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 forms(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 *WHO 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)

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

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 COIVD-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.

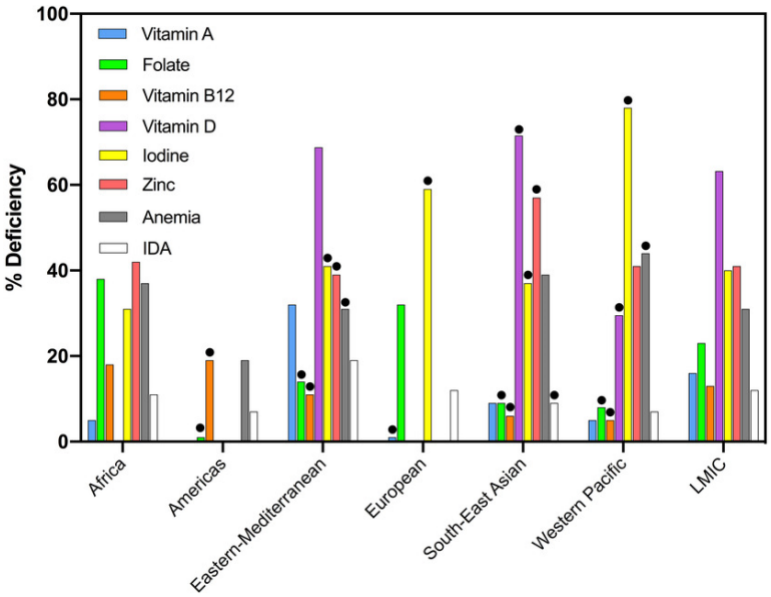

Figure 1. Regional estimates of micronutrient deficiencies and anaemia as reported in women of reproductive age. *Black circles are not representative (<3 countries). Data calculated from 52 national and regional surveys, published between 2013 and July 2017. Missing bars means no data were found for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

	Sub-Saharan Africa	Asia	South East Asia	South Asia	World
Preterm births (2014) (%)[a]	12.0	10.4	N/R	N/R	10.6
SGA (2012) (%)[b]	16.5	N/R	21.6	34.2	19.3
LBW (2015) (%)[c]	13.7*	17.3	12.3	26.4	14.6
Stillbirths (per 1000 total births)[d]	28.7	N/R	12.2	25.5	18.4
Neonatal mortality (per 1000 live births)[e]	27.7	N/R	13.5	27.6	18.6

[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)
** All 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)

Outcome	**Cochrane Review (19 RCTs) Relative Risks**	**IPD Meta-analysis (17 RCTs) Relative Risks**	
	Random effects	**Random effects**	**Fixed effects**
SGA (<10th percentile)	0.92 (0.88-0.97)[a]	0.94 (0.90-0.98)[b]	0.97 (0.96–0.99)[b]
LBW (<2500g)	0.88 (0.85-0.91)	0.86 (0.81-0.92)	0.88 (0.85–0.90)
VLBW (<2000g)	*Not Reported*	*Not Reported*	0.78 (0.72-0.85)
Preterm Birth (<37 weeks)	0.96 (0.90-1.01)	0.93 (0.97-0.98)	0.92 (0.88–0.95)
Very Preterm Birth (<34 weeks)	*Not Reported*	*Not Reported*	0.87 (0.79-0.95)
LGA (>90th percentile Oken)	*Not Reported*	1.04 (0.92-1.18)	1.05 (0.95–1.15)
LGA (>90th percentile INTERGROWTH)	*Not Reported*	*Not Reported*	1.11 (1.04-1.19)
Stillbirth	0.95 (0.86-1.04)	0.97 (0.85-1.11)	0.92 (0.86–0.99)
Neonatal Mortality (≤28 days)	1.00 (0.89-1.12)	0.99 (0.89-1.09)	0.98 (0.90–1.05)
Infant Mortality (≤365 days)	*Not Reported*	0.97 (0.88-1.06)	0.97 (0.88–1.06)

[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

Organization	2017	2018	2019
Kirk Humanitarian*	1,160,107	597,435	2,104,952
UNICEF**	2,090,000	2,090,000	2,090,000
Vitamin Angels	447,649	2,024,357	2,150,782
Total	**3,697,756**	**4,711,792**	**6,345,734**

** 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

Unit	Min	Max	Median
180 count	2.20 USD	3.15 USD	2.50 USD

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.

13. Availability of pharmacopeial standards (British Pharmacopoeia, International Pharmacopoeia, United States Pharmacopoeia, European Pharmacopeia)

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*.

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

Afghanistan
Angola
Belize
Bhutan
Bolivia
Botswana
Burkina Faso
Cambodia
Cameroon
Central African Republic
Chile
Congo
Cook Islands
Costa Rica
Côte d'Ivoire
Cuba
Democratic People's Republic of Korea
Democratic Republic of Congo
Djibouti
Dominica
Dominican Republic
Ecuador
El Salvador
Eritrea
Ethiopia
Gambia
Ghana
Grenada
Guyana
Honduras
India
Iran (Islamic Republic of)
Iraq
Jamaica
Jordan
Kenya
Kiribati
Lebanon
Liberia
Madagascar
Mali
Marshall Islands
Mauritania
Mexico
Mongolia
Mozambique
Myanmar
Namibia
Nicaragua
Niue
Papua New Guinea
Paraguay
Philippines
Saint Lucia
Senegal
Solomon Islands
Thailand
Timor-Leste
Togo
Tonga
Tunisia
Tuvalu
Uganda
Vanuatu
Viet Nam
Yemen
Zimbabwe

Appendix B. Clinical trial information

Study, as identified in Cochrane review	Location	Years of Study	Participants (n)	In IPD meta-analysis?	In 2019 Cochrane Review?	UNIMMAP formulation?
Ashorn, 2015	Mangochi District, Malawi	2011-2015	929	Yes	Yes	No
Bhutta, 2009a	Bilal colony, Karachi, Kot Diji, Sindh, Pakistan	2002-2004	2378	Yes	Yes	Yes
Biggs, 2010 (Hanieh 2013)	Ha Nam, Vietnam	2010-2012	1258	No	Yes	No
Christian, 2003	Sarlahi, Nepal	1998-2001	4926	Yes	Yes	No
Dewey 2009 (Adu-Afarwuah, 2015)	Somanya-Kpong, Ghana	2009-2011	1320	Yes	Yes	No
Fawzi, 2007	Dar es Salaam, Tanzania	2001-2004	8468	Yes	Yes	No
Friis, 2004	Harare, Zimbabwe	1996-1997	1669	Yes	Yes	No
Kaestel, 2005	Bissau, Guinea-Bissau	2001-2002	2100	Yes	Yes	Yes
Liu, 2013	Hebei Province, China	2006-2009	18775	No	Yes	Yes
Moore, 2009 (Johnson, 2017)	West Kiang, the Gambia	2009-2013	875	No	Yes	No
Osrin, 2005	Dhanusha and Mahottari Districts, Nepal	2002-2004	1200	Yes	Yes	Yes
Ramakrishnan, 2003	Cuernavaca, Mexico	1997-2000	873	Yes	Yes	No
Roberfroid, 2008	Hounde health district, Burkina Faso	2004-2006	1426	Yes	Yes	Yes
SUMMIT, 2008 (Shankar, 2008)	Lombok island, Indonesia	2001-2004	31290	Yes	Yes	Yes
Sunawang, 2009	Indramayu, West Java, Indonesia	2000-2003	843	No	Yes	Yes
Tofail 2008 (Persson, 2012)	Matlab, Bangladesh	2001-2003	4436	Yes	Yes	Yes
West, 2014	Gaibandha and Rangpur, Bangladesh	2007-2012	44567	Yes	Yes	No
Zagre, 2007	Maradi, Niger	2004-2006	2902	Yes	Yes	Yes
Zeng, 2008	Shaanxi Province, China	2002-2006	5828	Yes	Yes	Yes

Appendix C. GRADE assessment from 2019 Cochrane Review

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Multiple micronutrients compared to control (iron with or without folic acid) for women during pregnancy

Patient or population: women during pregnancy
Setting: low- and middle-income countries
Intervention: multiple micronutrients
Comparison: control (iron with or without folic acid)

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (trials)	Quality of the evidence (GRADE)	Comments
	Risk with control (iron with or without folic acid)	Risk with multiple micronutrients				
Preterm births	Study population		RR 0.95 (0.90 to 1.01)	91,425 (18 RCTs)	⊕⊕⊕◯ **Moderate**[a]	
	197 per 1000	188 per 1000 (178 to 199)				
Small-for-gestational age	Study population		RR 0.92 (0.88 to 0.97)	57,348 (17 RCTs)	⊕⊕⊕◯ **Moderate**[a]	
	337 per 1000	310 per 1000 (296 to 327)				
Low birthweight	Study population		RR 0.88 (0.85 to 0.91)	68,801 (18 RCTs)	⊕⊕⊕⊕ **High**	
	212 per 1000	187 per 1000 (181 to 193)				
Perinatal mortality	Study population		RR 1.00 (0.90 to 1.11)	63,922 (15 RCTs)	⊕⊕⊕⊕ **High**	Raw event and participant data were unavailable for Ramakrishnan 2003 and West 2014, so have not been included in No of participants column.
	39 per 1000	39 per 1000 (35 to 43)				
Stillbirths	Study population		RR 0.95 (0.86 to 1.04)	97,927 (17 RCTs)	⊕⊕⊕⊕ **High**	
	30 per 1000	28 per 1000 (26 to 31)				
Neonatal mortality	Study population		RR 1.00 (0.89 to 1.12)	80,964 (14 RCTs)	⊕⊕⊕⊕ **High**	Raw event and participant data were unavailable for Bhutta 2009a and Fawzi 2007 so have not been included in No of participants column.
	29 per 1000	29 per 1000 (26 to 32)				

*__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 effect

[a]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

Afghanistan
Armenia
Bangladesh
Barbados
Belize
Botswana
British Virgin Islands
Burkina Faso
Burundi
Cambodia
Cameroon
Chad
Colombia
Cote d'Ivoire
Democratic Republic of the Congo
Djibouti
Dominica
Dominican Republic
Ecuador
El Salvador
Ethiopia
Fiji
Gambia
Ghana
Greece
Guatemala
Guinea
Guyana
Haiti
Honduras
India
Iraq
Jamaica
Jordan
Kenya
Kosovo
Lebanon
Lesotho
Liberia
Macedonia
Madagascar
Malawi
Mali
Marshall Islands
Mexico
Micronesia
Mongolia
Mozambique
Nepal
Nicaragua
Niger
Nigeria
Pakistan
Palestine
Panama
Papua New Guinea
Paraguay
Peru
Philippines
Romania
Sierra Leone
Solomon Islands
Somalia
Somaliland
South Sudan
St. Lucia
Swaziland
Syria
Tanzania
Thailand
Togo
Turkey
Uganda
Ukraine
Uzbekistan
Vanuatu
Venezuela
Yemen
Zambia
Zimbabwe

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,▲ (USP 1-May-2020) vitamin D as Ergocalciferol (vitamin D_2) or Cholecalciferol (vitamin D_3), 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,▲ (USP 1-May-2020) Phytonadione (vitamin K_1), 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 retinol▲ (USP 1-May-2020) ($C_{20}H_{30}O$); vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$); vitamin E ▲as *2R*-alpha-tocopherol▲ (USP 1-May-2020) ($C_{29}H_{50}O_2$); phytonadione ($C_{31}H_{46}O_2$); and beta carotene ($C_{40}H_{56}$); and NLT 90.0% and NMT 150.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$); ▲▲ (USP 1-May-2020) biotin ($C_{10}H_{16}N_2O_3S$), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), folic acid ($C_{19}H_{19}N_7O_6$), niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), pyridoxine ▲▲ (USP 1-May-2020) ($C_8H_{11}NO_3$▲▲ (USP 1-May-2020)), riboflavin ($C_{17}H_{20}N_4O_6$), and thiamine ▲as thiamine ion ($C_{12}H_{17}N_4OS$)▲ (USP 1-May-2020) and NLT 90.0% and NMT 125.0% of the labeled amount of calcium (Ca), copper (Cu), iron (Fe), manganese (Mn), magnesium (Mg), phosphorus (P), potassium (K), and zinc (Zn); and NLT 90.0% and NMT 160.0% of the labeled amount of boron (B), chromium (Cr), fluorine (F), iodine (I), molybdenum (Mo), nickel (Ni), selenium (Se), tin (Sn), and vanadium (V). They may contain other labeled 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 polytef-lined 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 ($C_{20}H_{30}O$).

▲▲ (USP 1-May-2020)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin A as retinol ($C_{20}H_{30}O$)

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 ($C_{20}H_{30}O$)

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 ($C_{20}H_{30}O$)

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.

▲▲ (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 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:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of cholecalciferol or ergocalciferol from the *Sample solution*

r_S = peak area of cholecalciferol or ergocalciferol from the *Standard 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 ($C_{29}H_{50}O_2$), alpha-tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha-tocopheryl acid succinate ($C_{33}H_{54}O_5$) ▲as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$)▲ (USP 1-May-2020) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \text{ ▲}\times F\text{▲ (USP 1-May-2020)} \times 100$$

r_U = peak area of the relevant vitamin E form from the *Sample solution*

r_S = peak area of the relevant vitamin E form from the *Standard 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▲ (USP 1-May-2020) 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) in the *Sample solution* (mg/mL)

▲F = conversion factor for the content of *all-rac*-alpha-tocopherol to 2*R*-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 ($C_{29}H_{50}O_2$), alpha-tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha-tocopheryl acid succinate ($C_{33}H_{54}O_5$) as ▲2*R*-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 ($C_{29}H_{50}O_2$), alpha-tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha-tocopheryl acid succinate ($C_{33}H_{54}O_5$) as 2R-alpha-tocopherol ($C_{29}H_{50}O_2$) in the portion of Tablets taken as follows:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak area of the relevant vitamin E form from the *Sample solution*

r_S = peak area of the relevant vitamin E form from the *Standard 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 2*R*-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 ($C_{29}H_{50}O_2$), alpha-tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha-tocopheryl acid succinate ($C_{33}H_{54}O_5$) as ▲2*R*-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*

Calculate the percentage of the labeled amount of alpha-tocopherol ($C_{29}H_{50}O_2$), alpha-tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha-tocopheryl acid succinate ($C_{33}H_{54}O_5$) as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$) in the portion of Tablets taken as follows:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak area of the relevant vitamin E form from the *Sample solution*
r_S = peak area of the relevant vitamin E form from the *Standard solution*
C_S = concentration of *all-rac*-alpha-tocopherol from either USP Alpha Tocopherol RS and 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 2*R*-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 ($C_{29}H_{50}O_2$), alpha-tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha-tocopheryl acid succinate ($C_{33}H_{54}O_5$) as ▲2*R*-alpha-tocopherol▲ (USP 1-May-2020)

- **PHYTONADIONE, *Method 1***

[NOTE—Use low-actinic 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 Tocopheryl Acetate RS and 20 µg/mL of USP Phytonadione RS from the *Standard stock solution* diluted with methanol. [NOTE—Dissolve USP Alpha Tocopheryl 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-tocopheryl acetate and phytonadione are 0.68 and 1.0, respectively.]

Suitability requirements

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

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak areas. Calculate the percentage of the labeled amount of phytonadione ($C_{31}H_{46}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of phytonadione from the *Sample solution*
r_S = peak area of phytonadione from the *Standard solution*
C_S = concentration of USP Phytonadione RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of phytonadione in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–165.0% of the labeled amount of phytonadione ($C_{31}H_{46}O_2$)

- **PHYTONADIONE, *Method 2***

[NOTE—Use low-actinic 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_2) 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 2 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

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 as necessary, to meet the *System suitability* requirements.]
Flow rate: 1 mL/min
Injection volume: 25 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for the internal standard and phytonadione are 1.0 and 1.4, respectively.]
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 ($C_{31}H_{46}O_2$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phytonadione to the internal standard from the *Sample solution*
R_S = peak response ratio of phytonadione to the internal standard from the *Standard solution*
C_S = concentration of USP Phytonadione RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of phytonadione in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–165.0% of labeled amount of phytonadione ($C_{31}H_{46}O_2$)

Add the following:

▲• **VITAMIN A, VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL),** and **VITAMIN E, *Method 4*; PHYTONADIONE, *Method 3***
[NOTE—Where 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-actinic 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 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
19	0	100
27	0	100
28	100	0
30	100	0

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. Dissolve with the aid of sonication if necessary.
Vitamin E standard stock solution: 3.0 mg/mL of USP Alpha Tocopherol 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*
Suitability requirements
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

265 nm, respectively. [NOTE—This test is required because of possible interference with carotenoids (cryptoxanthin and lycopene) that may be present in the formulation. Use another procedure in the monograph for analysis of vitamin D and vitamin A (as retinyl palmitate) if the system is not suitable]

Analysis

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

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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

Calculate the percentage of the labeled amount of vitamin E, as *2R*-alpha-tocopherol ($C_{29}H_{50}O_2$), in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak area of the relevant vitamin E form from the *Sample solution*
r_S = peak area of the relevant vitamin E form from the *Standard 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 vitamin E, as *2R*-alpha-tocopherol, in the *Sample solution* (mg/mL)
F = conversion factor for the content of *all-rac*-alpha-tocopherol to *RRR*-alpha-tocopherol equivalent, 1/2 (for products labeled to contain *all-rac* vitamin E sources) and 1 (for products labeled to contain *RRR* vitamin E sources)

Calculate the percentage of the labeled amount of vitamin D, as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$), and phytonadione ($C_{31}H_{46}O_2$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the related vitamin from the *Sample solution*
r_S = peak area of the related vitamin from the *Standard solution*
C_S = concentration of related vitamin in the *Standard solution* (µg/mL)
C_U = nominal concentration of related vitamin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–165.0% of the labeled amount of each individual vitamin ▲ (USP 1-May-2020)

- **BETA CAROTENE**

[NOTE—Use low-actinic glassware throughout this procedure.]

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 ($C_{40}H_{56}$) in the portion of Tablets taken:

$$\text{Result} = (A_U/F) \times (100/C_U)$$

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 ($C_{40}H_{56}$)

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*.▲ (USP 1-May-2020)]

Acceptance criteria: 90.0%–150.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$), or its salts as calcium ascorbate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$) or sodium ascorbate ($C_6H_7NaO_6$)

• **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 ($C_{10}H_{16}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = 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_U = nominal concentration of biotin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$)

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. Store in a refrigerator.

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*,▲ (USP 1-May-2020) 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)

Acid-hydrolyzed casein solution	25 mL
Cystine–tryptophan solution	25 mL
Polysorbate 80 solution	0.25 mL
Dextrose, anhydrous	10 g
Sodium acetate, anhydrous	5 g
Adenine–guanine–uracil solution	5 mL
Calcium pantothenate solution	5 mL
Riboflavin–thiamine hydrochloride solution	5 mL
p-Aminobenzoic acid–niacin–pyridoxine hydrochloride solution	5 mL
Salt solution A	5 mL
Salt solution B	5 mL

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: [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 *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 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 (Σ_S) as the difference, $y = 2.00 - \Sigma_S$. 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, $y = 2.00 - \Sigma_U$, adding together the two transmittances (Σ_U) 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 $\overline{X}$, which equals the log-relative potency, M', of the *Sample solution*.

Determine the quantity, in µg, of biotin ($C_{10}H_{16}N_2O_3S$) in the portion of Tablets taken:

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

R = amount of biotin assumed to be present in the portion of the Tablets taken (µg)

Calculate the percentage of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$) in the portion of the Tablets taken:

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

N = nominal amount of biotin in the portion of the Tablets taken (µg)

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, $\overline{M}$, 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 ($C_{10}H_{16}N_2O_3S$)

• **BIOTIN, *Method 3***

[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 µg/mL of USP Biotin RS in water. [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 µg/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

[1] ATCC No. 8014 is suitable. This strain was formerly known as *Lactobacillus arabinosus* 17-5.

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% (w/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 ($C_{10}H_{16}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = 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_U = nominal concentration of biotin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$)

- **CYANOCOBALAMIN,** ***Method 1***

[NOTE—Use low-actinic 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 ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Cyanocobalamin (Crystalline) RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of cyanocobalamin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$)

Change to read:

- **CYANOCOBALAMIN,** ***Method 2***

[NOTE—Use low-actinic 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 ng/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, 1.29 g of dibasic sodium phosphate, 1.1 g of anhydrous citric acid, and 1.0 g of sodium metabisulfite. Autoclave the mixture at 121° 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 B_{12} 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°, 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 comparable to that obtained from the formula given herein.

Add the ingredients in the order listed in ▲*Table 3*,▲ (USP 1-May-2020)carefully dissolving cystine and tryptophan in the 1N hydrochloric acid before adding the next 8 solutions to the resulting solution. Add 100 mL of water, and dissolve the dextrose, sodium acetate, and ascorbic acid. Filter, if necessary. Add the *Polysorbate 80 solution*, adjust with 1 N sodium hydroxide to a pH of 5.5–6.0, and dilute with Purified Water to make 250 mL.

▲Table 3▲ (USP 1-May-2020)

L-Cystine	0.1 g
L-Tryptophan	0.05 g
1 N hydrochloric acid	10 mL
Adenine–guanine–uracil solution	5 mL
Xanthine solution	5 mL
Vitamin solution A	10 mL
Vitamin solution B	10 mL
Salt solution A	5 mL
Salt solution B	5 mL
Asparagine solution	5 mL
Acid-hydrolyzed casein solution	25 mL
Dextrose, anhydrous	10 g
Sodium acetate, anhydrous	5 g
Ascorbic acid	1 g
Polysorbate 80 solution	5 mL

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° 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° 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*.[3] [NOTE—Before first 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° and 40° held constant to within ±0.5°. 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° and 40° held constant to within ±0.5°. 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. Using 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.

[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).

Analysis

Samples: *Standard solution* and *Sample solution*

Because of the high sensitivity of the test organism to minute amounts of vitamin B_{12} activity and to traces of many cleansing agents, cleanse meticulously by suitable means, followed preferably by heating at 250° 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 to 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° 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°, for 16–24 h.

Terminate growth by heating to a temperature NLT 80° 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 (Σ_S) as the difference, $y = 2.00 - \Sigma_S$. 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, $y = 2.00 - \Sigma_U$, adding together the two transmittances (Σ_U) 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 $\overline{X}$, which equals the log-relative potency, M', of the *Sample solution*.

Determine the quantity, in µg, of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Tablets taken:

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

R = amount of cyanocobalamin assumed to be present in the portion of Tablets taken (µg)

Calculate the percentage of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of the Tablets taken:

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

N = nominal amount of cyanocobalamin in the portion of the Tablets taken (µg)

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, $\overline{M}$, is the assayed log-potency of the test material (see ▲ (USP 1-May-2020) *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 cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$)

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 ($C_{19}H_{19}N_7O_6$)

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 ($C_{19}H_{19}N_7O_6$)

- **CALCIUM PANTOTHENATE,** ***Method 1***

Mobile phase: Phosphoric acid and water (1:1000)

Internal standard solution: 80 mg of *p*-hydroxybenzoic acid in 3 mL of alcohol. 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 ($C_{18}H_{32}CaN_2O_{10}$) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = 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_S = 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 ($C_{18}H_{32}CaN_2O_{10}$)

Change to read:

• **CALCIUM PANTOTHENATE,** ***Method 2***

Standard stock solution: Dissolve 50 mg of USP Calcium Pantothenate RS ▲▲ (USP 1-May-2020) 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 4.0 mL 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°. 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 dilute with water to make 1000 mL. Store under toluene in a cool place at a temperature not below 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 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)

Acid-hydrolyzed casein solution	25 mL
Cystine–tryptophan solution	25 mL
Polysorbate 80 solution	0.25 mL
Dextrose, anhydrous	10 g
Sodium acetate, anhydrous	5 g
Adenine–guanine–uracil solution	5 mL
Riboflavin–thiamine hydrochloride–biotin solution	5 mL
p-Aminobenzoic acid–niacin–pyridoxine hydrochloride solution	5 mL
Salt solution A	5 mL
Salt solution B	5 mL

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 the 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 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, 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 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 (Σ_S) as the difference, $y = 2.00 - \Sigma_S$. 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, $y = 2.00 - \Sigma_U$, adding together the two transmittances (Σ_U) 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 $\overline{X}$, which equals the log-relative potency, M', of the *Sample solution*. Determine the quantity, in mg, of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of Tablets taken:

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

R = amount of calcium pantothenate assumed to be present in the portion of the Tablets taken (mg)

Calculate the percentage of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the Tablets taken:

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

N = nominal amount of calcium pantothenate in the portion of the Tablets taken (mg)

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, $\overline{M}$, 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 calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$)

• **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 the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of calcium pantothenate from the *Sample solution*
r_S = peak area of calcium pantothenate from the *Standard solution*
C_S = 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 ($C_{18}H_{32}CaN_2O_{10}$)

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.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*▲▲ (USP 1-May-2020)
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 ($C_{12}H_{17}N_4OS+$), vitamin B_2 as riboflavin ($C_{17}H_{20}N_4O_6$), vitamin B_3 as niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), vitamin B_6 as pyridoxine ($C_8H_{11}NO_3$) ▲▲ (ERR 1-May-2020) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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)

Change to read:

- **NIACIN,** ***Method 2***

▲Proceed as directed in *Niacin or Niacinamide Assay* ⟨441⟩, *Assay, Chromatographic Methods, Procedure 2*.▲ (USP 1-May-2020)

Acceptance criteria: 90.0%–150.0% of the labeled amount of niacin ($C_6H_5NO_2$)

Change to read:

- **NIACINAMIDE,** ***Method 2***

▲Proceed as directed in *Niacin or Niacinamide Assay* ⟨441⟩, *Assay, Chromatographic Methods, Procedure 2*.▲ (USP 1-May-2020)

Acceptance criteria: 90.0%–150.0% of the labeled amount of niacinamide ($C_6H_6N_2O$)

- **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*
Measure the peak areas of pyridoxine. Calculate the percentage of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of pyridoxine from the *Sample solution*
r_S = peak area of pyridoxine from the *Standard solution*
C_S = concentration of USP Pyridoxine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of pyridoxine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$)

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 ($C_{17}H_{20}N_4O_6$)

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 ($C_{12}H_{17}N_4OS+$)▲ (USP 1-May-2020)

Change to read:

- **NIACIN OR NIACINAMIDE, PYRIDOXINE HYDROCHLORIDE, RIBOFLAVIN,** and **THIAMINE,** ***Method 3***

[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, 15.0 mL of glacial acetic acid, and 350 mL of methanol to a 2000-mL volumetric flask. Dilute with 0.008 M sodium 1-hexanesulfonate to volume.

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°, 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°, 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°

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 B_1 as thiamine ion ($C_{12}H_{17}N_4OS+$), vitamin B_2 as riboflavin ($C_{17}H_{20}N_4O_6$), vitamin B_3 as niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), vitamin B_6 as pyridoxine ($C_8H_{11}NO_3$) ▲▲ (ERR 1-May-2020) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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 5

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	100	0
1.0	100	0
6.0	95	5
15.0	83	17
17.0	80	20
19.0	80	20
19.1	100	0
25.0	100	0

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°–70° 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 *Solution 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 ($C_{12}H_{17}N_4OS+$), vitamin B2 as riboflavin ($C_{17}H_{20}N_4O_6$), vitamin B3 as niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), vitamin B6 as pyridoxine ($C_8H_{11}NO_3$), calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), ▲ascorbic acid ($C_6H_8O_6$),▲ (ERR 1-May-2020) and folic acid ($C_{19}H_{19}N_7O_6$), in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

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)

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

[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.

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: Nitrous oxide–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 calcium, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (*C*), in µg/mL, of calcium in the *Sample solution*.

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

$$\text{Result} = (C/C_U) \times 100$$

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

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

Acceptance criteria: 90.0%–125.0% of the labeled amount of calcium (Ca)

• **CHROMIUM, *Method 1***

Chromium standard solution: 1000 µg/mL of chromium from potassium dichromate, previously dried at 120° for 4 h in water. Store in a polyethylene bottle.

Standard stock solution: 10 µg/mL of chromium from the *Chromium standard solution* diluted with 6 N hydrochloric acid and water (1 in 20)

Standard solutions: Transfer 10.0 and 20.0 mL of the *Standard stock solution* to separate 100-mL volumetric flasks, and transfer 15.0 and 20.0 mL of the *Standard stock solution* to separate 50-mL volumetric flasks. Dilute the contents of each of the 4 flasks with 0.125 N hydrochloric acid to volume to obtain concentrations of 1.0, 2.0, 3.0, and 4.0 µg/mL of chromium.

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

Instrumental conditions

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

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Chromium emission line at 357.9 nm

Lamp: Chromium 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 4 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:

$$\text{Result} = (C/C_U) \times 100$$

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

C_U = 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)

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

Acceptance criteria: 90.0%–125.0% of the labeled amount of copper (Cu)

- **FLUORIDE, *Method 1***

[NOTE—Store all solutions in plastic containers.]

3 M sodium acetate solution: Dissolve 408 g of sodium acetate in 600 mL of water contained in a 1000-mL volumetric flask. Allow the solution to equilibrate to room temperature, and dilute with water to volume. Adjust with a few drops of acetic acid to a pH of 7.0.

Sodium citrate solution: Dissolve 222 g of sodium citrate in 250 mL of water in a 1000-mL volumetric flask. Add 28 mL of perchloric acid, and dilute with water to volume.

Fluoride standard stock solution: 500 µg/mL of fluoride from a quantity of sodium fluoride, previously dried at 100° for 4 h and cooled in a desiccator in water

Intermediate stock solution A: 100 µg/mL of fluoride from the *Fluoride standard stock solution* diluted with water

Intermediate stock solution B: 10 µg/mL of fluoride from the *Fluoride standard stock solution* diluted with water

Standard solutions: To 5 separate 100-mL volumetric flasks transfer 3.0, 5.0, and 10.0 mL of *Intermediate stock solution B* and 5.0 and 10.0 mL of *Intermediate stock solution A*. To each flask add 10.0 mL of 1 N hydrochloric acid, 25 mL of *3 M sodium acetate solution*, and 25.0 mL of *Sodium citrate solution*. Dilute the contents of each flask with water to volume to obtain concentrations of 0.3, 0.5, 1.0, 5.0, and 10.0 µg/mL of fluoride.

Sample solution: Transfer a quantity of the finely powdered Tablets, equivalent to 200 µg of fluoride, to a 100-mL volumetric flask. Add 10.0 mL of 1 N hydrochloric acid, 25.0 mL of *3 M sodium acetate solution*, and 25.0 mL of *Sodium citrate solution*, and dilute with water to volume.

Analysis

Samples: *Standard solutions* and *Sample solution*

To separate plastic beakers, each containing a plastic-coated stirring bar, transfer 50.0 mL each of the *Standard solutions* and the *Sample solution*. Measure the potentials (see *pH* ⟨791⟩), in mV, of the *Standard solutions* and the *Sample solution*, with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride-specific ion-indicating electrode and a calomel reference electrode. [NOTE—When taking measurements, immerse the electrodes in the solution, stir on a magnetic stirrer having an insulated top until equilibrium is attained (1–2 min), and record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode.]

Plot the logarithms of fluoride concentrations, in µg/mL, of the *Standard solutions* versus potential, in mV. From the standard response curve and the measured potential of the *Sample solution*, determine the concentration (*C*), in µg/mL, of fluoride in the *Sample solution*.

Calculate the percentage of the labeled amount of fluorine (F) in the portion of Tablets taken:

$$\text{Result} = (C/C_U) \times 100$$

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

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

Acceptance criteria: 90.0%–160.0% of the labeled amount of fluorine (F)

- **FLUORIDE, *Method 2***

[NOTE—Use plastic containers and deionized water throughout this procedure.]

pH 10.0 buffer: Add 214 mL of 0.1 N sodium hydroxide to 1000 mL of 0.05 M sodium bicarbonate.

Mobile phase: Alcohol, 0.1 N sulfuric acid, and water (20:5:175)

Standard stock solution: 220 µg/mL of USP Sodium Fluoride RS in water. This solution contains 100 µg/mL of fluoride.

Standard solution: [NOTE—Condition the solid-phase extraction column specified for use in the *Standard solution* and the *Sample solution* in the following manner. Using a vacuum at a pressure not exceeding 5 mm of mercury, wash the column with 1 column volume of methanol followed by 1 column volume of *pH 10.0 buffer*. Do not allow the column top to dry. If the top of the column becomes dry, recondition the column.] Transfer 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask. Add 75 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 10.4 ± 0.1. Dilute with water to volume. Filter, discarding the first 15 mL of the filtrate. Transfer 25.0 mL of the filtrate to a 50-mL volumetric flask, add 15.0 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 10.0. Dilute with *pH 10.0 buffer* to volume. Elute a portion of this solution through a 3-mL solid-phase extraction column containing L1 packing that is connected through an adapter to a second solid-phase extraction column containing sulfonylpropyl strong cation-exchange packing. Discard the first 3 mL of the eluate, and collect the rest of the eluate in a suitable flask for injection into the chromatograph.

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of powdered Tablets, equivalent to 1 mg of fluorine, 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 fluorine (F) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = 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_U = nominal concentration of fluorine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–160.0% of the labeled amount of fluorine (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:

$$\text{Result} = V \times N_A \times F \times I_{me} \times (Aw/W) \times (100/L)$$

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_{me} = milliequivalent weight of iodine, 21.16 mg/meq
Aw = 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:

$$\text{Result} = (C/C_U) \times 100$$

C = measured concentration of iron in the *Sample solution* (µg/mL)
C_U = 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:

$$\text{Result} = (C/C_U) \times 100$$

C = measured concentration of magnesium in the *Sample solution* (µg/mL)
C_U = 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

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 manganese.

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

Lamp: Manganese 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 manganese, 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:

$$\text{Result} = (C/C_U) \times 100$$

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

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

Acceptance criteria: 90.0%–125.0% of the labeled amount of manganese (Mn)

- **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: Molybdenum emission line at 313.3 nm

Lamp: Molybdenum hollow-cathode

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 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:

$$\text{Result} = (C/C_U) \times 100$$

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

C_U = 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⟩.)▲ (ERR 1-May-2020)

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:

$$\text{Result} = (A_U/A_S) \times [(V \times C_S)/M_U] \times 100$$

A_U = absorbance of the *Sample*
A_S = 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_U = 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.]

Hydroquinone solution: 5 mg/mL of hydroquinone in water. Add 1 drop of sulfuric acid per 100 mL of solution.

Sodium bisulfite solution: 200 mg/mL of sodium bisulfite in water

Phosphorus standard stock solution: Weigh 4.395 g of monobasic potassium phosphate, previously dried at 105° for 2 h and stored in a desiccator, and transfer to a 1000-mL volumetric flask. Dissolve in water, add 6 mL of sulfuric acid as a preservative, dilute with water to volume, and mix to obtain a solution with a concentration of 1000 µg/mL of phosphorus.

Standard solution: 20 µg/mL of phosphorus from *Phosphorus standard stock solution* diluted with water

Sample solution: [NOTE—Finely powder and weigh a counted number of Tablets.] Transfer a portion of the powder, equivalent to 100 mg of phosphorus, to 25 mL of nitric acid, and digest on a hot plate for 30 min. Add 15 mL of hydrochloric acid, and continue the digestion to the cessation of brown fumes. Cool, and transfer the contents of the flask to a 500-mL volumetric flask with the aid of small portions of water. Dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

Instrumental conditions
(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
Mode: Vis
Analytical wavelength: 650 nm
Cell: 1 cm

Analysis
Samples: *Standard solution* and *Sample solution*
To 3 separate 25-mL volumetric flasks transfer 5.0 mL each of the *Standard solution*, the *Sample solution*, and water to provide the blank. To each of the 3 flasks add 1.0 mL each of *Ammonium molybdate solution*, *Hydroquinone solution*, and *Sodium bisulfite solution*, and swirl to mix. Dilute the contents of each flask with water to volume, and allow the flasks to stand for 30 min. Determine the absorbances of the solutions against the blank.
Calculate the percentage of the labeled amount of phosphorus (P) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of phosphorus in the *Standard solution* (µg/mL)
C_U = nominal concentration of phosphorus in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–125.0% of the labeled amount of phosphorus (P)

- **POTASSIUM**

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
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Potassium emission line at 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*), 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:

$$\text{Result} = (C/C_U) \times 100$$

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

C_U = 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 dissipate 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

Flame: Air–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 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:

$$\text{Result} = (C/C_U) \times 100$$

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

C_U = 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/mL 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

at 1000 rpm for 1 min to remove any remaining water. Determine the absorbances of the solutions obtained from the *Samples* against the solution obtained from the *Blank*.

Calculate the percentage of the labeled amount of selenium (Se) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times [(V \times C_S)/M_U] \times 100$$

A_U = absorbances of the cyclohexane layer from the *Sample solution*
A_S = absorbances of the cyclohexane layer from the *Standard solution*
V = volume of the *Standard stock solution* used to prepare the *Standard solution*, 10 mL
C_S = concentration of selenium in the *Standard stock solution* (µg/mL)
M_U = nominal amount of selenium in the *Sample solution* (µg)

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

- **ZINC, *Method 1***

Zinc standard stock solution: 1000 µg/mL of zinc from zinc oxide in 5 M hydrochloric acid (3.89 mg/mL) and diluted with water to final volume. [NOTE—Dissolve in 5 M hydrochloric acid by warming, if necessary, cool, and then dilute to final volume.]

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

Standard solutions: Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 mL of *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 concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 µg/mL of zinc.

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

Instrumental conditions

(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Zinc emission line at 213.8 nm
Lamp: Zinc 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 zinc, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of zinc in the *Sample solution*.

Calculate the percentage of the labeled amount of zinc (Zn) in the portion of Tablets taken:

$$\text{Result} = (C/C_U) \times 100$$

C = measured concentration of zinc in the *Sample solution* (µg/mL)
C_U = nominal concentration of zinc in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–125.0% of the labeled amount of zinc (Zn)

- **BORON, NICKEL, TIN,** and **VANADIUM, *Method 1*; CALCIUM, CHROMIUM, COPPER, IRON, MAGNESIUM, MANGANESE, PHOSPHORUS,** and **ZINC, *Method 2*; MOLYBDENUM** and **SELENIUM, *Method 3***

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 nylon syringe filter of 5-µm pore size. 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 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 5-µm pore size. If necessary, make any further dilutions using the *Diluent*.

Instrumental conditions

(See *Plasma Spectrochemistry* ⟨730⟩.)

Mode: Inductively coupled plasma spectrometry, 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	= measured concentration of the relevant element in the *Sample solution* (mg/L)
V	= volume of the *Sample solution* (L)
W	= sample weight (mg)
F	= dilution factor of the *Sample solution*
C_W	= average Tablet weight (mg)
L	= 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:**[4] 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 ▲*RRR*▲ (USP 1-May-2020) or ▲*all-rac*▲ (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
2*H*-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8,-tetramethyl-2-(4,8,12-trimethyltridecyl)-.
$C_{29}H_{50}O_2$ 430.70

USP Alpha Tocopheryl Acetate RS

USP Alpha Tocopheryl Acid Succinate RS

▲USP Ascorbic Acid RS▲ (USP 1-May-2020)

USP Biotin RS
1*H*-Thieno[3,4-*d*]imidazole-4-pentanoic acid, hexahydro-2-oxo-, 3a*S*-[(3aα,4β,6aα)]-.
$C_{10}H_{16}N_2O_3S$ 244.31

USP Calcium Pantothenate RS
β-Alanine, *N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1), (*R*)-.
$C_{18}H_{32}CaN_2O_{10}$ 476.53

USP Cholecalciferol RS
9,10-Secocholesta-5,7,10(19)-trien-3-ol, (3β,5*Z*,7*E*)-.
$C_{27}H_{44}O$ 384.64

USP Cyanocobalamin (Crystalline) RS
Vitamin B_{12}.
$C_{63}H_{88}CoN_{14}O_{14}P$ 1355.37

USP Ergocalciferol RS
9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol, (3β,5*Z*,7*E*,22*E*)-.
$C_{28}H_{44}O$ 396.65

[4] ▲▲ (USP 1-May-2020)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*-retinol (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 beta carotene = 0.6 µg of all-*trans*-beta carotene; 1 USP Vitamin D Unit = 0.025 µg of ergocalciferol or cholecalciferol; and 1 mg of ▲*all-rac*▲ (USP 1-May-2020)-alpha-tocopherol = 1.1 former USP Vitamin E Units; 1 mg of ▲*all-rac*▲ (USP 1-May-2020)-alpha-tocopheryl acetate = 1 former USP Vitamin E unit; 1 mg of ▲*all-rac*▲ (USP 1-May-2020)-alpha-tocopheryl acid succinate = 0.89 former USP Vitamin E Units; 1 mg of ▲*RRR*▲ (USP 1-May-2020)-alpha-tocopherol = 1.49 former USP Vitamin E Units; 1 mg of ▲*RRR*▲ (USP 1-May-2020)-alpha-tocopheryl acetate = 1.36 former USP Vitamin E Units; and 1 mg of ▲*RRR*▲ (USP 1-May-2020)-alpha-tocopheryl acid succinate = 1.21 former USP Vitamin E Units. In terms of ▲*RRR*▲ (USP 1-May-2020)-alpha-tocopherol equivalents, 1 mg of ▲*RRR*▲ (USP 1-May-2020)-alpha-tocopheryl acetate = 0.91; 1 mg of ▲*RRR*▲ (USP 1-May-2020)-alpha-tocopheryl acid succinate = 0.81; 1 mg of ▲*all-rac*▲ (USP 1-May-2020)-alpha-tocopherol = 0.74; 1 mg of ▲*all-rac*▲ (USP 1-May-2020)-alpha-tocopheryl acetate = 0.67; and 1 mg of ▲*all-rac*▲ (USP 1-May-2020)-alpha-tocopheryl acid succinate = 0.60. ▲Note that 1 mg of Institute of Medicine (IOM) alpha-tocopherol equivalent = 1 mg of 2*R*-alpha-tocopherol = 1 mg of *RRR*-alpha-tocopherol = 2 mg of *all-rac*-alpha-tocopherol.▲ (USP 1-May-2020)

USP Folic Acid RS
USP Niacin RS
3-Pyridinecarboxylic acid.
$C_6H_5NO_2$ 123.11
USP Niacinamide RS
3-Pyridinecarboxamide.
$C_6H_6N_2O$ 122.12
USP Phytonadione RS
USP Pyridoxine Hydrochloride RS
3,4-Pyridinedimethanol, 5-hydroxy-6-methyl-, hydrochloride.
$C_8H_{11}NO_3 \cdot HCl$ 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*E*,4*E*,6*E*,8*E*)-3,7-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.
$C_{17}H_{20}N_4O_6$ 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.
$C_{12}H_{17}ClN_4OS \cdot HCl$ 337.27
▲▲ (USP 1-May-2020)

Ann. N.Y. Acad. Sci. ISSN 0077-89

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Special Issue: Annals *Reports*
TECHNICAL REPORT

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,a] 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) formulation[2] has been shown to provide pregnant women and their offspring with a positive pregnancy and a healthy start to life in a safe[3] and cost-effective[4] 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

[a]See "Acknowledgments and author contributions" section for more information.

doi: 10.1111/nyas.14322

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 pharmacopoeia 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.[a]

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.[b] 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

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

[a]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

[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

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

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

[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.

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Table 3. Tablet characterization and purity

Test	Test method	Acceptance criteria
	Physical characteristics	
Appearance	Visual	TBD by manufacturer
Shape	Visual	TBD by manufacturer
Tablet thickness	Micrometer	TBD by manufacturer
Tablet length	Micrometer	TBD by manufacturer
Tablet friability	USP <1216>	TBD by manufacturer
Tablet breaking force	USP <1217>	TBD by manufacturer
	Performance	
Average tablet weight	USP <2091>	TBD by manufacturer
Weight variation		Each of the individual weights is within 95–105% of the average weight
Dissolution for vitamin A (index for oil-soluble vitamins)	USP <2040> Apparatus 2, at 75 rpm, in 0.05 M phosphate buffer pH 6.8, w/1% (w/v) sodium ascorbate and 1% (w/v) octoxynol 9, 900 mL	LT 75% of the labeled amount of vitamin A dissolved in 45 minutes
Dissolution for folic acid	USP <2040> Apparatus 2, at 75 rpm, in water or 0.05 M pH 6.0 citrate buffer, 900 mL	LT 75% of the labeled amount of folic acid dissolved in 1 hour
Dissolution for riboflavin (index for water-soluble vitamin)	USP <2040> Apparatus 2, at 75 rpm, in 0.1 N hydrochloric acid, 900 mL	LT 75% of the labeled amount of riboflavin dissolved in 1 hour
Dissolution for iron (index element)		LT 75% of the labeled amount of iron dissolved in 1 hour
	Elemental impurities	
Arsenic (inorganic)	USP <233> and USP <2232>	NMT 15 mcg/day
Cadmium		NMT 5 mcg/day
Lead		NMT 5 mcg/day
Mercury (total)		NMT 15 mcg/day
Methylmercury (as Hg)[e]		NMT 2 mcg/day
	Microbial contaminants	
Total aerobic microbial count (TAMC)	USP <2021>	NMT 3×10^3 CFU/g
Total combined yeast and mold (TCYM)	USP <2021>	NMT 3×10^2 CFU/g
Absence of *Escherichia coli*	USP <2022>	Absent in 10 g
Absence of *Salmonella* spp.	USP <2022>	Absent in 10 g
Absence of *Staphylococcus aureus*	USP <2022>	Absent in 10 g
Enterobacterial count (bile-tolerant Gram-negative bacteria)	USP <2021>	NMT 10 CFU/g

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)

Ingredient	Test method[a]	Label claim	USP	US-FDA[b]
Vitamin A (as retinyl acetate)	Vitamin A, method 1	800 mcg	LT 90.0% NMT 165.0%	LT 100.0% NMT 175.0%
Vitamin C (as ascorbic acid)	Vitamin C, assay <580>, method II	70 mg	LT 90.0% NMT 150.0%	LT 100.0% NMT 160.0%
Vitamin D (as cholecalciferol)	Cholecalciferol or ergocalciferol method 1	5 mcg (200 IU)	LT 90.0% NMT 165.0%	LT 100.0% NMT 175.0%
Vitamin E (as dl-alpha tocopheryl succinate)	Vitamin E, method 1	10 mg	LT 90.0% NMT 165.0%	LT 100.0% NMT 175.0%
Vitamin B1: thiamine (as thiamine mononitrate)	Niacin or niacinamide, pyridoxine hydrochloride, riboflavin, and thiamine; method 1	1.4 mg	LT 90.0% NMT 150.0%	LT 100.0% NMT 160.0%
Vitamin B2: riboflavin		1.4 mg	LT 90.0% NMT 150.0%	LT 100.0% NMT 160.0%
Vitamin B3: niacin (as niacinamide)		18 mg	LT 90.0% NMT 150.0%	LT 100.0% NMT 160.0%
Vitamin B6: pyridoxine hydrochloride		1.9 mg	LT 90.0% NMT 150.0%	LT 100.0% NMT 160.0%
Folate (as folic acid)	Folic acid, method 1	680 DFE (400 mcg folic acid)	LT 90.0% NMT 150.0%	LT 100.0% NMT 160.0%
Vitamin B12 (as cyanocobalamin)	Cyanocobalamin, method 1	2.6 mcg	LT 90.0% NMT 150.0%	LT 100.0% NMT 160.0%
Iodine (as potassium iodide)	Iodide	150 mcg	LT 90.0% NMT 160.0%	LT 100.0% NMT 170.0%
Iron (as ferrous fumarate)	Copper, iron, and zinc, method 2; selenium, method 3 plasma spectrochemistry <730>	30 mg	LT 90.0% NMT 125.0%	LT 100.0% NMT 135.0%
Zinc (as zinc oxide)		15 mg	LT 90.0% NMT 125.0%	LT 100.0% NMT 135.0%
Selenium (as sodium selenite)		65 mcg	LT 90.0% NMT 160.0%	LT 100.0% NMT 170.0%
Copper (as cupric oxide)		2 mg	LT 90.0% NMT 125.0%	LT 100.0% NMT 135.0%

NMT, not more than (≦); LT, less than (<).
[a]All tests to be performed per current USP-NF, oil- and water-soluble vitamins with minerals tablets.
[b]USP 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;

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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);
- Japanese Pharmacopoeia (JP).

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
- USP–NF general chapter ⟨2750⟩ Manufacturing Practices for Dietary Supplements.

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

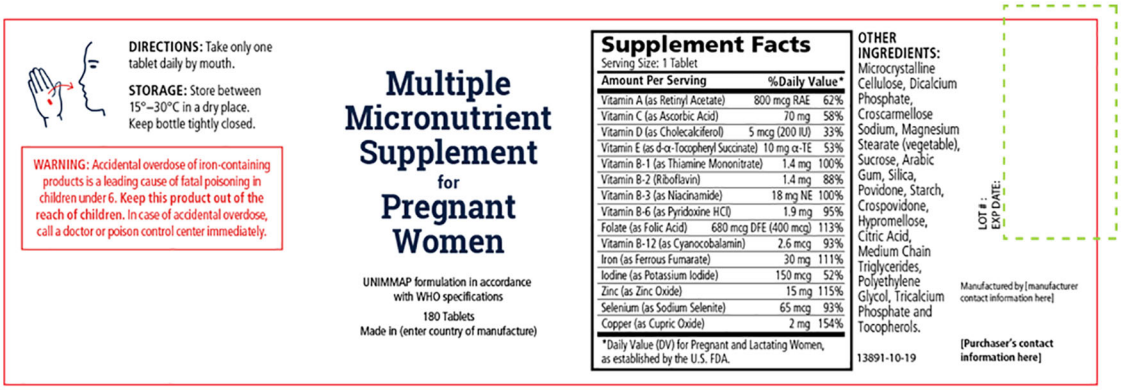

Figure 1. **Label illustrating the minimum recommended information that should appear on the label of the UNIMMAP–MMS.**

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.

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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 UNNIMAP-MMS product under adequate temperature and humidity control to prevent UNNIMAP-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).

Box 1. Definitions and acronyms

Term/abbreviation	Definition/long form
Accredited third-party certification body	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.
Article	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).
Batch	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.
Composition	Composition is the specified mix of food/dietary/nutritional ingredients and excipients in a food/dietary/nutritional supplement.

Continued

Box 1. ***Continued***

Term/abbreviation	Definition/long form
Code of Federal Regulations (CFR)	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.
Certificate of analysis (CoA)	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.
Country of destination	The country in which the product is intended to be marketed/used.
Excipients	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.
Food/dietary/nutritional ingredient	Food/dietary/nutritional ingredients are ingredients with an established nutritional value, namely, vitamins and minerals in their respective chemical entity.
Food/dietary/nutritional supplement ingredient	Food/dietary supplement ingredient includes food/dietary ingredients and excipients.
Food/dietary/nutritional supplement	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).
Globally recognized pharmacopeia compendial standard	The following international pharmacopoeia's official compendial standards are considered globally recognized: 1. British Pharmacopoeia (BP) 2. European Pharmacopoeia (Ph.Eur.) 3. International Pharmacopoeia (Ph.Int.) 4. Japanese Pharmacopoeia (JP) 5. United States Pharmacopeia (USP)
International Conference on Harmonization (ICH)	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.
Micronutrient Forum (MN Forum)	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.
Not less than (NLT)	NLT is equal to, but not less than a given value.

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Box 1. ***Continued***

Term/abbreviation	Definition/long form
Not more than (NMT)	NMT is equal to, but not more than a given value.
Pharmaceutical Inspection Co-operation Scheme (PIC/S)	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.
To be determined (TBD)	TBD is related to a variable that has not yet been determined.
United Nations International Multiple Micronutrient Antenatal Preparation (UNIMMAP)	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.
Vitamin Angel Alliance (VAA)	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 (www.vitaminangels.org).

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.

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