Tolerances for food supplements:

An introductory guide

IADSA

International Alliance of Dietary/
Food Supplement Associations

The International Alliance of Dietary/Food Supplement Associations (IADSA) brings together over 50 associations of dietary/food supplement manufacturers and distributors from across the world. IADSA's central goal is to ensure a greater exchange of information about the science and regulation of dietary supplements and ingredients across government, industry and the scientific community.

This publication has been developed by an international group of experts within the supplement industry. The lead authors are Peter Berry Ottaway and Sam Jennings, advisors to the UK Council for Responsible Nutrition.

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Introduction

Supplement Product Tolerances

All responsible supplement manufacturers use available technology and constantly strive to ensure that their products maintain their declared values throughout their shelf lives.

However, despite these efforts there are a number of factors, both singly and collectively, which can influence the declared values. Manufacturers are aware of these factors, but it is not practically possible to reduce or remove all the potential effects.

The combinations of factors that occur within a particular product formulation can result in assayed values falling either side of the declared value. However, what must be taken into account is that the declared value is only a target, and it cannot be considered to be absolute.

As the declared value is not an absolute value, it will not be achieved in every batch of product manufactured, and there will be minor deviations that can be both above and below the target value. The manufacturer aims to ensure that the product variation falls within a defined range, commonly referred to as 'manufacturing tolerances'.

The levels of certain quantified ingredients in food supplements could be measured in order to confirm the product's compliance with the levels declared on its label, and potentially to confirm compliance with the conditions for any quantified nutrition or health claims made for the product. The acceptable differences between the nutrient values declared on a label and those established in the course of official controls by the authorities, both above and below the declared values, are also often referred to as 'tolerances'. The measured value is expected to be within the tolerances around the declared value during the entire shelf life of the product.

Tolerances for food supplements need to include all factors for variation. In other words, the stated tolerance values need to take into account not only the shelf-life overage, raw material variation, the processing variation allowance and the range of variation around the average (mean) analytical result used for the declared value, but also the uncertainty of measurement that is associated with a measured value.

Tolerances on nutrient labelling do not mean that manufacturers can formulate to less than 100% of declared value at end of shelf life. On the contrary, the tolerances provide some protection to the manufacturer in the event that an externally obtained analytical result is less than the declared value. Tolerances on nutrient labelling are expected to allow for some batch-to-batch variation on ingredient specifications and any analytical variance resulting from appropriate testing methods, in addition to other factors for variation described in this publication. If external testing found the product to be around, for example, 80% of declared value, the company may be required to demonstrate that the product was correctly formulated for the intended period of shelf life. It may be necessary to demonstrate from batch records that the lower level found is due to, for example, analytical variation, or an ingredient being at its lower specification limit.

As tolerances are multi-factorial and are influenced by a disparate range of variables, they can be a difficult concept to easily understand.

1. Variability in raw material

A major factor affecting tolerances in raw materials is an inevitable variation in critical parameters such as moisture, assay levels and stability. These variations are found not only in raw materials derived from natural sources, such as botanicals, but also those that are chemically synthesised or highly processed.

As food supplements can often contain up to 40 ingredients and food additives (excipients), minor variations in each of these components can have a cumulative effect on the variability of the final product.

Some of the more common sources of variations in ingredients, that must be given due consideration for ingredients which are the subject of a quantitative declared value, are given below.

1.1 Moisture Content

The majority of ingredients used in supplements contain moisture to a greater or lesser degree. Where the cumulative moisture content is high, there is potentially a significant effect on the stability and shelf-life of a product and also on the calculations of the levels of active ingredients. This can therefore impact on the tolerance.

Moisture content can range from decimals of one percent in some components to double figure percentages in others. For example, some of the cereal powders such as rice flour used as carriers for ingredients in hard-gel capsules can have moisture levels in the range of 10 to 14%, and many dried and powdered botanical substances have specifications for moisture levels between 8 and 15%. The moisture in botanical extracts is usually more controlled, at around 6% or below.

When assessing the impact of moisture in an ingredient, or ingredients, on a product's stability and potential tolerances, the highest values given in the agreed specification should be taken into consideration. In the eventuality that all the moisture levels of the largest components of a product are at the maximum of the moisture range, the impact on the moisture content of the final product can be very significant. In the example given in Figure 1.1, the increase in moisture could be expected to have a significant effect on the shelf life of the product.

Some supplement ingredients can be hygroscopic and the storage conditions and length of time from the bulk containers being opened can have an effect on the moisture content. Thus, a hygroscopic ingredient in a part-used container stored at ambient humidity between infrequent batch productions can be expected to show an increase in moisture content. This is one of many difficulties that can be faced by manufacturers when aiming to ensure their finished product batches are as close to the declared value as possible.

Figure 1.1[1]: Example of the effect on total moisture content at upper moisture ranges of ingredients

Ingredient	% in Formula	Moisture range % in specification	'Average' moisture % from trend analysis	Increase on Average at Upper Limit %
1	50	8 - 12	9.0	3.0
2	25	4 - 8	4.4	3.6
3	20	5 - 9	6.0	3.0
Impact of 'Average' product moisture				
	From ingredient 1		1.5%	
	From ingredient 2		0.9%	
	From ingredient 3		0.6%	
	Increase in product moisture		3.0%	

1.1.1 Moisture Content and Loss on Drying

When interpreting raw material specifications and Certificates of Analysis, manufacturers are required to distinguish between the test methods and values that can relate to product moisture. The two most commonly referred to are 'moisture' and 'loss on drying', which do not necessarily give the same results for water content.

The actual water content of a product is determined by methods such as the Karl Fischer titration, which can quantify the water content of a substance down to trace amounts utilising the reaction of water with iodine.

Loss on drying (LOD) measures the total change in weight of a substance as a result of drying. Some supplement components such as alcohol, solvents or fat can evaporate with water. This means that the loss on drying method measures not only the water content but also the content of any volatile components and impurities.

With some supplement ingredients, the values obtained on the same substances from the Karl Fischer and LOD methods can differ significantly. These differences have to be recognised and taken into consideration when the manufacturer is formulating the supplement, adding to the complexity of determining appropriate declared values for the finished product.

The differences in results between the Karl Fischer and LOD methods can also result in discrepancies between the manufacturer's analyses and those carried out by official control laboratories.

1.2 Activity or Potency of an Ingredient Declared on Label

An important aspect of ingredient tolerances is what is often described as the activity level or 'potency' of ingredients on which declared values are based.

It is very rare to find an ingredient which is 100% of the pure substance being declared. Most commercial ingredients, including food additives (excipients), contain a number of substances in very low amounts in addition to moisture, such as other food additives, carriers and processing aids. In addition, many ingredients may be in the form of a compound, salt or ester of the active ingredient. The information on the actual levels of activity and their ranges is normally available from official monographs, such as Food Chemicals Codex and national Pharmacopeiae, and from ingredient manufacturer's specifications and Certificates of Analysis. These bibliographical and supplier sources should give, as a minimum, a range for the moisture content and for vitamins, nutrient minerals and trace elements and certain other substances, and the assay ranges for the active component(s), and should be the basis for the calculation of ingredient input.

The manufacturer uses the moisture range and the assay range (the range of the active component in the ingredient) for the calculation of the active level of the declared ingredients. To calculate an input level of an active ingredient there are a number of steps that need to be followed.

Step One

Assess the 'average' moisture content, ideally by trend analysis based on a number of production batches, but otherwise based on the maximum value given by the manufacturer of the ingredient.

Step Two

Check the assay range declared in the specification and use the lowest level given. The lowest level of any range is used to ensure that sufficient quantity of the active ingredient is always included in the product.

Step Three

Calculate the actual amount of declared active ingredient, using the convention applicable to the intended country or region of market. Across the globe there are different conventions for declaring the activity of certain vitamins, namely the B vitamins B1, B2, B6 and pantothenic acid. In some countries or regions, (for example, the USA), the whole salt can be taken as the basis for the calculation of the declared value. In some other countries or regions, (for example, the European Union), only the vitamin cations can be used for the declared value.

See Example 1.1 for an illustration of how these steps can be applied to a specification for a vitamin input declared on label.

Example 1.1: Application of Steps One to Three to an ingredient specification for thiamin hydrochloride, a common source of vitamin B1.

Step One

The maximum moisture stated in the specification is 5%.

Step Two

The assay range for thiamin hydrochloride stated in the specification is 98.0 to 102.0% calculated on the dry weight.

Step Three

(i) For countries or regions that declare the whole salt

Taking the lowest assay and highest moisture values, the active amount of thiamin hydrochloride for formulation would be 98.0 minus 5.0, which equates to 93.0%. The declared vitamin content would be 93.0% of the input amount of the salt.

(ii) For countries or regions where only the cation is declared

The value of 93.0% obtained for thiamin hydrochloride in (i) above is divided by 1.271 to obtain the pure thiamin content. Thus, the declared vitamin content would be 73.2% of the input amount of the salt.

Note: Steps One to Three are particularly important for ingredients such as ascorbic acid (vitamin C). In addition to taking into account the moisture and assay levels, much of the vitamin C powder now used in supplement tablets is direct compression (DC) grade, which can contain around 3% of other inert components needed to obtain the compressibility. These also have to be deducted from the total weight in order to determine the declared content of vitamin C.

The calculations to obtain elemental amounts from the mineral and trace element salts are more complex. Most of the minerals required for human nutrition can in each case be supplied from a number of sources, mainly organic and inorganic salts. Where these salts are used, the nutritional element has to be calculated from the salt. To accomplish this, both the molecular weight of the salt and the atomic weight of the desired element must be known. These calculations need to be undertaken before commencing Steps One to Three.

It should be noted that some ingredients that are not the main source of the mineral or trace element, may still contain minerals or trace elements and can impact on the analytical result. For example, the use of iron oxide colours can increase the total iron content of the product above that calculated from the iron source.

See Example 1.2 for an illustration of how this additional stage would apply to a specification for a mineral.

Example 1.2: Application of Steps One to Three to an ingredient specification for calcium carbonate, a common source of calcium used in supplements.

Preliminary calculation

The chemical formula for calcium carbonate is

CaCO3 with a molecular weight of 100.9

The atomic weight of calcium is 40.08.

The calcium carbonate formula breaks down into

12% carbon

47.96% oxygen

40.04% calcium

From these figures it can be seen that 100mg of the calcium carbonate salt only provides 40mg of calcium.

Step One

The maximum moisture stated on the specification is 2%.

Step Two

The specification gives an assay for calcium carbonate of not less than 98.5%.

Step Three

If the moisture at 2% is subtracted from the assay value of 98.5%, giving 96.5%, the calcium content of the food grade salt reduces to:

$$\frac{(40.04 \times 96.5)}{100} = 38.64\%$$

Thus, the declared value for the calcium would be 38.64% of the input amount of the salt.

For edible oils, such as omega-3 oils, an adjustment calculation is required if the fatty acid content is declared.

Oils, both plant and animal, are composed mainly of fatty acids in proportions consistent with their species. However, in addition to the fatty acids there are other components of the oil, particularly glycerol and unsaponifiable matter. These components can comprise between 3 and 5% of the oil, which means that the fatty acids collectively account for between 97 and 95%. This means that when fatty acids are the subject of a declared value, they can be given either as a percentage of the total oil or as a percentage of the total fatty acids. The basis for the calculation of the fatty acid percentages should be given on the supplier's specification for the oil and considered in the declared value.

Input calculations typically use the lower end of the supplier's specified active range of the ingredient, in order to always be in compliance of the declared value. At the time of manufacture, ingredients may assay at any point within the specification range, thereby introducing variability into the level of the active component in the finished product.

1.3 Raw Material Variations – Impact on Declared Value

As can be appreciated from the above sections (1.1 and 1.2) there are a number of factors that can affect the active levels of an ingredient. Many multi-vitamin and mineral supplements can contain over 30 declared nutrients, which means that all the declared ingredients require their input amounts into the formula to be individually calculated, based on quantitative information provided by the supplier, or derived from 'in-house' testing.

More information on ingredient manufacturer's specifications and Certificates of Analysis can be found in the following IADSA publication:

Certificates of Analysis for Supplement Ingredients: Guidelines on Their Preparation and Use[2]

2. Manufacturing / Processing variability

Another source of product variability is the processing of the product. This is particularly the case where the product is in tablet, hard gel capsule or powder form.

There are a number of stages during the manufacture of any supplement where variation in the product, and hence the declared values, can occur. These include the weighing and dispensing of the ingredients into batches, the mixing of the product to achieve homogeneity in the case of both solid and liquid products, and the handling of the mixed product between processing and packaging.

Manufacturers strive to put in place controls to reduce the variations caused during processing, but some variation is unavoidable.

More information on formulation considerations, quality control, manufacturing and processing can be found in the following IADSA publication:

Global Guide to Good Manufacturing Practice for Supplements[3]

2.1 Weighing and Dispensing of Ingredients

A critical stage in the manufacture of any supplement is the dispensing of the ingredients into batch weights from the bulk containers, and there are a number of potential variables that must be considered and controlled.

2.1.1 Equipment Calibration Variables

Supplements, particularly those containing vitamins and trace minerals, can contain some active ingredients at low microgram amounts per dose. This means that the accuracy of the weighing equipment is an important factor. A production batch of a supplement will contain ingredients in the weight range of tens to hundreds of kilograms, whilst at the same time have ingredients weighing only tens of grams. The weight of the largest ingredient can be many thousands of times greater than that of the smallest.

The manufacturer has to routinely check the accuracy and calibration of each piece of weighing equipment. Such checks take into account four components:

- Reproducibility: The instrument's ability to repeatedly deliver the same weight reading for a given object. The results are expressed as a standard deviation and should form part of the performance specification for the equipment.
- Linearity: The characteristic that quantifies the accuracy of the instrument at intermediate readings throughout its weighing range. This is similar to the assessment of the analytical measurement range of a laboratory test. As the instrument will be used to weigh items at all weights within its capacity, the knowledge of its linearity is a critical aspect of its overall accuracy.
- Calibration: A comparison of the weight reading of a given standard and the actual weight of the standard. This is carried out at different weights and particularly at full capacity.
- Cornerload errors: The errors that can occur when the mass to be weighed is placed in different positions on the weighing pan or platform of the instrument. The given mass should produce the same reading irrespective of where it is placed on the pan or platform.

2.1.2 Operator Variables

The training, skills and attention to detail of the operators tasked with the weighing and dispensing of the ingredients can have a significant influence on the variability of the batch of product and ultimately on the declared values.

The manufacturer can alleviate this influence to a great extent by ensuring that strict controls are not only in place, but are also adhered to continuously.

2.1.3 Transfer Losses

A small but still significant source of variation in a batch can be attributed to transfer losses, especially during the early stages of production. This particularly relates to the declared ingredients that are present in very small amounts in the final product, such as at microgram quantities.

Transfer losses can occur during the weighing and dispensing operations and when the weighed materials are transferred from the bags or tubs into the mixer. It has been demonstrated that residues of declared ingredients, such as some vitamins and trace elements, left in the transfer containers can affect the final levels in the product.

Manufacturers aim to reduce this problem by ensuring appropriate operator training and the selection of suitable transfer containers to minimise residues, but some variability due to transfer losses can be unavoidable.

Example 2.1 provides an illustration of the variance one company allows for potential weighing, operator and transfer losses, based on their operating experience.

Example 2.1: A Manufacturer's Experience

Based on their operating experience, Company A usually allows for a variance of 2-3% for potential weighing, operator and transfer losses.

Occasionally, for microgram quantities, they may accept a 6% variance, provided that safety is not a concern.

2.2 Mixing Variability

The greater proportion of supplements sold world-wide are in the form of tablets, powders and hard-gel capsules. All these forms require an accurate blending of multiple ingredients to a point where the powder is homogeneous, that is that all the components are equally and evenly distributed throughout the mass (see Figure 2.1). If homogeneity is not achieved, the resulting supplements will not be uniform in their content. Achieving homogeneity in food supplement powders is not easy, owing to the multiplicity of ingredients of disparate weights, particle sizes and morphology. The manufacturer is required to consider the different characteristics of each component in a mixture, as the incompatibility (in the form of incompatible physical characteristics) of one ingredient can affect the successful production of a product.

Figure 2.1: Representation of a Heterogeneous Mix versus a Homogeneous Mix

Heterogeneous (unmixed)

Homogeneous (uniform mix)

Powders vary widely in the physical characteristics of their particles. The size, shape, density and surface texture of the particles combine to impart the physical characteristics to the powder, and will affect a number of aspects of the formulation, such as the powder's ability to flow, mix and, in tablet technology, to compress.

As most tablets and hard-gel capsule products are combinations of powders and, in some cases, a large number of powders, the manufacturer is required to characterise each powder at an early stage of product development, as the powder characteristics can have an effect on the mixing variability.

The effects of the powder characteristics of the many ingredients of a complex supplement formulation cannot be over-estimated, as they can have an impact on the powder bulk density, the volume of powder in the mixer and also the ability of the formulated powder to achieve a homogeneous mix.

2.2.1 Powder Characteristics

The characterisation of an individual powder, and mixtures of powders, is an essential tool when formulating solid dose forms, so predictions can be made at laboratory scale about product performance at larger and more costly production scales.

Some of the reasons for producing free-flowing powders include:

- Uniform feed from bulk storage containers into the feed mechanisms of manufacturing equipment, allowing a homogeneous mix of particles and a consistent volume to mass ratio for consistent fill (dose) rates.
- Reproducible filling of tablet dies/capsule dosators, which improves weight uniformity and allows tablets and hard-gel capsules to be made with more consistent physico-mechanical properties.

Properties such as flowability (fluidity), powder mixing and powder densification can be characterised quickly and easily using the following simple processes that form the basis of investigations into powder properties:

i. Angle of repose

ii. Bulk density and tapped bulk density

iii. Flowability

Well established analytical tools are available for the measurement and quantification of these physical characteristics of a powder.

2.2.2 Particle Size

It should be noted that whilst good powder (granule) flow generally helps with mechanical processing, it can lead to an increase in segregation (separation) in some cases. Powder flow is therefore used in conjunction with particle size distributions when formulating the optimum system.

Particles which differ significantly in size have a tendency to segregate, which can lead to uneven distribution of the active components within the mix.

As a general rule, the tighter the distribution of particle size and weight, the better the chance of achieving homogeneity in a mix. Particle shape also has to be considered, as this can affect the adhesion and compression characteristics of the powder.

2.3 Mixer Characteristics

A critical stage of the manufacture of supplements is the mixing of the powders to obtain a homogeneous mix. The effect of the mixing process determines the quality of the product and the compliance with declared values.

The mixing stage is important, as the mixer type, operating weight and volume, and mixer speed and time can all affect the ability to achieve homogeneity in a powder.

The ideal mixer will produce a homogeneous mix of a number of individual powders in the shortest possible time and will enable full movement of all powder particles, with no 'dead' zones. However, in reality, companies must balance mixing quality with process compatibility. Selection of the 'optimal' mixer generally has to take into consideration some or all of the following:

- The purposes of the mixing operation;
- Powder properties;
- Rate of mixing;
- Efficiency;
- Power (electricity) requirements;
- Scale-up criteria;
- Maintenance problems;
- Unit and running costs.

In many production facilities the selection of the mixer type has to be a practical compromise and it is generally not possible to have an 'ideal' mixer for each individual product. Instead, the mixer(s) chosen may simply be the best one(s) to suit the range of supplements produced by the manufacturer.

2.3.1 Mixer Operating Volume

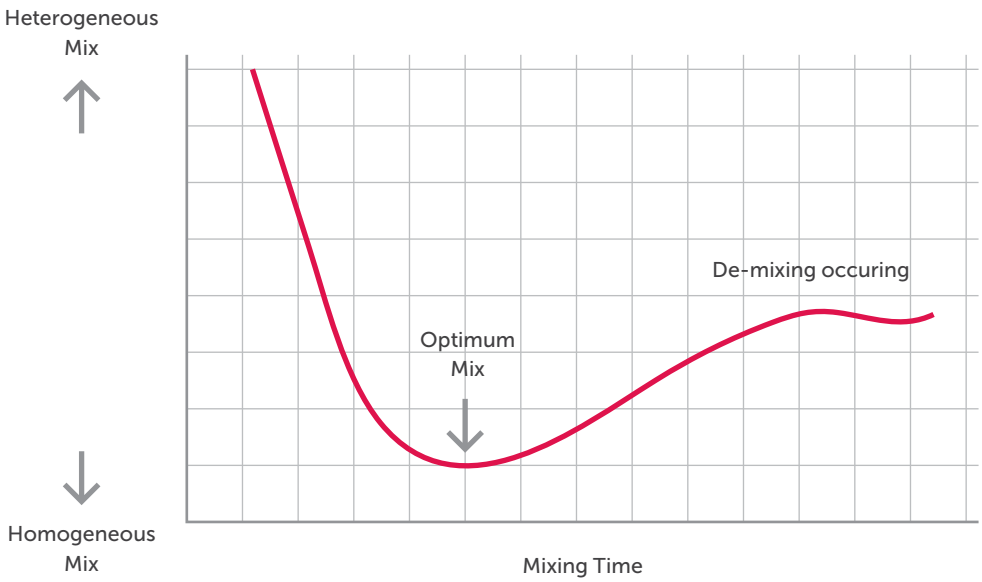
All mixers have an optimum operating volume, and the batch size of the product is ideally adjusted to the operating volume of the mixer. The bulk density determination of the powder is an important factor in the calculation of the volume.

2.3.2 Mixing Time

For all powders there is an optimum mixing time when homogeneity is achieved (Figure 3). Either side of this optimum time the powder will not be completely mixed, and this increases the further the actual mixing time is from the optimum point.

The optimum time is related to the composition of the powder, including the number of ingredients with different particle characteristics, the type of mixer and the volume of the batch in relation to the mixer's optimum operating capacity (see Figure 2.2).

Figure 2.2[4]: Graphical Illustration of Optimum Mixing

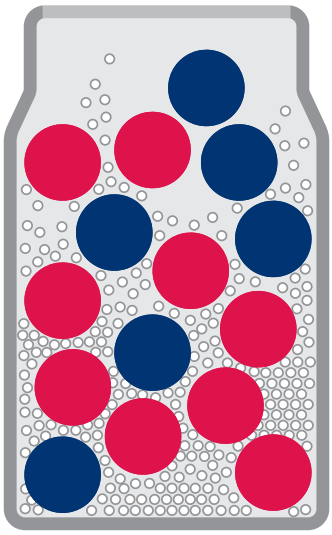


2.4 Handling of Powder After Mixing

There are a number of points after the mixing process has been completed where separation or de-mixing of the powder can occur.

De-mixing is most likely to be where there is a diversity of particle sizes from the different ingredients, that is, where there is a significant variation in particle size. For example, some particles are relatively large and heavy whilst others are small and light; similarly, others may be small and heavy, mixing with those that are large and light (see Figure 2.3).

Figure 2.3[5]: Representation of de-mixing occurring with different ingredient particle sizes



Parts of the post-mixing handling process which have been identified as being susceptible to de-mixing are:

- Gravity discharge from the mixer into a bulk container, particularly if there is a significant length of drop from the discharge point on the mixer to the bottom of the container.
- All screw-feed or air transported closed powder conveying systems are potential sources of powder separation.
- Discharge into hoppers above tabletting and encapsulating machines. This can be a particular problem where there is a distance between the discharge of the powder and the hopper. For example, in production facilities where the bulk powder is discharged from the floor above the production machine.
- Gravity feed from the hoppers into the platforms of the tabletting or encapsulating machines.
- Transfer of bulk powder between production areas or facilities, including loading and unloading of the powder.

2.5 Multi-ingredient Products

Supplements differ from most other food product sectors, in that almost all the products have quantified declared values that are expected to fall within the accepted tolerances throughout the life of the product. The only other foods with this requirement tend to be fortified food products, where vitamins, minerals and some other substances might be added to a standard food or beverage.

Supplements also tend to contain more than one declared ingredient, with the multi-vitamin and mineral supplements containing around 30 active substances. This can provide a challenge both when manufacturing and assaying supplement products. The higher the number of active ingredients, the more difficult it can be to ensure homogeneity in the powder mix during manufacture. It can also be difficult to assay certain active ingredients in the finished product matrix. This is particularly an issue when the ingredients vary dramatically in their required level of input.

A typical product will contain declared ingredients which are five or more logarithmic cycles apart (1000mg to <0.001mg). Examples of the variation that might be seen in a multi-vitamin and mineral supplement product are given in Table 2.1.

Table 2.1: Example of weight ranges of input of some vitamin and mineral ingredients in a single unit dose formula

> 100mg	–	Calcium, Magnesium, Vitamin C, Potassium
50mg → 10mg	–	Vitamin E, Iron, Zinc, Niacin
10mg → 1mg	–	Vitamin B_1, Vitamin B_2, Vitamin B_6, Pantothenic acid, Manganese, Copper
1mg → 0.1mg	–	Vitamin A, Folic acid, Iodine
0.1mg → 0.01mg	–	Biotin, Vitamin K, Selenium, Chromium, Molybdenum
0.01mg → 0.001mg	–	Vitamin B_{12}

2.6 Manufacturing Stress and Losses

Some powders can be affected by the stresses applied during the manufacturing process. These can include abrasion and breakdown of particles during the mixing process and subsequent powder handling.

Some ingredients, such as the vitamin A preparations, are microencapsulated to protect the active substance from deterioration, particularly from oxidation. Under certain mixing conditions, the microencapsulation can be broken down and there can be a loss of active material. In addition, the extra components required for the encapsulation can in some cases make full recovery of the active component difficult during assay. For example, beta-carotene beadlets have been observed to be impacted by compression loss.

2.7 Weight Variations within the Dosage Form

Every tabletting and encapsulating machine will have its own specification, which will include the tolerances around the target weight. This variation can depend upon the individual tablet size, weight and processing method.

The consequence of this is that there is an unavoidable variability of tablet and capsule fill weights, which will also impact on the manufacturing tolerance range for the final product, as even minor variations from the target will have an effect on the declared values.

2.8 Potential Impact of Processing on Declared values

As can be seen from the above, the manufacturing process for supplements, and all other processed foods, introduces inevitable variations from the target value. Some of these sources of variation are unavoidable; whilst others can be minimised, but not eradicated, by the application of good manufacturing practice (GMP).

These cumulative processing variables have to be taken into consideration when setting the official tolerance range for declared values.

3. Analytical variability and Measurement uncertainty

An area of variability that affects both supplement manufacturers and regulators alike is that surrounding the analysis of the products.

Ring trials, in which identical samples are sent to multiple accredited laboratories for analysis, have demonstrated significant variability in the analytical results for multivitamin supplements. Inconsistencies can be shown with certain vitamins in certain tablet matrices. For example, two supplement tablets with the same level of vitamins from the same sources, analysed using the same methodology but in different laboratories, will often give different results (see Annex II). In addition, any variations introduced during the sampling of a product will compound analytical variation between laboratories.

For many products containing micronutrients, it has been found to be very difficult to develop an analytical methodology that is not product specific, as the matrix effects of certain supplements can make such specificity essential.

It is imperative that method validation is carried out and documented for all assay methods intended to be used. Where 'in-house' modifications have to be included, they should be documented in detail and, where possible, it should be ensured that the modifications can be replicated by other laboratories.

3.1 Product Matrix

The matrix in which the micronutrients and other active ingredients are contained, that is the proportions and combinations of the essential carriers and additives (excipients), has a great influence on the accuracy of chemical analyses.

Due to the large differences in nutrient contents, the form of the product (tablet, capsule, powder), and the functions of the product (for example, tablets can be swallowable, chewable, dissolvable or effervescent), there can be no standard carriers or additives. Each combination has to be tailored to each product's form and function.

The matrix of a product can influence the sample preparation and extraction procedures, and these must be considered before the quantity of the micronutrients in the dose form can be accurately determined.

In addition, the extra components required for the encapsulation of certain ingredients, such as beta-carotene, can in some cases make full recovery of the active component difficult during assay.

In the determination of vitamins by chromatographic methods such as HPLC, a number of problems have been reported due to the effects of the matrix, and particularly the differential solubilities of the substances in a complex matrix.

Method verification and/or validation should be carried out on each product formulation and matrix to confirm that the analytical results are able to be replicated. In reality, whilst this can be achievable in the manufacturer's laboratory, it is normally not at all practical in an official control laboratory.

A significant aspect of matrix interference has been demonstrated in soft gelatin capsules and certain active ingredients, where there can be a migration of the active component into the gelatin shell during storage of the product. This can seriously affect the analysis of the product, by giving lower results if only the liquid component of a soft gelatin capsule is analysed. Similar problems have been observed with some coated ingredients, where the coating can affect the extraction stage of the analysis.

3.2 Variability within a Laboratory

There are a number of sources of variability that can be found within a laboratory. Apparently minor things, such as a change of technician carrying out the test, can affect the accuracy of an analysis, particularly at the preparation and extraction stages. This parameter should be taken into account during the validation check of the accuracy of the analytical method, and can be minimised by ensuring only certified personnel undertake the testing, but even official control laboratories can sometimes show a variability in results owing to changes in the technician carrying out the analysis. Ideally, test method validation would include conducting the analytical test in a number of different accredited laboratories. In practice, however, this is not always achievable.

Variations in equipment, such as changing a chromatography column or change of instrument, can affect the results, as can changes to the batches of reagents and standards. This is particularly important if the supplier of the reagent has to be changed.

Standard materials from different suppliers are not necessarily equivalent, even when obtained from suppliers that are globally well-known and recognised as being reputable. Thus, if a laboratory uses a standard material from Supplier A, then changes to a standard material of the same substance from Supplier B, there may be variation seen between the results obtained for the analyses, even if all other sources of variability within that laboratory are taken into account.

The environmental conditions, for example, temperature and humidity in a laboratory, may also contribute to an effect on the results.

3.3 Variability between Laboratories

It has been established that the factors outlined in sections 3.1 and 3.2 above result in significant differences in the results of analyses on the same sample when assayed by more than one accredited laboratory. These differences have become apparent even when the sample is analysed by two different official control laboratories. In most, if not all, cases of vitamin and mineral supplements, the problem can be traced to differences in amounts of micronutrients recovered from the extraction process.

On some occasions, erroneous calculations of the vitamin content from its source can affect the assay results. This is particularly relevant when the declared value is given in a generic form and the active component can be supplied from more than one source (see Example 3.1).

Example 3.1: Vitamin E

A generic declared value for 'vitamin E' means that the vitamin can be supplied from a number of sources. However, the vitamin E content of these various forms differ, as is shown by the officially recognised conversion factors for the activity of vitamin E from different sources:

1mg of vitamin E is equivalent to:

1mg	dl-α-tocopheryl acetate
0.909mg	dl-α-tocopherol
0.826mg	d-α-tocopheryl acid succinate
0.735mg	d-α-tocopheryl acetate
0.671mg	d-α-tocopherol

Similar effects of variability in analysis between laboratories have also been seen for some substances other than vitamins and minerals, such as botanical powders and extracts. Differences in standard materials obtained from separate suppliers, and divergence in how the laboratories might approach testing of the active substances in various matrices, can all have an effect on the results of analysis of a sample.

3.4 Measurement Uncertainty

The true value of an analytical analysis could only be obtained by a perfect measurement, but such a measurement cannot exist in reality. A doubt about the measurement result will always be present, and this is known as Measurement Uncertainty.

The international Joint Committee for Guides in Metrology (JCGM), in their Guide to the Expression of Uncertainty in Measurement, defines uncertainty in relation to measurement as a "parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand". This definition is followed by three notes:

1. The parameter may be, for example, a standard deviation (or a given multiple of it), or the half width of an interval having a stated level of confidence.
2. Uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of the results of series of measurements and can be characterised by experimental standard deviations. The other components, which also can be characterised by standard deviations, are evaluated from assumed probability distributions based on experience or other information.
3. It is understood that the result of the measurement is the best estimate of the value of the measurand, and that all components of uncertainty, including those arising from systematic effects, such as components associated with corrections and reference standards, contribute to the dispersion.

In simple terms, it can never be known whether the result obtained is the exact measurement. There is always the possibility that it will be slightly above or below the exact measurement.

The analytical variabilities referred to in sections 3.1 to 3.3 above can all impact on the degree of measurement uncertainty that is naturally present.

Products containing live bacteria (probiotic products) are particularly affected by measurement uncertainty, as a variation in the Colony Forming Unit measurement of up to 30% has been reported as typical.

3.5 Impact on Declared Value

The variability introduced during the analysis of a product is outside of the control of the manufacturer. So many factors can potentially influence the accuracy of the analysis of micronutrients and other substances, particularly in a multi-active product, that realistic tolerances around the declared value have to be set, taking into account the analytical variability and associated measurement uncertainty.

In cases of discrepancies, the manufacturer should be given the opportunity to investigate the inconsistent analytical result and produce the documentation relating to the product's manufacture and their own analyses to substantiate the product's declared content.

4. Stability of active ingredients

Many of the active ingredients found in supplements are inherently unstable and will deteriorate and lose their activity over time.

The vitamins are the most important group of nutrients used in supplements and they are relatively unstable. They are also one of the few groups of food constituents in which it is possible to demonstrate quantitatively a deterioration in content over a period of time.

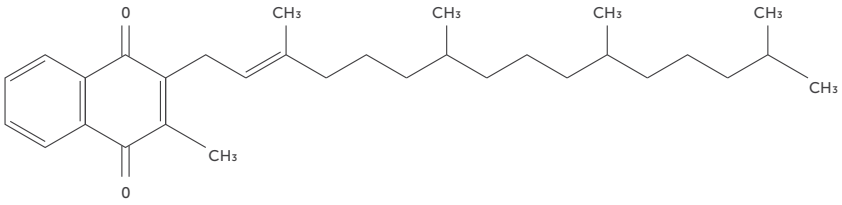
The stability of substances other than vitamins can be widely variable and has to be reviewed on an individual basis. The same diversity of molecules and loss of activity owing to external factors can also apply to plant-derived ingredients. For example, carotenoids can lose their activity in a manner similar to vitamins, and some other plant-derived components, such as polyphenols, may also show a loss of activity over time. Conversely, in general, the nutrient minerals and trace elements do not show a loss on storage.

4.1 Vitamin Instability

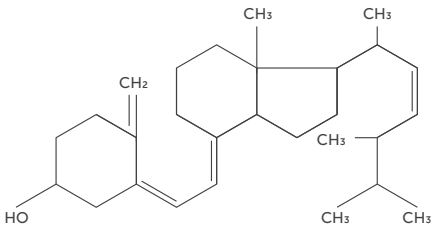
Chemically the vitamins are a heterogeneous group of compounds with very diverse chemical structures and no common structural attributes (see Figure 4.1). Of the thirteen recognised vitamins, some are single entities, such as biotin, whilst others, such as vitamin E, are large groups of compounds each exhibiting vitamin E activity. This very diverse group of substances is only linked together by an impure definition relating to their biological importance in human physiology.

Figure 4.1: Structural formulae of a few of the vitamins, showing the wide diversity in structure

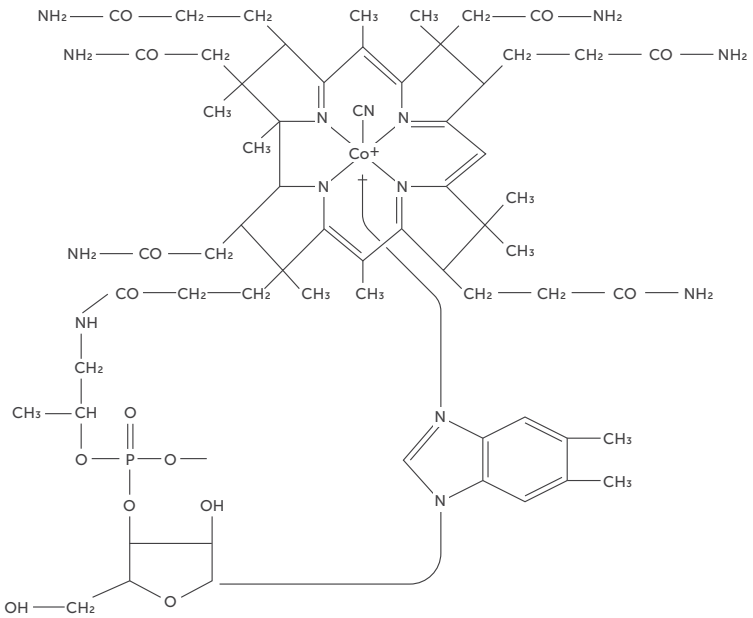
Vitamin A



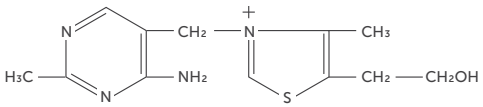
Vitamin D



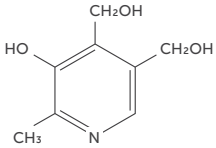
Vitamin B12



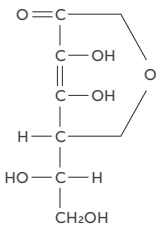
Thiamin



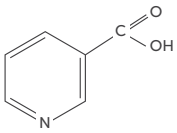
Vitamin B6



Vitamin C



Niacin



As the vitamins do not have common chemical attributes and are all inherently unstable, with some being more unstable than others, they provide a number of challenges for both the manufacturer and the regulator.

The manufacturer has to develop a product in which all the vitamins maintain their declared value at the end of a commercially feasible shelf life, and any official system of tolerances must take into consideration that the deterioration of the vitamins in a product will take place at different rates, and therefore between manufacture and the end of shelf life they could be present in the product at a level which is above the declared value.

4.1.1 Factors Affecting Vitamin Stability

There are a number of factors that are common to the degradation of all vitamins. The most important of these are temperature, moisture, oxygen, light and pH. Other factors include oxidising and reducing agents, the presence of metallic ions (e.g. iron, copper and zinc), other components in the product, such as sulphites (sulphur dioxide) and the presence of other vitamins. Invariably, the cause is a combination of all of the above during a certain time span rather than one specific factor.

For example, vitamin C is affected by oxygen, metallic ions, particularly copper and iron ions, and pH. Vitamin A is rapidly oxidised by oxygen, and is sensitive to ultra-violet light and metallic ions. Vitamin B_1 (thiamin) can be destroyed by oxidising and reducing agents and sulphur dioxide (sulphites), whilst vitamin D is affected by both light and atmospheric oxygen. In addition, many of these reactions are temperature related, and the higher the storage temperature, the faster the reaction will take place.

4.2 Stability of Other Ingredients

Whilst the stability of vitamins in a range of matrices of foods and supplements has been researched and published in the scientific literature over several decades, far less has been documented in relation to the stability of many of the other ingredients commonly used in supplement products.

Inorganic ingredients such as minerals and trace elements are relatively stable and are not expected to change over the shelf life of the product (e.g. calcium, magnesium etc). Industry best practise is to include these minerals in the long-term stability study, minimally, at the beginning and end (expiry) of the study. Whilst the minerals are not expected to degrade over time, the inherent variability of the mineral assay in finished products is typically included in the tolerance limits evaluation.

The one trace element that can show instability in supplements is iodine, where some salts (for example, potassium iodide) can exhibit losses in certain products during storage. In addition, it has been reported that the migration of iodine from the fill to the capsule shell in soft gelatine capsules can occur, resulting in low recoveries if only the capsule contents and not the whole capsule are assayed.

In addition to beta carotene, which is classified as 'pro vitamin A', the other carotenoids such as lutein and lycopene can exhibit instability under certain conditions.

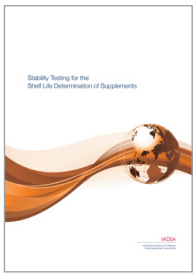
A number of the plant polyphenols have also been shown to be relatively unstable in products, as can some other botanically derived substances exhibiting a physiological effect in humans.

The stability of each supplement product containing other ingredients should be assessed on a case-by-case basis when determining the need for any overages and if considering the tolerances around the declared value.

Products containing live microorganisms (probiotic products) and which make quantitative declarations on the label need special consideration with respect to product stability, as the stability and hence shelf life of such products can be affected by a number of factors. A comprehensive stability profile needs to be established using nationally or internationally recognised microbiological test methods appropriate for the specific microorganisms and matrices. This data is also necessary to establish the tolerances for the product.

Detailed information on stability and determination of shelf-life can be found in the following IADSA publications:

Shelf-Life Recommendations for Supplements[6]



Stability Testing for Shelf Life Determination of Supplements[7]

4.3 Interactions between Ingredients

It has been found that in certain combinations of ingredients there can be interaction between the ingredients that can reduce the shelf life of the product. In products containing vitamins, the interactions can be between two vitamins, between vitamins and mineral salts and between vitamins and other ingredients.

If an interaction between ingredients has been identified from stability data or could be predicted from bibliographical sources, the formulation should be modified so as to remove or reduce the effect of the interaction. For example, many of the ingredients of concern could be coated or micro-encapsulated to prevent direct contact with the other reactive ingredients. In some cases, less reactive chemical forms of one or both of the reactive ingredients could be investigated. Sometimes input overages for these particular ingredients may have to be considered.

4.3.1 Vitamin – Vitamin Interactions

The vitamins that are known to interact with other vitamins are vitamin C (ascorbic acid), thiamin (vitamin B_1), riboflavin (vitamin B_2), folic acid and vitamin B_{12}.

Some of these reactions take place in liquid, syrup type or moist supplements at specific pH ranges, whilst others can be instigated by other factors, such as light or, in the case of thiamin and vitamin B_{12}, the decomposition of thiamin can increase the rate of the breakdown of the B_{12} due to substances formed during the thiamin cleavage.

Even when the known interactions are taken into account when formulating, some interactions leading to stability losses may still occur.

4.3.2 Vitamin – Mineral Interactions

Some vitamins, and particularly ascorbic acid (vitamin C), thiamin (vitamin B_1) and retinol (vitamin A) have their stability seriously affected by the presence of metallic ions from the trace minerals in the product. Combinations that include botanical ingredients may be particularly affected. Trace minerals in botanicals may vary from lot to lot (harvest to harvest), making it difficult to accommodate this affect when formulating.

Example 4.1: Ascorbic acid, retinol and thiamin

The oxidation of ascorbic acid can be catalysed by copper ions in concentrations as low as 0.85mg/kg (ppm).

The order of effect of metallic ions from trace minerals on ascorbic acid is generally $Cu^{+2} > Fe^{+2} > Zn^{+2}$

A similar effect can be found with copper and other trace mineral ions, particularly iron, in relation to retinol and thiamin.

4.3.3 Interactions between Vitamins and Other Ingredients

Interactions between vitamins and other ingredients have also been identified. For example, thiamin is very sensitive to sulphites and bisulphites, both of which can cleave the thiamin molecule. The presence of even trace quantities of sulphites carried over into glucose powder or naturally occurring in some botanical ingredients is sufficient to destroy the thiamin activity in a product.

4.4 Packaging

The choice of packaging can make a large difference to the stability of a product and hence the declared value. The ideal packaging should protect the product against the ingress of moisture, oxygen from ambient air and light. This means that it should have impervious barriers to all three factors. The packaging and its seals also need to be designed to minimise the risk of excess moisture and air entering the pack after it has been opened by the consumer.

4.4.1 Packaging Material

The manufacturer has to select both the material used for the pack and the pack design with a focus on product stability. For example, supplements with light sensitive active components are most stable in opaque or dark containers, whilst those which are moisture sensitive are more stable in packaging with a good moisture barrier and possibly the inclusion of a desiccant pack.

In most cases, the manufacturer has to rely on the packaging suppliers providing data on the important parameters, such as moisture and oxygen permeation rates and light (ultra-violet) transmittance, to help with the choice of materials.

4.4.2 Packaging Headspace

The amount of air trapped in a sealed container after filling it with product is known as the 'headspace'. The amount of headspace can be critical to the stability of a product as over one fifth of the air will be oxygen, which can affect the stability of many vitamins. For example, it has been shown that 11.2mg of ascorbic acid is destroyed by 0.1mg of oxygen, so it is essential that any headspace in a package is minimised.

Manufacturers therefore have to select the appropriate packaging container for their product that provides minimum headspace, but which still provides a sufficient outer surface for a legible label to be applied containing any mandatory information required in the country or region of market.

4.5 Climatic Conditions

As a generalisation, the rate of a chemical reaction doubles with approximately every 10°C rise in temperature. This means that a supplement product designed to be sold in a temperate climate with an average temperature of around 25°C will have a considerably reduced shelf life if stored and distributed in a tropical environment where the average temperature is about 35°C.

The International Conference on Harmonisation (ICH) has produced guidance on the stability testing of products for the different climatic zones. If the product is contract manufactured, it is advisable that the contract manufacturer is made aware of the potential markets and their climatic conditions.

It is recommended that a manufacturer takes climatic conditions of potential markets into consideration when formulating and setting shelf life expiry dates, as it is often not practical to use the same formula and shelf lives in both temperate and tropical climates.

The season during which a product is produced and transported affects the degree to which climate impacts the stability of the product and potentially the levels of overages that will be required.

4.6 Processing and Storage Losses

It must be appreciated that from the moment the vitamin containing product is mixed until the time the product container is opened by the consumer, there will be a gradual loss of the vitamins in the product. These losses have to be estimated by the manufacturer when setting the expiry dates.

4.6.1 Losses during Manufacture

Some vitamins, and particularly vitamins A and D in microencapsulated form, can be damaged by the shear and heat generated in the powder during the mixing and tabletting of a product. Appropriate compensation has to be made for these losses, and therefore the input amounts in the manufacturing formula are usually adjusted to take them into consideration.

With liquid products particular attention needs to be given to the vitamin stability in cases where the product undergoes heating during processing.

4.6.2 Losses during Distribution

With some vitamins it is almost inevitable that some loss of activity will occur after packaging of the product and during storage and distribution. The manufacturer has to try and estimate such losses and take these into account when formulating and setting the expiry date.

4.6.3 Losses at Point of Retail

Once a product leaves the manufacturer's storage area and enters the distribution chain its storage conditions become unpredictable and out of the direct control of the manufacturer. The stability of a product can be affected by high temperatures during transportation if the vehicle does not have climate control, and the storage conditions in the wholesalers' and retailers' premises may also not be ideal.

The storage temperatures, climatic conditions and source of lighting in a retail outlet can have an effect on product stability. For example, the light spectra of the lighting in a retail cabinet has been shown to seriously affect the levels of the light sensitive vitamins such as vitamin A, the carotenoids, riboflavin (vitamin B_2) and folic acid if the product is kept under a strong light for a period of time.

4.7 Potential Impact on Declared Value

All the factors outlined in sections 4.1 to 4.6 above show that declared values, particularly those relating to vitamins and other quantified substances, are subject to many influences, some of which are outside the direct control of the product manufacturer, (for example, once the product has entered the distribution chain). The consequence of these various influences is that whilst responsible manufacturers strive to ensure that their product meets its declared values throughout the declared shelf life of the product, there will inevitably be some small variations from the target values. These various factors have to be taken into consideration when assessing product compliance.

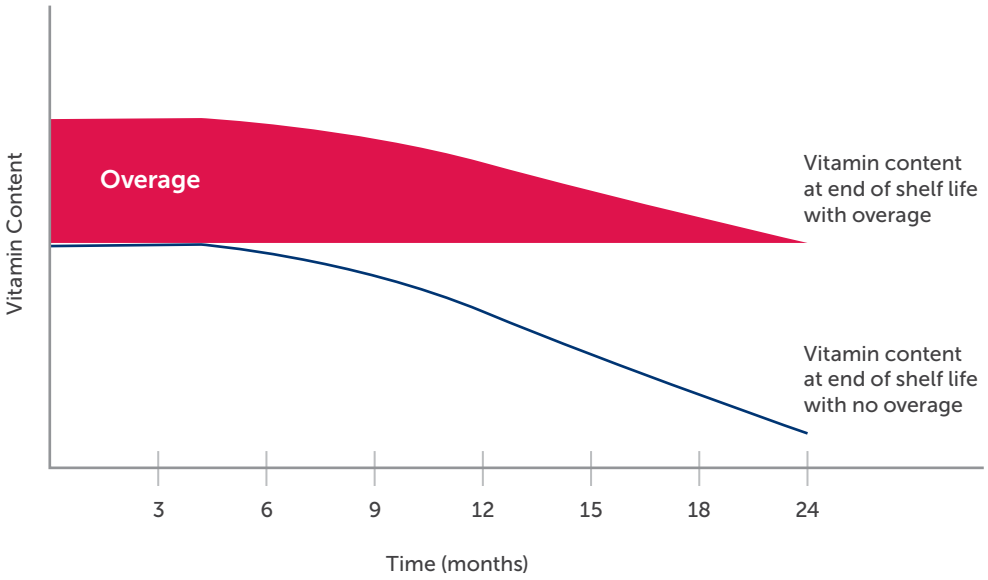
5. Vitamin overages

As the composition of each product is different, and even apparently similar products can differ in moisture content and other parameters (for example, the manufacturing process), manufacturers cannot always rely on general predictions of shelf life. In such cases, properly conducted stability tests have to be carried out on the product in the proposed packaging (or range of packaging) under a range of storage conditions that have been selected for the climatic conditions in the intended markets for the product.

If the storage tests indicate that the ingredients in the product degrade at a rate determined by the various factors that affect their stability (for example, temperature, moisture, oxygen etc.), and that each ingredient deteriorates at a different rate, the varying rates of degradation in supplements containing a number of such components can pose considerable problems in the assessment of a commercial shelf life for a product.

To meet declared values during a realistic shelf life, the amounts of some active ingredients in the product may need to be above the amounts stated on the label. The difference between the formulated and the declared levels of ingredient, known as the **'overage'**, will vary according to the inherent stability of each ingredient; the conditions under which the product is prepared and packed; and the anticipated shelf life of the product (see Figure 5.1).

Figure 5.1[8]: Graphical representation of how the overage decreases over time to ensure sufficient content of the vitamin is still present to meet the declared value at the end of shelf life.



Overages are normally expressed as a percentage of the declared level:

$$\frac{\text{Amount of vitamin present in product} - \text{amount declared}}{\text{Amount declared on product label}} \times 100$$

Thus an input level of 45mg of vitamin C and a declared level of 30mg would give an overage of 50%.

The overages for supplements, where the added vitamins are the only significant source of those nutrients, are usually calculated as a percentage of the amount required in the product to meet the label declaration at the end of the required shelf life.

The stability of the vitamin in the supplement and the length of required shelf life will govern the amount of overage selected. The more unstable vitamins such as vitamins C, A and B12 generally require relatively high overages, whilst those that are inherently more stable, such as niacin and vitamin E, generally only need small overages.

The shelf life of a product is often dictated by external factors, which include the time taken to reach the consumer; the range of temperatures that it is likely to be subjected to between production and consumption; and the length of time that it is likely to be stored by the supplier before purchase by the consumer. This information can be obtained by studying the distribution patterns of similar products already on the market, and generally includes estimates of the longest distribution chain and the rate of sale through the smaller retailer. Data on the ambient temperatures and seasonal fluctuations in the countries in which the product is to be sold are also taken into consideration, as shelf lives based on vitamin levels can vary significantly between products kept under tropical conditions and those stored in more temperate climes.

5.1 Assessment of Overages

Once the basic criteria have been established for the shelf life, the overages have to be assessed, taking into consideration all the information available. The estimation of the overages during product development is difficult and may have to be based initially on historic information from products of a similar composition. Factors that can also affect the estimation are any legal/regulatory requirements surrounding declared values and allowances in the countries or regions of intended sale; and any legal/regulatory requirements on upper levels of additions of certain vitamins for safety reasons.

There is currently no harmonisation across the world on the legal upper levels of vitamins and minerals in products. Some countries base their controls on multiples of the national recommended intake, whilst others require that scientifically assessed safety levels are not exceeded. In the latter case, these upper limits include stability overages.

Example 5.1:

If the safety limit has been assessed as	100mg / day
and the stability overage required is	20%
Then the maximum declared value would be	83mg / day
and the overage	17mg / day
Thus the 100mg safety limit is not exceeded	

Another legal/regulatory issue that is accepted by most authorities across the world is that, whilst the overage forms part of the total vitamin content of the product, it is not counted in the declared value for the product during intake safety assessments, as the overage reduces during the life of the product.

An important principle consistent with good manufacturing practice that must be applied during the estimation of overages, is that the quantity of overage added should be based on scientific evidence and be at the minimum level necessary to achieve its purpose. An over-arching requirement is that input amounts, including overage (stability and/or production), do not lead to an amount in the final product for release to market that exceeds the safety limit and could jeopardise the safety of the consumer.

Although there are notable exceptions, stability overages for vitamins generally fall within a range of 5-30%. A maximum overage of 50% should cover overage requirements for vitamins in most solid dosage forms. In certain cases, overages for multivitamin supplements sold in a liquid dosage form can be somewhat higher.

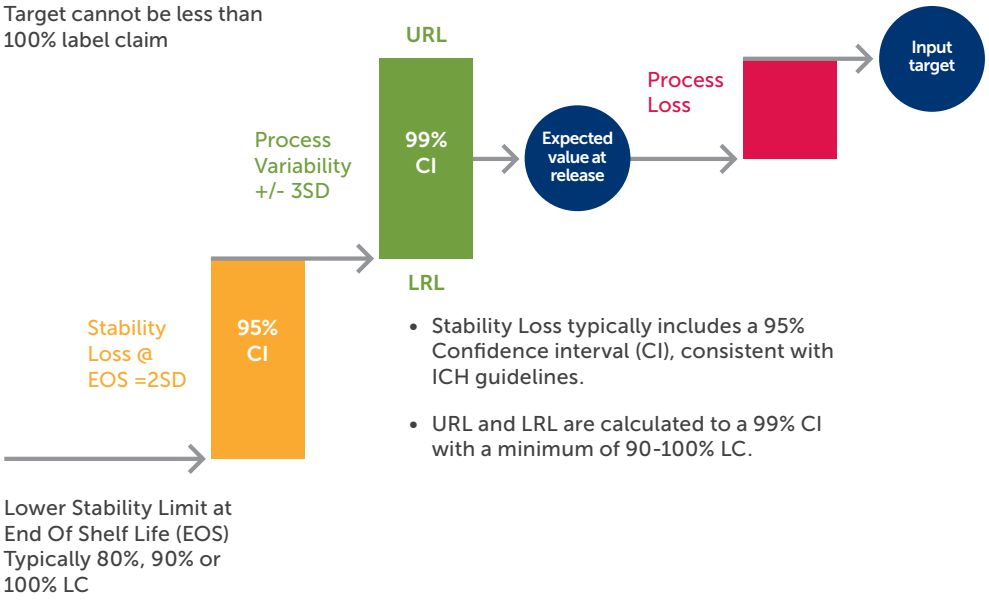
5.2 Overages for Other Ingredients

The principle of overages can be applied to those other ingredients, (for example some carotenoids, certain microorganisms etc.), which also exhibit loss of activity over time in the same way as the vitamins. The same cautions regarding safety are applied when assessing overages for non-vitamin ingredients.

6. Tolerances for declared values

From the large number of sources of variation that can be identified as affecting the quantities of micronutrients in a supplement during the manufacture and storage of the product, it can be appreciated that very few declared values will be exactly the same from batch to batch of the same product. Samples from each batch are likely to show small variations above and below the target value. Variations introduced during the sampling and analysis of the product will further compound these differences. Even within a sample of tablets or capsules there are likely to be small but detectable variations. See Figure 6.1 for an example of the typical methodology used to consider manufacturing tolerance limits.

Figure 6.1[9]**:** Typical methodology to consider manufacturing tolerance limits in setting input target, upper and lower release limits at time of manufacture, and end of shelf life lower limits, taking process variability, manufacture process loss and stability loss over time into account. Analytical (assay) variability is implicit in the other sources of variance.



URL:	Upper Release Limit
LRL:	Lower Release Limit
CI:	Confidence Interval
EOS:	End Of Shelf Life
SD:	Standard Deviation
LC:	Declared Value (Label Claim)

When considering the manufacturing tolerances shown in Figure 6.1 it should be noted that very few assay results will ever test near the lower stability limit at end of shelf life, for the following reasons:

1. The stability loss in the diagram starts at the lower release limit at time of manufacture. In other words, at the worst-case scenario.
2. The end of shelf life aligns with the confidence band around the mean, such that 95% of the time the values would be above the lower stability limit at end of shelf life.

A declared value cannot be taken as an absolute value, but rather as an average (mean) of a number of values, based on available scientific evidence.

With vitamins, the issue is further compounded by the need for overages to compensate for the gradual loss of activity throughout the declared shelf life of the supplement.

This means that assays carried out on a product should generally show vitamin levels higher than the declared values, with the highest values being achieved immediately after the product was packed and entered distribution, and the lowest values at the expiry date. The levels determined by official controls will vary depending upon the point in the product's life that the sample was taken.

In order to establish a product's compliance with the relevant requirements, the enforcement authority has to take all the sources of variation, and particularly the overage levels, into consideration.

As it is unlikely that the enforcement authority will be able to determine the actual point in the product's life, and hence the remaining overage, without information from the manufacturer, a system must be in place which accommodates this legitimate variance. This system must include accepted tolerances above and below the label declaration. Products where all the assays for the declared active ingredients fall within the tolerance ranges should be deemed acceptable. Where any results are above or below these ranges, the manufacturer should be requested to demonstrate that all due diligence had been undertaken to achieve compliance.

The concept of tolerances is already being applied as part of the official controls in a number of countries and economic blocs, but the variability in stability of the different vitamins, and hence the need for higher or lower overage amounts, is not always taken into consideration.

Official tolerances for declared values need to take into account the range of overages required for the different vitamins. They also need to allow for batch to batch variations on ingredients, processing variables and the analytical variations found within the test methods.

6.1 Supplements versus Drug (medicinal) Products

The concepts of both overages and tolerances set supplements and fortified foods apart from the conventions that apply to drugs.

Supplements are generally considered to be foods supplying measured amounts of nutrients to supplement a diet, and they are measured against the national recommendations for nutrient intakes, such as recommended daily allowances (RDAs) or nutrient reference values (NRVs). Supplements are not designed to alleviate medical conditions. Supplements can provide 30 or more active substances in a single dose.

By contrast, drugs are designed to deliver carefully measured and targeted doses of substances (usually no more than two or three together) for the treatment of specific medical conditions. A drug, therefore, has to be manufactured and stored with the intention of it being used within its shelf life without the benefit of an overage. This means that the tolerances have to be controlled by tighter limits in the drug industry, as the focus is on maintaining an accurate dosage.

By their nature, drugs are used for the treatment of medical conditions and diseases. The quantities of the drug used are assessed on the risk : benefit principle, and there is an inherent safety risk if the drug delivery does not meet the tight controls.

It is inappropriate to attempt to treat tolerances for the nutrient levels in supplements in a similar manner to the stringent requirements for the active levels in drugs, as the two product categories are designed for completely different reasons and with completely different safety profiles.

6.2 Consumer Expectations

An important consideration with respect to supplements is the consumer expectation for the products. Unlike many foods, where the consumer can see, smell, feel and taste what they are actually buying with regard to the quality of the product, supplement consumers do not have any indication of the content of the product and consequently are totally reliant on the descriptions and quantifications given on the label. The consumer's only knowledge of the product is derived from the list of ingredients and the quantitative declarations for the content of the nutrients being delivered by the product.

As the consumer has to place total faith in the label, it is important that the product delivers against expectations throughout the time it is on the market. Reliance has to be placed on the manufacturer to ensure that the expectations can be consistently achieved. A scientific approach to tolerances is an essential part of ensuring that consumer expectations are met.

6.3 Upper and Lower Tolerances

A tolerance range requires that there are scientifically established upper and lower limits to the range. The establishment of the upper limit is the most complex, as it not only has to incorporate all the anticipated variables, but it also has to take into consideration any stability overage.

An essential consideration when developing a tolerance range is that both the upper and lower limits must compensate for all unavoidable variability affecting the product, as described in Sections 1 to 5.

As a general rule, the ranges for tolerances for active ingredients in supplements need to be acceptable for all forms of supplements, including solid forms (for example, tablets, hard-gel capsules and powders) and liquid forms (for example, syrups, oils and emulsions).

Whilst the ideal tolerance range should fit all eventualities, it has been found in practice that this is not the case. Tolerance ranges are not "one size fits all". Owing to the wide variation in supplement product forms and composition, and the impact of other factors as described in the previous sections, there are situations where it is not possible for a product to fit within an externally applied standard tolerance range. In such cases, the manufacturer should be able to provide data to demonstrate the appropriateness of their declared value and selected shelf life.

7. References

1	Berry Ottaway & Associates Ltd., Hereford, UK (2016) private communication.
2	Certificates of Analysis for Supplement Ingredients: Guidelines on Their Preparation and Use (2014). International Alliance of Dietary/Food Supplement Associations (IADSA).
3	Global Guide to Good Manufacturing Practice for Supplements (2011). International Alliance of Dietary/Food Supplement Associations (IADSA).
4	Berry Ottaway & Associates Ltd., Hereford, UK (2016) private communication.
5	IADSA member (2018) DSM Nutritional Products, private communication.
6	Shelf-Life Recommendations for Supplements (2014). International Alliance of Dietary/Food Supplement Associations (IADSA).
7	Stability Testing for Shelf Life Determination of Supplements (2016). International Alliance of Dietary/Food Supplement Associations (IADSA).
8	Berry Ottaway & Associates Ltd., Hereford, UK (2014) private communication.
9	IADSA member (2017) Pfizer Consumer Healthcare, private communication.

ANNEX I

Glossary of terms

Active ingredient	A biologically active vitamin, mineral, herb or other botanical, amino acid or other nutritional substance for use by man to supplement the diet by increasing the total dietary intake (e.g., enzymes or tissues from organs or glands), or a concentrate, metabolite, constituent or extract.
Ambient humidity	Ambient humidity relates to Relative humidity (RH) in a particular climatic zone as humidity is related to temperature. Relative humidity is the ratio of the actual amount of water vapour in the air compared to the maximum water the air is able to hold at a given temperature. RH is reported as a percentage.
Analytical Method	A detailed description of the procedures to be followed in performing tests for assessing conformity with the specification.
Analytical result	Quantitative data obtained following the performance of tests for assessing conformity with the specification.
Analytical variance	A statistical measure of the spread of repeated measurements either side of the mean.
Angle of repose	The steepest angle at which a sloping surface of a mound of powder is stable.
Assay	See Test.
Assayed value	The quantitative value obtained from an assay/test.
Batch	See Lot.
Botanical	A plant, part of a plant or a substance obtained from a plant.
Botanical extract	A substance produced as a result of either a liquid or gaseous, solvent acting on a plant material and dissolving some of its components.

Catalyst	A substance that increases the rate of a chemical reaction without undergoing any permanent change.
Cation	An ion (atom) or group of ions that has more protons than electrons, so it is positively charged.
Certificate of Analysis (COA):	A document relating specifically to the results of testing a representative sample drawn from the batch of ingredient to be delivered.
Characteristic	Distinguishing feature.
Colony forming unit (CFU)	A CFU is one viable cell with the capacity to multiply, and thus CFUs are used as a measure of the number of microorganisms present in or on the surface of a sample.
Commercial ingredient	An ingredient in the final form in which it is sold to the manufacturer.
Compound ingredient	An ingredient formed of two or more ingredients.
Confidence interval	An estimated range of values with a specific probability that the true value of a parameter is contained within it. For analytical methods 95% is commonly selected, but any level between 0-100% can be selected. A 95% confidence interval is the range of values that can give 95% certainty that it contains the true value of the parameter.
Contract	Legally binding agreement.
Contract Manufacture	Manufacture or partial manufacture ordered by one person or organisation (the contract giver) and carried out by a separate person or organisation (the contract acceptor).
Critical parameter	A key variable that can have a major impact on the success of a process, assay, ingredient or product.

Declared value	The amount of active ingredient stated on the label of the supplement as being provided per defined daily intake.
Direct compression	Where a dry ingredient mixture is formed into tablet shapes under high pressure, without a previous granulation or other process to increase the compression ability of the mixture.
Documentation	All written procedures, instructions and records, quality control procedures and recorded test results involved in the manufacture of a supplement.
Dry weight	The mass of the ingredient once all water has been removed.
Emulsion	A homogenised mixture of an oil-based ingredient and a water-based ingredient.
Equipment calibration	The checking and/or adjustment of an instrument against a standard in order to maintain its accuracy.
Expiry Date	The date after which the supplement manufacturer cannot ensure potency or quality of the product.
Finished Product	A supplement which has undergone all the stages of manufacture.
Flowability	The ease with which a powder will move continuously in one direction under a specified set of conditions
Food grade	A substance that is intended for safe human consumption.
Hygroscopic ingredient	An ingredient that attracts water from its surroundings via absorption or adsorption.

Homogeneity	Uniform in structure or composition throughout. When applied to mixing, all parts of a multi-component mix should have the same composition.
In-house testing	Testing that is undertaken within the manufacturer's own organisation, without input or assistance from an external laboratory.
Information	Meaningful data.
Ingredient	Any substance that is used in the manufacture of a supplement and that is intended to be present in the finished product.
Karl Fischer Titration	An analytical method that measures only the water content, to trace amounts, within a sample of a substance.
Loss on drying (LOD)	A percent measure of the total change in weight of a substance as a result of drying. For some substances, LOD measures both the water and volatile impurity (e.g. fat or alcohol) content.
Lot	A quantity of any supplement produced during a given cycle of manufacture and from a specific formulation order, that is uniform in character and quality (the essence of a manufacturing lot is its homogeneity).
Manufacture	The complete cycle of production and quality control of a supplement from the acquisition of all materials through all stages of subsequent processing, packaging and storage to the distribution or release of the finished product.
Manufacturer	The person or business that is involved in the manufacture of a finished product.
Manufacturing tolerance	The permissible limit or limits of variation as a result of the processing of a product.

Measurand	A physical quantity or property that is to be measured.
Measurement uncertainty	The difference between a measurement result and the actual or true value. The set of values that could reasonably be obtained from a measurement of the measurand and is determined by the precision and accuracy of the measurement. Any measurement is an estimation and the true value will remain unknown.
Microencapsulation	The process by which individual particles or droplets of solid or liquid material are surrounded by a thin polymeric coating to produce capsules in the micrometer to millimetre range.
Moisture content	The ratio of the mass of water in a substance to the mass of solids in the substance, expressed as a percentage.
Operator	A person who operates equipment or a machine.
Overage	The quantity of a substance above the amount claimed on the label that is added to the supplement during manufacture to cover losses that may occur from degradation during processing and storage of the product.
Packaging	All operations, including filling and labelling, that a bulk product has to undergo in order to become a finished product.
Packaging Materials	Any material, including printed material, employed in the packaging of a supplement, such as containers, closures, bags, packing, label materials (labels, inserts, etc.), seals, binding materials, adhesives and tapes.
Permeation rate	The speed at which a liquid or gas moves into or through a porous or permeable solid over a specified period of time.

Potency	In the context of supplements, the measurable activity of a substance with a nutritional or physiological effect in a defined weight/dose of a product.
Procedure	Specified way to carry out an activity or a process.
Process	Set of interrelated or interacting activities which transform one or more of the properties (physical, chemical, microbiological, sensory) of the raw materials.
Processing variation	The cumulative percentage variations around a mean that occur at the various stages of the processing of a product.
Product	Result of a process.
Product Matrix	The physical and chemical attributes that make up and define a product.
Quality	Degree to which a set of inherent characteristics fulfils requirements.
Quality Control	Part of quality management focussed on fulfilling quality requirements. Includes all measures undertaken during manufacture designed to ensure the uniform output of supplements that conform to established specifications of identity, purity, strength and other characteristics.
Raw Materials	All materials whether active or inactive that are employed in the processing of supplements.
Record	Document stating results achieved or providing evidence of activities performed.
Reducing agent	An element or compound that loses (or donates) an electron to another chemical species in a redox chemical reaction. (Also called a reducer or reductant)

Release limit	A defined range of values surrounding the lot mean, formed on the basis of given specifications and real time stability data, so that a future lot whose measured value at time of manufacture falls within these limits has a high level of assurance that its mean will remain within specifications throughout shelf life.
Requirement	Need or expectation that is stated, generally implied or obligatory.
Review	Activity undertaken to determine the suitability, adequacy and effectiveness of the subject matter to achieve established objectives.
Ring trial	Inter laboratory comparison where identical samples are sent to multiple accredited laboratories for analysis and the analytical results are subsequently compared.
Shelf-life	The period during which a finished product retains its specific properties when properly stored.
Specification	A document giving the description of a starting material, intermediate, bulk or finished product in terms of its chemical, physical and (if any) biological characteristics. A specification describes in detail the requirements with which the products or materials used or obtained during manufacture have to conform and normally includes descriptive clauses and numerical clauses, stating standards and permitted tolerances. It serves as a basis for quality evaluation.
Stability	Ability of a substance to remain unchanged over time under stated, or reasonably expected, conditions of storage and use.
Storage Area	Designated area within the manufacturing premises where all raw materials and/or quarantined material/ product and/or finished product can be kept safe until utilised, disposed of or distributed as applicable.

Supplement ingredient	Any substance intended for use in the manufacture of a supplement, including those that may not appear in the finished lot of the supplement. Ingredients include active ingredients and other ingredients (such as excipients, preservatives and colorants) which may be included in a supplement.
Supplier	Organisation or person that provides materials or services needed for the manufacture of a product.
System	Set of interrelated or interacting elements.
Test	Determination of one or more quantitative or objective characteristics according to a specified procedure.
Transfer loss	Small losses of material (ingredients of mixed product) that occur at the different stages of a production process.
Trend analysis	A method of detecting a pattern, or changes in a pattern, in data that have been collected over a period of time. The trends, or patterns, can be visualized by plotting the test results over time.
Unsaponifiable matter	All the substances present in the ingredient which, after saponification of the latter by potassium hydroxide and extraction by hexane, are not volatile under the specified operating conditions (from ISO 18609:2000).
Validation	Confirmation, through the provision of objective evidence, that the specific intended use or application of a procedure, process, equipment, material, activity or system leads to the expected results.
Verification	Confirmation, through the provision of objective evidence, that the requirements for any procedure, process, equipment, material, activity or system have been fulfilled.

ANNEX II

Laboratory ring trial

Comparison of Analytical Results Obtained from Different Laboratories for Vitamin Levels in a Multi-vitamin and Multi-mineral Tablet Product.

Background

Following several discrepancies between the manufacturers' formulated and assayed levels of vitamins in food supplement tablets and those carried out by third party checks, an exercise was carried out using five laboratories with accreditation for the relevant vitamin analyses to evaluate the inherent inter-laboratory variability.

Methodology

A batch of multivitamin/multimineral tablets was carefully monitored at all stages of production. Approximately 2000 consecutive tablets were removed from the dedusting stage of the tabletting process. This aliquot was mixed and divided into subunits of 100 tablets each. The sub-units were packed into containers and sent to five accredited laboratories for the analysis of 10 vitamins. Each laboratory received the same information on the composition of the tablets.

To examine intra-laboratory variation, a second sample was sent to laboratory A with a different code number. The results of the two samples from Laboratory A are reported as A-1 and A-2.

Results

The results from each of the laboratories for the 10 vitamins are given in Table A1. The range of variation of the results from the input amount of each vitamin are given in Table A2 plus or minus the input level, expressed as a percentage of the input. For example, the range for thiamin was –20% to +16% of the input of 1.6mg.

Discussion

The sampling and sample preparation procedures were designed to minimise the variability in tablet content.

All five laboratories were in possession of the relevant national accreditation for vitamin analysis and considered themselves competent in the assays requested. Laboratory D originally reported riboflavin and niacin by method 1 (Table A1). They subsequently offered to repeat these two assays using a modified method of extraction (Method 2).

The results of the assays showed that, for a number of vitamins, there can be a significant intra-and inter- laboratory variation among accredited laboratories. This emphasises that even under the best conditions, the analysed value of an analyte is an approximation of the true value of the contents of the sample.

Testing variation inherent to the analytical test methods contributes to the variation that can be seen in raw material and finished product testing.

Acknowledgement

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Comparison of Analytical Results Obtained from Different Laboratories for Vitamin levels in a Multi-vitamin and Multi-mineral Tablet Product

Vitamin	Input / Tablet	Laboratory					
		A - 1	A - 2	B	C	D	E
Thiamin mg/ tablet	1.6	1.63	1.56	1.28	1.85	1.57	1.41
Riboflavin mg/tablet	1.76	1.46	1.79	1.65	1.34	1.37 method 1 1.54 method 2	1.19
Niacin mg/ tablet	19.8	20.5	20.5	19.5	21.9	18.5 method 1 21.09 method 2	20.5
Pantothenic acid mg/ tablet	7.2	7.4	7.3	6.3	–	7.39	7.53
Vitamin B_6 mg/tablet	2.2	2.10	2.11	2.3	2.02	2.02	2.00
Vitamin B_{12} µg/tablet	1.5	0.55	–	1.4	–	1.52	0.76
Vitamin C mg/tablet	69	72	72	44.7	62.6	68.85	68.9
Folic acid µg/tablet	300	195	201	275	266	294	250
Vitamin A µg RE/tablet	1200	1113	1054	1000	850	1081.5	923
Vitamin E mg TE/tablet	10.5	13.5	12.8	10.9	–	9.77	10.72

Inter-Laboratory Study on Vitamin Content in Tablets
Range of Variation from Input

Vitamin	Input Amount per Tablet	Label Claim per Tablet	Assay % Variation from Input
Thiamin	1.6mg	1.4mg	-20 to +16
Riboflavin	1.76mg	1.6mg	-32 to +2
Niacin	19.8mg	18mg	-15 to +10.5
Pantothenic acid	7.2mg	6mg	-12.5 to +4.5
Vitamin B_6	2.2mg	2mg	-10 to +4.5
Vitamin B_{12}	1.5µg	1µg	-63 to +1
Vitamin C	69mg	60mg	-35 to +4
Folic acid	300µg	200µg	-35 to -2
Vitamin A	1200µg RE	800µg RE	-23 to -7
Vitamin E	10.5mg TE	10mg TE	-7 to +2

ANNEX III

Useful resources

Certificates of Analysis for Supplement Ingredients:
Guidelines on Their Preparation and Use (2014)

International Alliance of Dietary/Food Supplement Associations (IADSA).

Global Guide to Good Manufacturing Practice for Supplements (2011)

International Alliance of Dietary/Food Supplement Associations (IADSA).

Shelf-Life Recommendations for Supplements (2014)

International Alliance of Dietary/Food Supplement Associations (IADSA).

Stability Testing for Shelf Life Determination of Supplements (2016)

International Alliance of Dietary/Food Supplement Associations (IADSA).

Technical Aspects of Manufacturing Food Supplements:
A Guide for Food Supplement Manufacturers (2017)

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