actual normality of the sodium thiosulfate solution used
- \( F \) = correction factor to convert mg to µg, 1000 µg/mL
- \( I_{eq} \) = milliequivalent weight of iodine, 21.16 mg/meq
- \( A/w \) = average weight of the Tablets
- \( W \) = weight of the portion of the Tablets taken
- \( L \) = labeled amount of iodine (µg/Tablet)

**Acceptance criteria:** 90.0%–160.0% of the labeled amount of iodine (I)

• **IRON, Method 1**

Iron standard stock solution: Transfer 100 mg of iron powder to a 1000-mL volumetric flask. Dissolve in 25 mL of 6 N hydrochloric acid, and dilute with water to volume.

**Standard solutions:** To separate 100-mL volumetric flasks, transfer 2.0, 4.0, 5.0, 6.0, and 8.0 mL of the *Iron standard stock solution*. Dilute the contents of each flask with water to volume to obtain concentrations of 2.0, 4.0, 5.0, 6.0, and 8.0 µg/mL of iron.

**Sample solution:** Prepare as directed for *Calcium, Method 1*, except obtain a concentration of 5 µg/mL of iron and omit the use of the *Lanthanum chloride solution*.

**Instrumental conditions**

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** Iron emission line at 248.3 nm

**Lamp:** Iron hollow-cathode

**Flame:** Air–acetylene

**Blank:** 0.125 N hydrochloric acid

**Analysis**

**Samples:** Standard solutions and Sample solution

Determine the absorbances of the solutions against the Blank. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of iron, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of iron in the *Sample solution*.

Calculate the percentage of the labeled amount of iron (Fe) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{C}{C_0} \right) \times 100
\]

- \( C \) = measured concentration of iron in the *Sample solution* (µg/mL)
- \( C_0 \) = nominal concentration of iron in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–125.0% of the labeled amount of iron (Fe)

• **MAGNESIUM, Method 1**

Lanthanum chloride solution: Prepare as directed in *Calcium, Method 1*.

**Magnesium standard solution:** Transfer 1.0 g of magnesium ribbon to a 1000-mL volumetric flask, dissolve in 50 mL of 6 N hydrochloric acid, dilute with water to volume, and mix to obtain a solution with a known concentration of 1000 µg/mL of magnesium.

**Standard stock solution:** 20 µg/mL of magnesium from the *Magnesium standard solution* diluted with 0.125 N hydrochloric acid

**Standard solutions:** To separate 100-mL volumetric flasks transfer 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the *Standard stock solution*. To each flask add 1.0 mL of the *Lanthanum chloride solution*, and dilute with 0.125 N hydrochloric acid to volume to obtain concentrations of 0.2, 0.3, 0.4, 0.5, and 0.6 µg/mL of magnesium.

**Sample solution:** Prepare as directed for *Calcium, Method 1*, except obtain a concentration of 0.4 µg/mL of magnesium.

**Instrumental conditions**

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** Magnesium emission line at 285.2 nm

**Lamp:** Magnesium hollow-cathode

**Flame:** Air–acetylene

**Blank:** 0.125 N hydrochloric acid containing 1 mL of *Lanthanum chloride solution* per 100 mL

**Analysis**

**Samples:** Standard solutions and Sample solution

Determine the absorbances of the solutions against the Blank. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of magnesium, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of magnesium in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium (Mg) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{C}{C_0} \right) \times 100
\]

- \( C \) = measured concentration of magnesium in the *Sample solution* (µg/mL)
- \( C_0 \) = nominal concentration of magnesium in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–125.0% of the labeled amount of magnesium (Mg)

• **MANGANESE, Method 1**

Manganese standard stock solution: Transfer 1.00 g of manganese to a 1000-mL volumetric flask. Dissolve in 25 mL of 6 N hydrochloric acid, and dilute with water to volume.
20 mL of nitric acid, dilute with 6 N hydrochloric acid to volume, and mix to obtain a solution with a concentration of 1000 µg/mL of molybdenum.

**Standard stock solution:** 50 µg/mL of manganese from the *Manganese standard stock solution* diluted with 0.125 N hydrochloric acid

**Standard solutions:** To separate 100-ml volumetric flasks transfer 1.0, 1.5, 2.0, 3.0, and 4.0 mL of the **Standard stock solution**. Dilute the contents of each flask with 0.125 N hydrochloric acid to volume to obtain solutions with known concentrations of 0.5, 0.75, 1.0, 1.5, and 2.0 µg/mL of manganese.

**Sample solution:** Prepare as directed for *Calcium, Method 1*, except obtain a concentration of 1 µg/mL of manganese and omit the use of the *Lanthanum chloride solution*.

### Instrumental conditions

(See Atomic Absorption Spectroscopy (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** Manganese emission line at 279.5 nm

**Flame:** Nitrous oxide–acetylene

**Blank:** Diluent and perchloric acid (20:1)

### Analysis

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

Determine the absorbances of the solutions against the **Blank**. Plot the absorbances of the **Standard solutions** versus the concentration, in µg/mL, of molybdenum, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of manganese in the **Sample solution**.

Calculate the percentage of the labeled amount of manganese (Mn) in the portion of Tablets taken:

\[
\text{Result} = \left(\frac{C}{C_0}\right) \times 100
\]

\(C\) = measured concentration of manganese in the **Sample solution** (µg/mL)

\(C_0\) = nominal concentration of manganese in the **Sample solution** (µg/mL)

**Acceptance criteria:** 90.0%–125.0% of the labeled amount of molybdenum (Mo)

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**MOLYBDENUM, Method 1**

**Diluent:** 20 mg/mL of ammonium chloride in water

**Molybdenum standard solution:** Transfer 1.0 g of molybdenum wire to a 1000-ml volumetric flask, and dissolve in 50 mL of nitric acid, warming if necessary. Dilute with water to volume, and mix to obtain a solution with a concentration of 1000 µg/mL of molybdenum.

**Standard stock solution:** 100 µg/mL of molybdenum from the *Molybdenum standard solution* diluted with water

**Standard solutions:** To separate 100-ml volumetric flasks transfer 2.0, 10.0, and 25.0 mL of the **Standard stock solution**, and add 5.0 mL of perchloric acid to each flask. Gently boil the solution in each flask for 15 min, cool to room temperature, and dilute each with **Diluent** to volume to obtain concentrations of 5.0, 10.0, and 25.0 µg/mL of molybdenum.

**Sample solution:** Transfer a portion of the powder, equivalent to 1000 µg of molybdenum, to a suitable flask, and add 12 mL of nitric acid. [NOTE—The volume of nitric acid may be varied to ensure that the powder is uniformly dispersed.] Carefully swirl the flask to disperse the test specimen. Sonicate for 10 min or until the test specimen is completely dissolved. Gently boil the solution for 15 min, and cool to room temperature. Carefully add 8 mL of perchloric acid, heat until perchloric acid fumes appear, and swirl the flask to dissipate the fumes. Repeat the heating and swirling until the fumes appear again. Cool to room temperature. Quantitatively transfer the contents of the flask to a 100-ml volumetric flask with the aid of the **Diluent**, and dilute with **Diluent** to volume.

### Instrumental conditions

(See Atomic Absorption Spectroscopy (852).)

**Mode:** Atomic absorption spectrophotometry

#### Analytical wavelength

313.3 nm

**Flame:** Molybdenum hollow-cathode

**Blank:** Diluent and perchloric acid (20:1)

### Analysis

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

Determine the absorbances of the solutions against the **Blank**. Plot the absorbances of the **Standard solutions** versus the concentration, in µg/mL, of molybdenum, and draw the straight line best fitting the 3 plotted points. From the graph, determine the concentration (C), in µg/mL, of molybdenum in the **Sample solution**.

Calculate the percentage of the labeled amount of molybdenum (Mo) in the portion of Tablets taken:

\[
\text{Result} = \left(\frac{C}{C_0}\right) \times 100
\]

\(C\) = measured concentration of molybdenum in the **Sample solution** (µg/mL)

\(C_0\) = nominal concentration of molybdenum in the **Sample solution** (µg/mL)

**Acceptance criteria:** 90.0%–160.0% of the labeled amount of molybdenum (Mo)

---

**Change to read:**

- **MOLYBDENUM, Method 2**

**Sodium fluoride solution:** Add 200 mL of water to 10 g of sodium fluoride, stir until the solution is saturated, and filter. Store in a polyethylene bottle.

**Ferrous sulfate solution:** 4.98 mg/mL of ferrous sulfate in water

**Potassium thiocyanate solution:** 200 mg/mL of potassium thiocyanate in water

**20% stannous chloride solution:** Transfer 40 mg of stannous chloride to a beaker, add 20 mL of 6.5 N hydrochloric acid solution, and heat the solution until the stannous chloride is dissolved. Cool, and dilute with water to 100 mL.

**Diluted stannous chloride solution:** 20% stannous chloride solution diluted with water (1 in 25). Prepare this solution fresh at the time of use.

**Standard solution:** 20 µg/mL of molybdenum in water

**Sample:** A portion of finely powdered Tablets equivalent to 40 µg of molybdenum

### Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

**Mode:** Vis

**Analytical wavelength:** 465 nm

**Cell:** 1 cm

**Blank:** Amyl alcohol

### Analysis

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

Transfer the **Sample** and 2.0 mL of the **Standard solution** to separate 200-mL beakers. Add 20 mL of nitric acid to each beaker. Cover each beaker with a watchglass, and boil slowly on a hot plate for 45 min. Cool to room temperature. Add 6 mL of perchloric acid, cover the beakers with a watchglass, and continue the heating until digestion is complete, as indicated when the liquid becomes colorless or pale yellow. Evaporate the solutions
in the beakers to dryness. Rinse the sides of the beakers and the watchglasses with water, and add more water to complete 50 mL in each beaker. Gently boil the water solution for a few min. Cool to room temperature. Add 2 drops of methyl orange TS, and neutralize with ammonium hydroxide. Add 8.2 mL of hydrochloric acid. Quantitatively transfer the contents of the beakers to separate 100-mL volumetric flasks, rinse the beakers with water, transfer the rinsings to the corresponding volumetric flasks, and dilute with water to volume. Transfer 50.0 mL of each solution to separatory funnels. To each separatory funnel add 1.0 mL of Sodium fluoride solution, 0.5 mL of Ferrous sulfate solution, 4.0 mL of Potassium thiocyanate solution, 1.5 mL of 20% stannous chloride solution, and 15.0 mL of amyl alcohol, and shake the separatory funnel for 1 min. Allow the layers to separate, and discard the aqueous layers. Add 25 mL of Diluted stannous chloride solution to each separatory funnel, and shake gently for 15 s. Allow the layers to separate, and discard the aqueous layers. Transfer the organic layer from each separatory funnel to a centrifuge tube, and centrifuge at 2000 rpm for 10 min. Determine the absorbances of the organic phases obtained from the Standard solution and the Sample, and correct with the Blank.

Calculate the percentage of the labeled amount of molybdenum (Mo) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{A_s}{A_0} \right) \times \left[ \left( V \times C_s \right) / M_0 \right] \times 100
\]

where

- \( A_s \) = absorbance of the Sample
- \( A_0 \) = absorbance of the Standard solution
- \( V \) = volume of the Standard solution analyzed, 2.0 mL
- \( C_s \) = concentration of molybdenum in the Standard solution (µg/mL)
- \( M_0 \) = nominal amount of molybdenum in the Sample (µg)

Acceptance criteria: 90.0%–160.0% of the labeled amount of molybdenum (Mo)

• PHOSPHORUS, Method 1

Sulfuric acid solution: Cautiously add sulfuric acid to water (37.5: 100), and mix.

Ammonium molybdate solution: 50 mg/mL of ammonium molybdate in the Sulfuric acid solution and water (2:3). [Note—Dissolve in water first, and then dilute with the Sulfuric acid solution to volume.] (See Ultraviolet-Visible Spectroscopy (857).) Mode: Vis

Ammonium molybdate solution: 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

Phosphorus standard stock solution: 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

Potassium standard solution: 100 µg/mL of potassium from potassium chloride, previously dried at 105° for 2 h, in water

Standard stock solution: 10 µg/mL of potassium from the Potassium standard solution diluted with 0.125 N hydrochloric acid

Standard solutions: Transfer 5.0, 10.0, 15.0, 20.0, and 25.0 mL of the Standard stock solution to separate 100-mL volumetric flasks. Dilute the contents of each flask with 0.125 N hydrochloric acid to volume to obtain solutions containing 0.5, 1.0, 1.5, 2.0, and 2.5 µg/mL of potassium.

Sample solution: Prepare as directed for Calcium, Method 1, except obtain a concentration of 1 µg/mL of potassium and omit the use of the Lanthanum chloride solution.

Instrumental conditions

Analytical wavelength: 766.5 nm

Lamp: Potassium hollow-cathode

Flame: Air–acetylene

Blank: Water

Analysis

Samples: Standard solutions and Sample solution

Determine the absorbances of the solutions against the Blank. Plot the absorbances of the Standard solutions versus the concentration, in µg/mL, of potassium, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration \( C_s \), in µg/mL, of potassium in the Sample solution.

Calculate the percentage of the labeled amount of potassium (K) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{C}{C_s} \right) \times 100
\]

where

- \( C \) = measured concentration of potassium in the Sample solution (µg/mL)
C₀ = nominal concentration of potassium in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–125.0% of the labeled amount of potassium (K)

**Selenium, Method 1**

Diluent: Prepare as directed in Molybdenum, Method 1.

Selenium standard solution: [Caution—Selenium is toxic; handle it with care.] Dissolve 1 g of metallic selenium in a minimum volume of nitric acid. Evaporate to dryness. Add 2 mL of water, and evaporate to dryness. Repeat the addition of water and the evaporation to dryness 3 times. Dissolve the residue in 3 N hydrochloric acid, transfer to a 1000-mL volumetric flask, and dilute with 3 N hydrochloric acid to volume to obtain a concentration of 1000 µg/mL of selenium.

Standard stock solution: 100 µg/mL of selenium from the Selenium standard solution diluted with water

Standard solutions: To separate 100-mL volumetric flasks transfer 5.0, 10.0, and 25.0 mL of the Standard stock solution, and add 5.0 mL of perchloric acid to each flask. Gently boil the solutions for 15 min, cool to room temperature, and dilute each with Diluent to volume to obtain solutions with concentrations of 5.0, 10.0, and 25.0 µg/mL of selenium.

Sample solution: Transfer a portion of the powder, equivalent to 1000 µg of selenium, to a suitable flask, and add 12 mL of nitric acid. [Note—The volume of nitric acid may be varied to ensure that the powder is uniformly dispersed.] Carefully swirl the flask to disperse the test specimen. Sonicate for 10 min or until the test specimen is completely dissolved. Gently boil the solution for 15 min, and cool to room temperature. Carefully add 8 mL of perchloric acid to the flask, heat the flask until perchloric acid fumes appear, and swirl the flask to disperse the fumes. Repeat the heating and swirling until the fumes appear again. Cool to room temperature. Transfer the contents of the flask to a 50-mL volumetric flask with the aid of the Diluent, and dilute with Diluent to volume.

**Instrumental conditions**

(See Atomic Absorption Spectroscopy [852],) Mode: Atomic absorption spectrophotometry

Analytical wavelength: Selenium emission line at 196.0 nm

Lamp: Selenium hollow-cathode

Blank: Diluent and perchloric acid (20:1)

**Analysis**

Samples: Standard solutions and Sample solution Determine the absorbances of the solutions against the Blank. Plot the absorbances of the Standard solutions versus the concentration, in µg/mL, of selenium, and draw the straight line best fitting the 3 plotted points. From the graph, determine the concentration (C), in µg/mL, of selenium in the Sample solution. Calculate the percentage of the labeled amount of selenium (Se) in the portion of Tablets taken:

\[
\text{Result} = \left( \frac{C}{C₀} \right) \times 100
\]

C = concentration of selenium in the Sample solution from the graph (µg/mL)

C₀ = nominal concentration of selenium in the Sample solution (µg/mL)

Acceptance criteria: 90.0%–160.0% of the labeled amount of selenium (Se)

**Selenium, Method 2**

Hydrochloric acid solution: Hydrochloric acid diluted with water (1 in 10)

50% ammonium hydroxide solution: Ammonium hydroxide diluted with water (1 in 2)

Reagent A: 9 mg of edetate disodium and 25 mg/mL of hydroxylamine hydrochloride in water. [Note—Dissolve edetate disodium in a portion of water first, then add hydroxylamine hydrochloride, and dilute with water to volume.]

Reagent B: Transfer 200 mg of 2,3-diaminonaphthalene to a 250-mL separatory funnel, and add 200 mL of 0.1 N hydrochloric acid. Wash the solution with 3 40-mL portions of cyclohexane, and discard the cyclohexane layer. Filter the solution into a brown bottle, and cover the solution with a 1-cm layer of cyclohexane. This solution is stable for 1 week if stored in a refrigerator.

Standard stock solution: [Caution—Selenium is toxic; handle it with care.] Dissolve 1 g of metallic selenium in a minimum volume of nitric acid. Evaporate to dryness. Add 2 mL of water, and evaporate to dryness. Repeat the addition of water and evaporation to dryness 3 times. Dissolve the residue in 3 N hydrochloric acid, transfer to a 1000-mL volumetric flask, and dilute with 3 N hydrochloric acid to volume to obtain a solution with a concentration of 1000 µg/mL of selenium. Dilute a volume of the solution with 0.125 N hydrochloric acid to obtain a concentration of 2.0 µg/mL of selenium.

Standard solution: Transfer 10 mL of the Standard stock solution to a glass-stoppered flask. Add 1 mL of perchloric acid and 1 mL of Hydrochloric acid solution, and dilute with water to 20 mL.

Sample solution: Transfer a portion of finely powdered Tablets, equivalent to 20 µg of selenium, to a suitable flask. Add 10 mL of nitric acid, and warm gently on a hot plate. Continue heating until the initial nitric acid reaction has subsided, then add 3 mL of perchloric acid.

[Caution—Exercise care at this stage because the perchloric acid reaction becomes vigorous.]

Continue heating on the hot plate until the appearance of white fumes of perchloric acid or until the digest begins to darken. Add 0.5 mL of nitric acid and resume heating, adding additional amounts of nitric acid if further darkening occurs. Digest for 10 min after the first appearance of perchloric acid fumes or until the digest becomes colorless. Cool the flask, add 2.5 mL of Hydrochloric acid solution, and return the flask to the hot plate to expel residual nitric acid. Heat the mixture for 3 min after it begins to boil. Cool the flask to room temperature, and dilute with water to 20 mL.

**Instrumental conditions**

(See Ultraviolet-Visible Spectroscopy [857],) Mode: UV

Analytical wavelength: 380 nm

Cell: 1 cm

Blank: 1 mL of perchloric acid and 1 mL of Hydrochloric acid solution diluted with water to 20 mL

Analysis Samples: Standard solution and Sample solution Treat the Sample solution, the Standard solution, and the Blank as follows. Add 5 mL of Reagent A to each flask, and swirl gently to mix. Adjust the solution in each flask with 50% ammonium hydroxide solution to a pH of 1.1 ± 0.1. Add 5 mL of Reagent B to each flask, and swirl gently to mix. Place the flasks in a water bath maintained at 50°, and equilibrate for 30 min, taking care that the flasks are covered to protect them from light. Cool to room temperature, and transfer the contents of each flask to separate separatory funnels. Transfer 10.0 mL of cyclohexane to each separatory funnel, and extract vigorously for 1 min. Discard the aqueous layer. Transfer the cyclohexane layer to a centrifuge tube, and centrifuge...
PHOSPHORUS, and ZINC, Method 2; MOLYBDENUM and SELENIUM, Method 2

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.

System suitability solution: Prepare a mixture of 1000 mg/L of yttrium in 5% nitric acid solution, 1000 mg/L of scandium in 5% nitric acid solution, and Diluent (1:1:198), and mix.

Standard stock solution 1 (Ca, Cu, Fe, Mg, Mn, P, and Zn) : [Note—It is only necessary to include the minerals of interest in the solution.] Using commercially available element standard (single- or multi-element) solutions in 5% nitric acid solution, pipet the appropriate amount of element standard solution into a volumetric flask, and dilute with 5% nitric acid solution to obtain a solution having final concentrations of about 1000 mg/L of calcium, 100 mg/L of copper, 250 mg/L of iron, 500 mg/L of magnesium, 100 mg/L of manganese, 800 mg/L of phosphorus, and 250 mg/L of zinc.

Standard stock solution 2 (B, Cr, Mo, Ni, Se, Sn, and V): [Note—It is only necessary to include the minerals of interest in the solution.] Using commercially available element standard (single- or multi-element) solutions in 20% hydrochloric acid solution, pipet the appropriate amount of element standard solution into a volumetric flask, and dilute with 20% hydrochloric acid solution to obtain a solution having final concentrations of about 200 mg/L of boron, and 100 mg/L each of chromium, molybdenum, nickel, selenium, tin, and vanadium.

Standard solutions: Prepare a mixture of Standard stock solution 1 and Standard stock solution 2, as required, in the Diluent to prepare a 6-point calibration curve to bracket the concentration range of each mineral of interest.

Sample solution 1 (for Tablets containing minerals in Standard stock solution 1 and Standard stock solution 2): Weigh and finely powder NLT 20 Tablets. Transfer a portion, equal to 3.5 times the average Tablet weight, to a 250-mL volumetric flask. Slowly add 25 mL of the Stock aqua regia solution in 5-mL increments, followed by mixing. [Note—If the sample contains a carbonate, bubbling will occur. Wait until bubbling ends to proceed.] Bring the solution to a boil on a hot plate. Continue to heat gently until fumes cease (about 1 h). [Note—If the sample contains selenium, digest for NMT 15 min.] Remove from heat, cool, and dilute with water to volume. Pass about 30 mL into a centrifuge tube using a 5-µm pore size nylon syringe filter. If necessary, make any further dilutions using the Diluent.

Sample solution 2 (for Tablets containing minerals only in Standard stock solution 2): Weigh and finely powder NLT 20 Tablets. Transfer a portion, equal to 3.5 times the average Tablet weight, to a 250-mL volumetric flask. Slowly add 25 mL of the Stock aqua regia solution in 5-mL increments followed by mixing. [Note—If the sample contains a carbonate, bubbling will occur. Wait until bubbling ends to proceed.] Bring the solution to a boil on a hot plate. Continue to heat gently until fumes cease (about 1 h). [Note—If the sample contains selenium, digest for NMT 15 min.] Remove from heat, cool, and dilute with water to volume. Pass about 30 mL into a centrifuge tube using a 5-µm pore size nylon syringe filter. If necessary, make any further dilutions using the Diluent.

Sample solution 3 (for Tablets containing minerals only in Standard stock solution 1): Weigh and finely powder NLT 20 Tablets. Transfer a portion, equal to 3.5 times the average Tablet weight, to a 250-mL volumetric flask. Slowly add 25 mL of the Stock aqua regia solution in 5-mL increments followed by mixing. [Note—If the sample contains a carbonate, bubbling will occur. Wait until bubbling ends to proceed.] Bring the solution to a boil on a hot plate. Continue to heat gently until fumes cease (about 1 h). [Note—If the sample contains selenium, digest for NMT 15 min.] Remove from heat, cool, and dilute with water to volume. Pass about 30 mL into a centrifuge tube using a 5-µm pore size nylon syringe filter. If necessary, make any further dilutions using the Diluent.
20 Tablets. Transfer a portion, equal to the average Tablet weight, to a 250-mL volumetric flask. Slowly add 25 mL of the Stock aqua regia solution in 5-mL increments, followed by mixing. [NOTE—If the sample contains a carbonate, bubbling will occur. Wait until bubbling ends to proceed.] Bring the solution to a boil on a hot plate. Continue to heat gently (about 1 h) until fumes cease. Remove from heat, cool, and dilute with water to volume. Pass about 30 mL into a centrifuge tube using a nylon syringe filter of 3-µm pore size. If necessary, make any further dilutions using the Diluent.

Instrumental conditions
(See Plasma Spectrochemistry (730.).)
Mode: Inductively coupled plasma spectroscopy, using a spectrometer 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 solutions and Sample solution
Determine the emission of each mineral of interest in the Standard solutions and Sample solution with an inductively coupled plasma system using the Diluent as the blank. Plot the emission of the Standard solutions versus the concentration, in mg/L, of the minerals of interest, and draw the straight line best fitting the plotted points. From the graph, determine the concentration (C), in mg/L, for each mineral of interest in the Sample solution.
Calculate the percentage of the labeled amount for each mineral taken:

\[
\text{Result} = C \times (V/W) \times F \times (C_{W}/L) \times 100
\]

\[
C = \text{measured concentration of the relevant element in the Sample solution (mg/L)}
\]

\[
V = \text{volume of the Sample solution (L)}
\]

\[
W = \text{sample weight (mg)}
\]

\[
F = \text{dilution factor of the Sample solution}
\]

\[
C_{W} = \text{average Tablet weight (mg)}
\]

\[
L = \text{labeled amount (mg/Tablet)}
\]

Acceptance criteria: 90.0%–125.0% of the labeled amount of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), and zinc (Zn); and 90.0%–160.0% of the labeled amount of boron (B), chromium (Cr), molybdenum (Mo), nickel (Ni), selenium (Se), tin (Sn), and vanadium (V)

Performance tests
• Disintegration and Dissolution (2040), Dissolution: Meet the requirements
• Weight Variation (2091): Meet the requirements

Contaminants
• Microbial Enumeration Tests (2021): The total aerobic microbial count does not exceed 3 × 10^3 cfu/g, and the combined molds and yeasts count does not exceed 3 × 10^2 cfu/g.
• Absence of Specified Microorganisms (2022), Test Procedures, Test for Absence of Salmonella Species and Test for Absence of Escherichia coli: Meet the requirements

Additional requirements
• Packaging and Storage: Preserve in tight, light-resistant containers.

Change to read:
• Labeling: The label states that the product is Oil- and Water-Soluble Vitamins with Minerals Tablets. The label also states the quantity of each vitamin and mineral in mg/Tablet or µg/Tablet (USP 1-May-2020) 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 is the \text{\textit{RRR}} or \text{\textit{all-rrr}} (USP 1-May-2020) form. Where more than one assay method is given for a particular vitamin, the labeling states with which assay method the product complies only if Method 1 is not used.

Change to read:
• USP reference standards (11)
  USP Alpha Tocopherol RS
  2H1-Benzopyran-6-ol, 3, 4, dihydro-2, 5, 7, 8- tetramethyl-2-(4, 8, 12-trimethyltridecyl)-
  C_{92}H_{18}O_{4} 430.70
  USP Alpha Tocopherol Acetate RS
  USP Alpha Tocopherol Acid Succinate RS
  USP Ascorbic Acid RS (USP 1-May-2020)
  USP Biotin RS
  1H-Thieno[3, 4-d]imidazole-4-pentanoic acid, hexahydro-2-oxo-, 3α-(3α, 4β, 6αa)-
  C_{92}H_{18}N_{2}O_{5} 244.31
  USP Calcium Pantothenate RS
  β-Alanine, N-(2, 4-dihydroxy-3, 3-dimethyl-1-oxobutyl)-, calcium salt (2:1), (α)-
  C_{10}H_{20}CaN_{2}O_{10} 476.53
  USP Cholecalciferol RS
  9, 10-Secosterols, 5, 7, 10-(19)- trien-3-ol, (3p, 5z, 7e)-
  C_{27}H_{44}O 384.64
  USP Cyanocobalamin (Crystalline) RS
  Vitamin B_{12}, C_{59}H_{80}CoN_{2}O_{18}P 1355.37
  USP Ergocalciferol RS
  9, 10-Secosterols, 5, 7, 10-(19), 22-tetraen-3-ol, (3p, 5z, 7e, 22d)-
  C_{27}H_{44}O 396.65

Where articles are labeled in terms of Units in addition to the required labeling, the relationship of the USP Units or IU to mass is as follows. One USP Vitamin A Unit = 0.3 µg of all-trans-retinyl vitamin A alcohol or 0.344 µg of all-trans-retinyl acetate (vitamin A acetate) or 0.55 µg of all-trans-retinyl palmitate (vitamin A palmitate), and 1 µg of retinol (3.3 USP Vitamin A Units) = 1 retinol equivalent (RE). 1 IU of vitamin E = 0.6 µg of all-trans-beta carotene; 1 USP Vitamin D Unit = 0.025 µg of ergocalciferol or cholecalciferol; and 1 mg of \text{\textit{all-rrr}} (USP 1-May-2020) alpha-tocopherol = 1.1 former USP Vitamin E Units; 1 mg of \text{\textit{all-rrrr}} (USP 1-May-2020) alpha-tocopherol acid succinate = 0.89 former USP Vitamin E Units; 1 mg of \text{\textit{all-rrrr}} (USP 1-May-2020) alpha-tocopherol = 1.49 former USP Vitamin E Units; 1 mg of \text{\textit{all-rrrr}} (USP 1-May-2020) alpha-tocopherol acid succinate = 1.36 former USP Vitamin E Units; and 1 mg of \text{\textit{all-rrrr}} (USP 1-May-2020) alpha-tocopherol acid succinate = 1.21 former USP Vitamin E Units. In terms of \text{\textit{RRR}} (USP 1-May-2020) alpha-tocopherol equivalents, 1 mg of \text{\textit{RRR}} (USP 1-May-2020) alpha-tocopherol acid acetate = 0.91; 1 mg of \text{\textit{RRR}} (USP 1-May-2020) alpha-tocopherol acid succinate = 0.81; 1 mg of \text{\textit{all-rrrr}} (USP 1-May-2020) alpha-tocopherol = 0.74; 1 mg of \text{\textit{all-rrrr}} (USP 1-May-2020) alpha-tocopherol acid acetate = 0.67; and 1 mg of \text{\textit{all-rrrr}} (USP 1-May-2020) alpha-tocopherol acid succinate = 0.60. Note that 1 mg of Institute of Medicine (IOM) alpha-tocopherol equivalent = 1 mg of \text{\textit{RRR}} alpha-tocopherol = 1 mg of \text{\textit{RRR}} alpha-tocopherol = 2 mg of \text{\textit{all-rrrr}} alpha-tocopherol. (USP 1-May-2020)
USP Folic Acid RS
USP Niacin RS
  3-Pyridinecarboxylic acid.
  \( \text{C}_6\text{H}_5\text{NO}_2 \)  123.11
USP Niacinamide RS
  3-Pyridinecarboxamide.
  \( \text{C}_6\text{H}_6\text{N}_2\text{O} \)  122.12
USP Phytonadione RS
USP Pyridoxine Hydrochloride RS
  3,4-Pyridinedimethanol, 5-hydroxy-6-methyl-,
  hydrochloride.
  \( \text{C}_8\text{H}_{11}\text{NO}_3 \cdot \text{HCl} \)  205.64
▲ USP Retinyl Acetate RS
  3,7-Dimethyl-9-(2,6,6-trimethyl-1-
  cyclohexen-1-yl) 2,4,6,8-nonatetraen-1-ol acetate.
USP Retinyl Palmitate RS
  \([2(\text{E},4\text{E},6\text{E},8\text{E})-3,7\text{-Dimethyl-9-(2,6,6-trimethyl-1-
  cyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl}]\)
  hexadecanoate.▲ (USP 1-May-2020)

USP Riboflavin RS
Riboflavine.
  \( \text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_6 \)  376.36
USP Sodium Fluoride RS
Sodium fluoride.
  NaF  41.99
USP Thiamine Hydrochloride RS
Thiazolium, 3-\{(4-amino-2-methyl-5-pyrimidinyl) methyl]-5-(2-hydroxyethyl)-4-methyl-,
  chloride, monohydrochloride.
  \( \text{C}_{12}\text{H}_{17}\text{ClN}_4\text{OS} \cdot \text{HCl} \)  337.27
▲ ▲ (USP 1-May-2020)
Expert consensus on an open-access United Nations International Multiple Micronutrient Antenatal Preparation–multiple micronutrient supplement product specification

The Multiple Micronutrient Supplement Technical Advisory Group (MMS-TAG)1,2 and The Micronutrient Forum (MNF)2

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The multiple micronutrient supplement (MMS) based on the United Nations International Multiple Micronutrient Antenatal Preparation (UNIMMAP) formula provides women and their offspring with a healthy start to life in an efficacious, safe, and cost-effective way. To date, however, no precise and transparent specifications exist to support the manufacturing and distribution of UNIMMAP–MMS globally. To palliate for this need, the MMS Technical Advisory Group at the New York Academy of Sciences and the Micronutrient Forum convened a technical consultation to develop an open access UNIMMAP–MMS Product Specification for the manufacturing of this product. The specifications offered in this paper cover: ingredients, excipients, and processing aids used in the manufacturing of UNIMMAP–MMS; stability studies recommended under different testing conditions and climatic zones; packaging considerations; manufacturing standards, including pharmacopeia standards, manufacturing practices, certificates of analysis, change control, and quality agreement; finished product specifications, including tablet characterization and purity, potency assay; analytical test methods; and storage and transportation requirements.

Keywords: product specification; multiple micronutrient supplement (MMS); UNIMMAP; packaging; manufacturing; quality; standards

Introduction

Micronutrient deficiencies can have deleterious impacts on maternal health and pregnancy outcomes.1 The United Nations International Multiple Micronutrient Antenatal Preparation–multiple micronutrient supplement (UNIMMAP–MMS) formulation2 has been shown to provide pregnant women and their offspring with a positive pregnancy and a healthy start to life in a safe3 and cost-effective4 manner. The strength of the evidence, combined with global advocacy and the availability of technical assistance, drives an increased demand globally for the UNIMMAP–MMS product. The surge in demand, however, far exceeds global supply: most of the nearly 200 million pregnancies that occur annually in low- and middle-income countries are likely to benefit from the use of MMS; yet, current production covers only about five millions of those pregnancies. Reaching an adequate level of supply will bring several new manufacturers into production. This paper, which results from the deliberations of a panel of international experts assembled in Washington DC, November 11–12, 2019, was commissioned by the MMS Technical Advisory Group at the New

See “Acknowledgments and author contributions” section for more information.
York Academy of Sciences and the Micronutrient Forum to ensure consistency in the manufacturing of quality and affordable UNIMMAP–MMS across antenatal care programs, while allowing for variances in national regulatory and pharmacopeia standards. Further, the paper provides the basis for a quality agreement between a purchaser and a manufacturer by providing both parties with a clear technical understanding of the manufacturing requirements for the UNIMMAP–MMS product; and the means and methods to verify that the product delivered meets the quality that was expected by the purchaser.

1. Product description

The product defined by the following specification conforms to the UNIMMAP) formula and is an MMS for pregnant women that is delivered in the form of a film coated tablet.  

2. Ingredients

2.1. Food/dietary/nutritional ingredients

Table 1 shows the food/dietary/nutritional ingredients used in the UNIMMAP formulation and should be prepared from ingredients that meet United States Pharmacopeia (USP) or other globally recognized pharmacopeia compendial standards. Where such standards do not exist, ingredients may be used in the UNIMMAP formulation if they have been shown to be of acceptable food grade quality using other suitable procedures.

![Table 1. Recommended food/dietary/nutritional ingredients](image)

<table>
<thead>
<tr>
<th>Component</th>
<th>Chemical entity</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Retinyl acetate</td>
<td>800 mcg RAE</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Ascorbic acid</td>
<td>70 mg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Cholecalciferol</td>
<td>5 mcg (200 IU)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Alpha tocopheryl succinate</td>
<td>10 mg α-TE</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>Thiamine mononitrate</td>
<td>1.4 mg</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>Riboflavin</td>
<td>1.4 mg</td>
</tr>
<tr>
<td>Vitamin B3</td>
<td>Niacinamide</td>
<td>18 mg NE</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Pyridoxine HCl</td>
<td>1.9 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Folic acid</td>
<td>680 mcg DFE (400 mcg)</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Cyanocobalamin</td>
<td>2.6 mcg</td>
</tr>
<tr>
<td>Iron</td>
<td>Ferrous fumarate</td>
<td>30 mg</td>
</tr>
<tr>
<td>Iodine</td>
<td>Potassium iodide</td>
<td>150 mcg</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zinc oxide</td>
<td>15 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>Sodium selenite</td>
<td>65 mcg</td>
</tr>
<tr>
<td>Copper</td>
<td>Cupric oxide</td>
<td>2 mg</td>
</tr>
</tbody>
</table>

These chemical entities may be replaced by other chemical entities if they demonstrate equal or better performance (e.g., stability).

2.2. Excipients

Excipients used in the UNIMMAP formulation generally are prepared from ingredients that meet USP, National Formulary (NF), Food Chemical Codex, or other globally recognized pharmacopeia compendial standards. Where such standards do not exist, ingredients may be used in the UNIMMAP formulation if they have been shown to be of acceptable food grade quality using other suitable procedures.

Ingredients may be added to the UNIMMAP formulation provided that the ingredients comply with applicable regulatory requirements, and do not interfere with the assay and tests prescribed for determining compliance with the bulk or finished UNIMMAP–MMS product specification.

2.3. Processing aids or other materials

Processing aids or other materials used in the manufacture of the UNIMMAP formulation that do not end up in the finished product should be of acceptable food grade quality using suitable procedures.

Potable water must meet, at minimum, all the requirements for drinking water promulgated in the U.S. Environmental Protection Agency’s National Primary Drinking Water Regulations (40 CFR Part 141), and any applicable state and local drinking water requirements that are more stringent. For

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*a*This specification states a preference for a film coated tablet, which provides certain benefits, including lower cost and better performance and stability under expected conditions of high temperature and humidity. However, capsules that demonstrate equal or better performance may also be considered.

*b*Readers should consider the following: (1) UNIMMAP–MMS might be considered a medicinal product in some countries, and if so, the product must comply with the respective regulatory requirements of that country; (2) no reference is made in this document to “pharmaceutical” or “medicinal” ingredients or products, although the product might be considered a medicinal product in some countries; and (3) this specification outlines the minimum requirements for the manufacture of a UNIMMAP–MMS; as previously indicated, if a country has stricter requirements, they must be met.
Table 2. Recommended International Conference on Harmonization Testing conditions for all climatic zones

<table>
<thead>
<tr>
<th>Climatic zone</th>
<th>Climate definition</th>
<th>Mean temperature/mean partial water vapor pressure</th>
<th>Derived climatic conditions</th>
<th>Long-term stability</th>
<th>Accelerated stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Temperate</td>
<td>NMT 15 °C/LT 11 hPa</td>
<td>21 °C/45% RH</td>
<td>25 °C ± 2 °C/60% RH ± 5% RH</td>
<td>40 °C ± 2 °C/75% RH ± 5% RH</td>
</tr>
<tr>
<td>II</td>
<td>Subtropical, Mediterranean</td>
<td>GT 15 °C and NMT 22 °C/GT 11 hPa and NMT 18 hPa</td>
<td>25 °C/60% RH</td>
<td>25 °C ± 2 °C/60% RH ± 5% RH</td>
<td>40 °C ± 2 °C/75% RH ± 5% RH</td>
</tr>
<tr>
<td>III</td>
<td>Hot, dry</td>
<td>GT 22 °C/LT 15 hPa</td>
<td>30 °C/35% RH</td>
<td>30 °C ± 2 °C/35% RH ± 5% RH</td>
<td>40 °C ± 2 °C/NMT 25%</td>
</tr>
<tr>
<td>IVa</td>
<td>Hot, humid</td>
<td>GT 22 °C/GT 15 hPa and NMT 27 hPa</td>
<td>30 °C/65% RH</td>
<td>30 °C ± 2 °C/65% RH ± 5% RH</td>
<td>40 °C ± 2 °C/75% RH ± 5% RH</td>
</tr>
<tr>
<td>IVb</td>
<td>Hot and very humid</td>
<td>GT 22 °C/GT 27 hPa</td>
<td>30 °C/75% RH</td>
<td>30 °C ± 2 °C/75% RH ± 5% RH</td>
<td>40 °C ± 2 °C/75% RH ± 5% RH</td>
</tr>
</tbody>
</table>

NMT, not more than (≤); LT, less than (<); GT, greater than (>); RH, relative humidity.

Manufacturers outside the United States, potable water meeting equivalent requirements may be acceptable with justification, for example, the drinking water regulations of the European Union (European Commission Directive 98/93/EC) or Japan Drinking Water Quality Standards. Water not meeting such requirements should not be permitted for use in the water purification system for purified water.

### 3. Stability studies

The UNIMMAP–MMS finished product labeling must state a shelf-life (expiration) date that is indicative of the date before which the product is ensured to meet applicable specifications of identity, strength, quality, and purity when stored under labeled conditions. The shelf-life (expiration) date must be supported by suitable stability data, following the guidelines in International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q1A.

A documented ongoing testing program must be designed to monitor the stability characteristics of the UNIMMAP–MMS, and the results must be used to establish appropriate storage conditions and shelf-life (expiration) dates for the UNIMMAP–MMS finished product. Test procedures used in stability testing must be validated and indicate stability. Stability samples should be stored in container-closure systems that simulate the packaging proposed to distribute the finished UNIMMAP–MMS for consumer/patient use. Stability studies should include testing of those attributes of the dietary supplement that are susceptible to change during storage and that influence the quality of the dietary supplement.

The first three production batch(es) should be placed on the stability monitoring program to establish the product shelf-life (expiration) date. The lots should be those that are manufactured at the regular manufacturing scale; however, two of the three production batches can be at least 1/10th the size of the manufacturing scale. Thereafter, at least one batch per year of manufactured UNIMMAP–MMS should be added to the stability monitoring program. All batches must comply with the finished product specification throughout the product shelf-life (expiration) date.

As appropriate, the stability storage conditions for temperature and relative humidity for product intended for use globally should be for Climate Zone IVb, hot and very humid. However, actual climatic conditions in the country of destination of the UNIMMAP–MMS may require stability studies to be carried out under the conditions of a different climatic zone (e.g., Climatic Zone III, hot and dry). Table 2 shows the recommended testing conditions appropriate to each climatic zone that might...
be required in a given country of destination of the product.

The frequency of testing should be sufficient to establish the stability profile of the dietary supplement. The storage conditions and length of studies chosen should be sufficient to cover storage, shipment, and subsequent use. Data from the accelerated storage condition can be used to evaluate the effect of short-term excursions outside the label storage conditions, such as might occur during shipping. The shelf-life (expiration) period of the UNIMMAP–MMS should be 30 months, at minimum. The following testing frequencies are recommended:

- **Long term** = 0, 3, 6, 9, 12, 18, 24, 30, and 36 months;
- **Accelerated** = 0, 3, and 6 months.

Where an expectation (based on development experience) exists that results from accelerated studies are likely to approach significant change criteria, increased testing should be conducted by adding samples at the 1- and 2-month time points. In general, a significant change for a dietary supplement is defined as a 5% change in the assay from its initial value; or failure to meet any of its product specification acceptance criteria.

4. **Packaging**

4.1. **Package types and tablet count**

This specification focuses on the use of UNIMMAP–MMS packaged in bottles containing 180 tablets per bottle. Bottles (or blister packaging) containing 30 tablets are acceptable, but packaging options/tablet counts should be considered in light of cost and environmental implications, and evidence that may come later as to the impact that a particular packaging option or tablet count may have on distribution, uptake, adherence rates, or clinic attendance. c

Bulk packaging for business-to-business transactions is acceptable with demonstrated stability. Bulk packaging for clinics (e.g., 500, 1000, etc., count bottles) should be avoided due to health and safety concerns to avoid inadvertent child exposure to UNIMMAP–MMS; and to avoid repackaging by clinic staff of MMS into other temporary less desirable packaging (e.g., plastic bags and newspaper), which may cause premature product deterioration.

4.2. **Bottles**

Bottles must be:

- White/opaque
- Screw cap
- High-density polypropylene (HDPE) material (complying with internationally recognized pharmacopoeia standards)
- Tamper evident
- Child resistant

The need for desiccant depends on tablet formulation and must be determined by the manufacturer based on experience and supporting stability data.

4.3. **Blister packaging**

If blister packaging is used, a thermoformable moisture barrier film, such as Aclar®, should be used to ensure the stability of the product throughout the shelf-life of the product. Using a child-resistant version of a blister pack is deemed impractical, as the “child-resistant” requirement makes a blister pack unusually difficult to dispense package contents.

4.4. **Labeling**

Labeling must comply with applicable country of destination regulatory requirements for the food/dietary/nutritional supplement. Quantitative label claims for the product must be truthful and accurately reflect the contents of the declared food/dietary/nutritional ingredients; fortified or fabricated nutrients must meet 100% of the quantitative label claim throughout the shelf-life of the product for products that can be distributed in the United States. For products distributed outside the United States, fortified or fabricated nutrients must meet 100% of the quantitative label claim throughout the shelf-life of the product for products that can be distributed in the United States. For products distributed outside the United States, fortified or fabricated nutrients must meet 100% of the quantitative label claim throughout the shelf-life of the product for products that can be distributed in the United States.

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c No evidence currently exists yet in the literature indicating that packaging type/tablet count increases uptake, adherence rates, or clinical attendance. However, the 180-count bottled product makes it less expensive per tablet to dispense and has a lesser environmental impact than other packaging. For these reasons, the 180-count-HDPE-bottled product was selected as the packaging option of choice in the preparation of this document. If future implementation research, or if program needs indicate otherwise, other packaging options may be considered with appropriate modifications to these technical specifications.
### Table 3. Tablet characterization and purity

<table>
<thead>
<tr>
<th>Test</th>
<th>Test method</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>Visual</td>
<td>TBD by manufacturer</td>
</tr>
<tr>
<td>Shape</td>
<td>Visual</td>
<td>TBD by manufacturer</td>
</tr>
<tr>
<td>Tablet thickness</td>
<td>Micrometer</td>
<td>TBD by manufacturer</td>
</tr>
<tr>
<td>Tablet length</td>
<td>Micrometer</td>
<td>TBD by manufacturer</td>
</tr>
<tr>
<td>Tablet friability</td>
<td>USP &lt;1216&gt;</td>
<td>TBD by manufacturer</td>
</tr>
<tr>
<td>Tablet breaking force</td>
<td>USP &lt;1217&gt;</td>
<td>TBD by manufacturer</td>
</tr>
<tr>
<td><strong>Performance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average tablet weight</td>
<td>USP &lt;2091&gt;</td>
<td>TBD by manufacturer</td>
</tr>
<tr>
<td>Weight variation</td>
<td></td>
<td>Each of the individual weights is within 95–105% of the average weight</td>
</tr>
<tr>
<td>Dissolution for vitamin A (index for oil-soluble vitamins)</td>
<td>USP &lt;2040&gt;</td>
<td>LT 75% of the labeled amount of vitamin A dissolved in 45 minutes</td>
</tr>
<tr>
<td>Dissolution for folic acid</td>
<td>USP &lt;2040&gt;</td>
<td>LT 75% of the labeled amount of folic acid dissolved in 1 hour</td>
</tr>
<tr>
<td>Dissolution for riboflavin (index for water-soluble vitamin)</td>
<td>USP &lt;2040&gt;</td>
<td>LT 75% of the labeled amount of riboflavin dissolved in 1 hour</td>
</tr>
<tr>
<td>Dissolution for iron (index element)</td>
<td></td>
<td>LT 75% of the labeled amount of iron dissolved in 1 hour</td>
</tr>
<tr>
<td><strong>Elemental impurities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic (inorganic)</td>
<td>USP &lt;233&gt; and USP &lt;2232&gt;</td>
<td>NMT 15 mcg/day</td>
</tr>
<tr>
<td>Cadmium</td>
<td></td>
<td>NMT 5 mcg/day</td>
</tr>
<tr>
<td>Lead</td>
<td></td>
<td>NMT 5 mcg/day</td>
</tr>
<tr>
<td>Mercury (total)</td>
<td></td>
<td>NMT 15 mcg/day</td>
</tr>
<tr>
<td>Methylmercury (as Hg)</td>
<td></td>
<td>NMT 2 mcg/day</td>
</tr>
<tr>
<td><strong>Microbial contaminants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total aerobic microbial count (TAMC)</td>
<td>USP &lt;2021&gt;</td>
<td>NMT 3 × 10³ CFU/g</td>
</tr>
<tr>
<td>Total combined yeast and mold (TCYM)</td>
<td>USP &lt;2021&gt;</td>
<td>NMT 3 × 10² CFU/g</td>
</tr>
<tr>
<td>Absence of Escherichia coli</td>
<td>USP &lt;2022&gt;</td>
<td>Absent in 10 g</td>
</tr>
<tr>
<td>Absence of Salmonella spp.</td>
<td>USP &lt;2022&gt;</td>
<td>Absent in 10 g</td>
</tr>
<tr>
<td>Absence of Staphylococcus aureus</td>
<td>USP &lt;2022&gt;</td>
<td>Absent in 10 g</td>
</tr>
<tr>
<td>Enterobacterial count (bile-tolerant Gram-negative bacteria)</td>
<td>USP &lt;2021&gt;</td>
<td>NMT 10 CFU/g</td>
</tr>
</tbody>
</table>

NMT, not more than (≦); LT, less than (<); CFU, colony forming unit.

USP <233> Elemental Impurities-Procedures.

USP <2232> Elemental Contaminants in Dietary Supplements.

USP <2021> Microbial Enumeration Tests – Nutritional and Dietary Supplements.

USP <2022> Microbiological Procedures for Absence of Specified Microorganisms – Nutritional and Dietary Supplements.
Table 4. Potency assay (per tablet)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Test methoda</th>
<th>Label claim</th>
<th>USP</th>
<th>US-FDA b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (as retinyl acetate)</td>
<td>Vitamin A, method 1</td>
<td>800 mcg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin C (as ascorbic acid)</td>
<td>Vitamin C, assay &lt;580&gt;, method II</td>
<td>70 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin D (as cholecalciferol)</td>
<td>Cholecalciferol or ergocalciferol method 1</td>
<td>5 mcg (200 IU)</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin E (as dl-alpha tocopheryl succinate)</td>
<td>Vitamin E, method 1</td>
<td>10 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin B1: thiamine (as thiamine mononitrate)</td>
<td>Niacin or niacinamide, pyridoxine hydrochloride, riboflavin, and thiamine; method 1</td>
<td>1.4 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin B2: riboflavin</td>
<td></td>
<td>1.4 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin B3: niacin (as niacinamide)</td>
<td></td>
<td>18 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin B6: pyridoxine hydrochloride</td>
<td></td>
<td>1.9 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Folate (as folic acid)</td>
<td>Folic acid, method 1</td>
<td>680 DFE (400 mcg folic acid)</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Vitamin B12 (as cyanocobalamin)</td>
<td>Cyanocobalamin, method 1</td>
<td>2.6 mcg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Iodine (as potassium iodide)</td>
<td>Iodide</td>
<td>150 mcg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Iron (as ferrous fumarate)</td>
<td>Copper, iron, and zinc, method 2; selenium, method 3 plasma spectrochemistry &lt;730&gt;</td>
<td>30 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Zinc (as zinc oxide)</td>
<td></td>
<td>15 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Selenium (as sodium selenite)</td>
<td></td>
<td>65 mcg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
<tr>
<td>Copper (as cupric oxide)</td>
<td></td>
<td>2 mg</td>
<td>LT 90.0%</td>
<td>LT 100.0%</td>
</tr>
</tbody>
</table>

NMT, not more than (≤); LT, less than (<).
aAll tests to be performed per current USP-NF, oil- and water-soluble vitamins with minerals tablets.
bUSP General Notices 4.10.20. Acceptance Criteria: An official product shall be formulated with the intent to provide 100% of the quantity of each ingredient declared on the label. Where the minimum amount of a substance present in a dietary supplement is required by law to be higher than the lower acceptance criterion allowed for in the monograph, as per 21 CFR 101.36(f)(1) and 21 CFR 101.9(g)(3) and (g)(4), the upper acceptance criterion in the monograph may be increased by a corresponding amount.

fabricated nutrients must meet USP compendial assay acceptance criteria (see Tables 3 and 4 for details).

The UNIMMAP–MMS product label (see Fig. 1) should list:

- The term “food supplement,” “dietary supplement,” or “nutritional supplement”;
- The quantity of each dietary ingredient and the correct reference daily intake, listed as % Daily Value, as necessary;
- List the common or usual name of each ingredient in descending order of predominance by weight, except that dietary ingredients listed in the nutrition label supplement facts need not be repeated in the ingredient list;
incidental additives, including water, present in a dietary supplement at insignificant levels are exempted from this requirement.

Labels for the UNIMMAP–MMS containing iron or iron salts for use as an iron source must include the following required cautionary statement: “WARNING: Accidental overdose of iron-containing products is a leading cause of fatal poisoning in children under 6-years of age. Keep this product out of reach of children. In case of accidental overdose, call a doctor or poison control center immediately.”

The label must include a statement of the necessary storage requirements for the UNIMMAP–MMS. The label must accurately state the country of origin for any product of foreign origin imported into the country of destination.

The name and place of business of the manufacturer, packer, or distributor, and the expiration date must be located to the right of the principle display panel. When the name appearing on the label is not that of the actual manufacturer, the name should be qualified in a manner to accurately reflect this relationship (e.g., “Manufactured for ___,” “Distributed by ___”). The label must include a domestic address or phone number through which an adverse event report for a dietary supplement may be received.

5. Manufacturing standards and certificates

5.1. Pharmacopoeia standards

Compliance to the following international pharmacopoeial standards is acceptable for food/dietary/nutritional supplement ingredients and finished product:

- United States Pharmacopoeia (USP);
- European Pharmacopoeia (Ph.Eur.);
- International Pharmacopoeia (Ph.Int.);
- British Pharmacopoeia (BP);

5.2. Manufacturing practices and conditions

The UNIMMAP–MMS must be manufactured under current good manufacturing practice (cGMP) regulations promulgated by an internationally recognized regulatory authority (e.g., U.S. FDA and MHRA), or other GMP guidelines by a stringent authority (e.g., World Health Organization (WHO)), by a PIC/S member, or by a globally recognized pharmacopeia (e.g., USP), including but not limited to:

- U.S. FDA 21 CFR Part 111 Current Good Manufacturing Practice in Manufacturing, Packing, Labeling, or Holding Operations for Dietary Supplements; and

The manufacturing site and operations should be audited by an accredited third-party certification body.

5.3. Certificates of analysis

Certificates of analysis (CoA) must be issued for each batch of UNIMMAP–MMS product. The CoA should list the name and item code of the UNIMMAP–MMS product, batch number, tested

Figure 1. Label illustrating the minimum recommended information that should appear on the label of the UNIMMAP–MMS.
and released dates, and the expiration date. The CoA should list each test performed, the specific identity of the test procedure, the acceptance limits or criteria, and the results with numerical units, as appropriate. The CoA should be dated and signed by authorized quality unit personnel. For CoAs issued for external use, the CoA should list the name, address, and telephone number of the manufacturer.

The testing protocol should include the performance of full specification testing. However, a reduced level of testing (or sampling) for particular specified parameters may be allowed based upon one or more of the following: statistical analysis of an adequate quantity of historical test data; statistical confidence in the capability of the manufacturing process as determined by suitable verification; or ongoing monitoring of the process using recognized statistical process control techniques. The manufacturer must notify the purchaser of the UNIMMAP–MMS product in writing of any change to the testing protocol.

5.4. Halal certification
The UNIMMAP–MMS product may be manufactured meeting Halal requirements. The exact requirements shall be obtained from local authorities or from an accredited source.

5.5. Change control
A manufacturer might make changes to the UNIMMAP–MMS product’s specification, raw material source, manufacturing processing steps and/or equipment, testing protocols, or any other criteria deemed to be essential or significant to product quality. Manufacturers must notify the purchaser of the UNIMMAP–MMS product of any significant changes that might affect product quality, in writing upon implementation, along with the rationale for the change(s).

A manufacturer must also notify the purchaser of the UNIMMAP–MMS product of any change to its manufacturing site, including any changes to the certification status held by the manufacturer from a GMP issuing authority.

5.6. Quality agreement
There should be a written and approved contract or quality agreement between the contract giver and the contract acceptor that defines in detail the GMP responsibilities, including the quality measures, of each party. The contract should permit the purchaser to audit the manufacturer’s (seller’s) facilities for compliance with GMPs. Where subcontracting is allowed, the seller should not pass to a third party any of the work entrusted to it under the contract without the buyer’s prior evaluation and approval of the arrangements.

6. Finished product specification
The UNIMMAP is a formulation for use by pregnant women. UNIMMAP–MMS was developed during a workshop of experts organized by the WHO, UNICEF, and the United Nations University in 1999 specifically to identify an MMS formula for efficacy clinical trials. It contains 15 micronutrients at dosages that approximate the recommended dietary allowances for pregnancy.

Tables 3 and 4 show criteria and requirements for tablet characterization and purity, as well as the potency assay requirements.

7. Analytical test methods
Tests and examinations that are used to determine whether UNIMMAP–MMS specifications are met must be appropriate for their intended use, and scientifically validated methods. Test methods or procedures must meet proper standards of accuracy and reliability.

If the test procedure is not in an official compendium, the procedure must be validated according to USP general chapter <1225> Validation of Compendial Procedures or ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology. Method performance characteristics include specificity, linearity, range accuracy, precision, detection limit, and quantitation limit, and those of interest may vary depending on the type of test: identification, assay, impurities, or performance.

If the test procedure is in an official compendium, such as USP-NF, the procedure only needs to be verified for its suitability under actual conditions of use, according to USP general chapter <1226> Verification of Compendial Procedures. Verification requirements should be based on an assessment of the complexity of both the procedure and material to which the procedure is applied. Verification is not required for basic compendial procedures, such as loss on drying, residue on ignition, and simple instrumental determinations, such as pH measurements.
An alternative method or procedure is defined as any method or procedure other than the compendial method or procedure for the article in question. The alternative method or procedure must be fully validated and must produce comparable results to the compendial method or procedure within allowable limits established on a case-by-case basis. Alternative methods or procedures can be developed for any number of reasons not limited to simplification of sample preparation, enhanced precision and accuracy, improved (shortened) run time, or being better suited to automation than the compendial method or procedure. Only those results obtained by the methods and procedures given in the compendia are conclusive.

8. Storage and transportation requirements

The UNIMMAP–MMS product must be held under appropriate conditions of temperature, humidity, and light so that its identity, purity, strength, and composition are not affected (e.g., NLT 15°C and NMT 30°C, protected from humidity and light).

The UNIMMAP–MMS product must be distributed under conditions that will protect it against contamination and deterioration. The manufacturer and purchaser need to work together to ensure that this requirement is met.

All transportation operation must be conducted under such conditions and controls necessary to prevent UNIMMAP–MMS product from becoming adulterated during transportation. Responsibility for ensuring that transportation operations are carried out adequately must be assigned to competent supervisory personnel. Shippers, receivers, loaders, and carriers engaged in transportation must conduct all transportation operations under such conditions and controls necessary to protect the UNNIMAP-MMS product from becoming adulterated during transportation. Such operations include, but are not limited to, taking effective measures such as:

- Segregation, isolation, or the use of packaging to protect the UNNIMAP-MMS product from contamination from other articles in the same load;
- Use of vehicles and transportation equipment that are adequately designed and maintained in a sanitary condition to prevent the UNIMMAP–MMS product from becoming contaminated during transportation operations; and
- Use of vehicles and transportation equipment that are adequately designed, maintained, and equipped to transport UNIMMAP-MMS product under adequate temperature and humidity control to prevent UNIMMAP-MMS product from becoming adulterated during transportation.

The first manufactured batch of the UNIMMAP–MMS product should be distributed first. Distributing operations must be designed to facilitate its recall, if necessary.

9. Definitions and acronyms (Box 1).

<table>
<thead>
<tr>
<th>Term/abbreviation</th>
<th>Definition/long form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accredited</td>
<td>An accredited third-party certification body means a third-party certification body that meets the applicable requirements of ISO/IEC 17020:2012 and/or ISO/IEC 17065:2012 and is accredited to conduct audits or inspections according to the applicable standard or regulatory requirements.</td>
</tr>
<tr>
<td>Article</td>
<td>Article includes substances (such as excipients, food/dietary/nutritional ingredients, and in-process material), products (such as food/dietary/nutritional supplements), and materials (such as packaging containers and closures, and labels).</td>
</tr>
<tr>
<td>Batch</td>
<td>Batch is a specific quantity of a food/dietary/nutritional supplement or other article that is intended to be uniform; that is intended to meet specifications for identity, purity, strength, and composition; and that is produced during a specified time period according to a single manufacturing record during the same cycle of manufacture.</td>
</tr>
<tr>
<td>Composition</td>
<td>Composition is the specified mix of food/dietary/nutritional ingredients and excipients in a food/dietary/nutritional supplement.</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Code of Federal Regulations (CFR)</td>
<td>The CFR annual edition is the codification of the general and permanent rules and regulations published in the Federal Register by the executive departments and agencies of the Federal Government of the United States. It is structured into 50 subject matter titles; title 21 applies to food and drugs. Titles are broken down into parts, subparts, sections, and paragraphs.</td>
</tr>
<tr>
<td>Certificate of analysis (CoA)</td>
<td>CoA is a document relating specifically to the results of testing a representative sample drawn from a batch of material. The CoA should list each test performed in accordance with compendial or manufacturer requirements, including reference to the test procedure, acceptance limits, and the results obtained.</td>
</tr>
<tr>
<td>Country of destination</td>
<td>The country in which the product is intended to be marketed/used.</td>
</tr>
<tr>
<td>Excipients</td>
<td>Excipients are substances other than food/dietary ingredients that have been appropriately evaluated for safety and are intentionally included in a food/dietary supplement to do one or more of the following: aid in the manufacture of a food/dietary supplement; protect, support, or enhance stability, bioavailability, or user acceptability; assist in product identification; and/or enhance any other attribute of the overall safety or delivery of the food/dietary supplement during storage or use. The term excipient is sometimes used synonymously with the term inactive ingredients and other ingredients.</td>
</tr>
<tr>
<td>Food/dietary/nutritional ingredient</td>
<td>Food/dietary/nutritional ingredients are ingredients with an established nutritional value, namely, vitamins and minerals in their respective chemical entity.</td>
</tr>
<tr>
<td>Food/dietary/nutritional supplement ingredient</td>
<td>Food/dietary/nutritional supplement is a product intended to supplement the diet that contains one or more food/dietary/nutritional ingredients, that is intended for ingestion in a tablet, capsule, or liquid form, that is not represented for use as a conventional food or as the sole item of a meal or the diet, and is labeled as a food/dietary/nutritional supplement, and that sometimes referred to as a multi-micronutrient supplement (MMS).</td>
</tr>
<tr>
<td>Globally recognized pharmacopeia</td>
<td>The following international pharmacopoeia’s official compendial standards are considered globally recognized:</td>
</tr>
<tr>
<td>compendial standard</td>
<td>1. British Pharmacopoeia (BP)</td>
</tr>
<tr>
<td></td>
<td>2. European Pharmacopoeia (Ph.Eur.)</td>
</tr>
<tr>
<td></td>
<td>3. International Pharmacopoeia (Ph.Int.)</td>
</tr>
<tr>
<td></td>
<td>5. United States Pharmacopeia (USP)</td>
</tr>
<tr>
<td>International Conference on</td>
<td>The International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use brings together regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration.</td>
</tr>
<tr>
<td>Harmonization (ICH)</td>
<td></td>
</tr>
<tr>
<td>Micronutrient Forum (MN Forum)</td>
<td>The Micronutrient Forum serves as a global catalyst and convener for sharing expertise, insights, and experience relevant to micronutrients in all aspects of health promotion and disease prevention, with special emphasis on the integration with relevant sectors.</td>
</tr>
<tr>
<td>Not less than (NLT)</td>
<td>NLT is equal to, but not less than a given value.</td>
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</tbody>
</table>

**Box 1. Continued**
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<table>
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<tbody>
<tr>
<td>Not more than (NMT)</td>
<td>NMT is equal to, but not more than a given value.</td>
</tr>
<tr>
<td>Pharmaceutical Inspection Co-operation</td>
<td>PIC/S is a nonbinding, informal co-operative arrangement between regulatory authorities in the field of good manufacturing practice (GMP) of medicinal products for human or veterinary use. PIC/S currently consists of 52 participating authorities and aims at harmonizing inspection procedures.</td>
</tr>
<tr>
<td>To be determined (TBD)</td>
<td>TBD is related to a variable that has not yet been determined.</td>
</tr>
<tr>
<td>United Nations International Multiple</td>
<td>UNIMMAP is a formulation for a prenatal micronutrient supplement intended for use in developing countries that was developed in 1999 by UNICEF, the United Nations University (UNU), and the World Health Organization (WHO). It contains 15 micronutrients at dosages that approximate the recommended dietary allowances for pregnancy.</td>
</tr>
<tr>
<td>Micronutrient Antenatal Preparation</td>
<td></td>
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<tr>
<td>(UNIMMAP)</td>
<td></td>
</tr>
<tr>
<td>Vitamin Angel Alliance (VAA)</td>
<td>The Vitamin Angel Alliance, Inc. is a 501(c)(3) tax exempt organization that aims to reduce health and economic disparities across the life span of underserved populations by effectively delivering evidence-based nutrition interventions to hard-to-reach populations globally (<a href="http://www.vitaminangels.org">www.vitaminangels.org</a>).</td>
</tr>
</tbody>
</table>

Acknowledgments and authorship

This consensus open-access UNIMMAP–MMS product specification is based upon and adapted, with permission, from the open access MMS product specification originally created by the Vitamin Angel Alliance and revised for global use by a technical consultation of experts convened in Washington, DC on November 11–12, 2019, and hosted jointly by the New York Academy of Science’s Multiple Micronutrient Supplementation Technical Advisory Group (MMS-TAG) and the Micronutrient Forum (MN Forum) with funding from the Bill & Melinda Gates Foundation. For more information or assistance in understanding how to apply this product specification, contact the New York Academy of Sciences MMS-TAG, The Micronutrient Forum, or the Vitamin Angel Alliance.

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UNICEF’s role in this technical consultation was solely as an observer and limited to sharing UNICEF’s technical standards/specifications and its process for internal evaluation. Rajiv Kshirsagar participated as an observer only for the purpose of delivering a presentation on UNICEF’s technical standards and its internal evaluation process. Nita Dalmiya participated in person as an observer. UNICEF does not endorse, or imply endorsement of the content in this publication, or outcomes of this technical consultation.

Competing interests

The authors declare no competing interests.

References