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Measurement uncertainty from validation and duplicate analysis results in HPLC analysis of multivitamin preparations and nutrients with different galenic forms

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Abstract

An approach to calculate the measurement uncertainty in the HPLC analysis of several hydro- and liposoluble vitamins in multivitamin preparations with different galenic composition and properties is described. In the first instance it is examined if duplicate analysis results, obtained with a fully validated analysis method on different lots of an effervescent tablet preparation spread over several points of time, might contribute to calculate the measurement uncertainty of the HPLC method used and if the established uncertainty is acceptable in the assessment of compliance with the legal content limits. Analysis of variance (ANOVA) and precision calculations, based on the ISO 5725-2 norm are applied on the analysis results obtained to estimate precision components, necessary to derive the measurement uncertainty. In the second instance it is demonstrated to which extent the fully validated method of analysis for effervescent tablets is applicable to other galenic forms as e.g. capsules with oily emulsions, tablets, coated tablets, oral solutions, ... and which specific modifications in the analysis steps are involved. By means of duplicate analysis results, acquired from a large series of real samples over a considerable period of time and classified according to their similarity in content, galenic forms and matrices, estimations of measurement uncertainty calculations are shown.

Keywords: Hydro- and lipo-soluble vitamins; Reversed-phase high performance liquid chromatography; Duplicate analysis results; Measurement uncertainty

1. Introduction

As an analytical laboratory, involved in official quality control of multivitamin preparations and nutrients on the Belgian market, an EN-45001 accreditation is required for the analysis methods applied. This implies full method validation as well as a quality assurance laboratory environment, assuring the equipment's good performance, the validity of the reagents, glassware, reference standards etc. and the ability and skill of analysts. Classical validation parameters for assay methods are selectivity, linearity, range, accuracy, precision

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with repeatability, intermediate precision and reproducibility.

In 2003 the ISO standard 17025 is obligatory and replaces the EN-45001 standard. This new ISO standard prescribes the estimation of the uncertainty for calibration and measurements and specifies detailed requirements concerning this estimation and how it should be stated in test reports.

According to the international vocabulary of basic and general terms in metrology the uncertainty of a measurement is defined as "a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand". This parameter could be a standard deviation or another part of an interval, indicating a certain confidence range. This means that a measurement cannot be properly interpreted without knowledge of its uncertainty. Knowledge of the uncertainty of measurement of testing results is fundamentally important for laboratories, their clients and all institutions using these results for comparative purposes.

However, very different approaches are published in literature and international guidelines to measure the uncertainty, related to analytical results obtained. Uncertainty can be obtained either by calculating all the sources of uncertainty individually or by grouping different sources of uncertainty whenever possible. The first way is known as the "bottom-up" approach and was proposed by ISO [1]. However, identifying and quantifying all individual sources of uncertainty is not practical in e.g. HPLC-analysis methods, so that calculating uncertainty using information from validation results is more suitable. The relationship between validation and uncertainty statement is not always demonstrated in practice by means of existing validation data. Important parts of uncertainty may be obtained by interpreting the statistical spread of results of a series of measurements. In a clear survey article Hunt et al. [2] deal with the problem for analytical chemists how to determine uncertainty in their specific situations. Different reasons are described why different approaches are found in literature and guidelines. The treatment of systematic error is a point of discordance as well as what is considered to be the truth: a relative or absolute truth. A third reason is related to the way error is determined in practice. Analytical chemists prefer determining uncertainty as much as possible from method validation results. Here the proposed strategy within one laboratory of in duplicate analysis on different lots of two types of effervescent tablets, spread over several points of time, allows the determination of the repeatability variance (s_r^2), as well as the between-day variance (s_{BD}^2) as the intermediate variance. Both important parts of measurement uncertainty might be calculated, as demonstrated, by means of analysis of variance (ANOVA) as well as by applying the ISO-5725 guide [14]. In addition, repeatability variances from historical in duplicate analysis results, obtained with the same validated method on several different other galenic forms as e.g. capsules with oily emulsions, tablets, coated tablets, ... might be evaluated by comparison with the repeatability variances, measured for the two types of effervescent tablets.

2. Experimental

2.1. Uncertainty and validation of methods of analysis

Methods of analysis must be validated before they are used to daily practice and so systematic errors are estimated from the accuracy, which is "the closeness of agreement between the test result and the accepted reference value" [3]. The measure of accuracy is usually expressed in terms of bias but describes a combination of random components and a common systematic error or bias component. One can distinguish the method bias and the laboratory bias, which is a systematic error for an individual laboratory or a random error in a population of laboratories, participating in an interlaboratory study.

A further term of uncertainty considers the variation of the applied method of analysis for routine samples. Therefore, this uncertainty depends on the intermediate precision of the procedure (time effects) and also takes into account the

fact that results depend on the matrix of routine samples. As well constant as proportional matrix effects may affect this uncertainty. It also contains the repeatability error, which is a random error occurring between replicate determinations performed within one run.

As uncertainty one should consider all these sources of error for an analytical error and calculate it in a general way by grouping them in different terms

$$U = t_{\alpha/2,\mathrm{eff}} \sqrt{(u)^2_{\mathrm{methodbias}} + (u)^2_{\mathrm{labbias}} + (u)^2_{\mathrm{runeffect}} + (u)^2_{\mathrm{repeatability}}} \quad (1)$$

where $t_{\alpha/2,\mathrm{eff}}$ is the two-sided t-tabulated value for the effective degrees of freedom [4] which can be replaced by the coverage factor k. A restriction of measurement of uncertainty by method validation could be that there might be a tendency to underestimate the importance of sources of error not linked to the analytical method as e.g. sampling or the lack of homogeneity of the sample. In a series of articles Maroto et al. [5–7] propose procedures to estimate uncertainties of analytical results using information from the validation process, using regression techniques and spiked samples and accuracy assessments from recovery assays.

In this paper we consider the terms of uncertainty $(u)^2_{\mathrm{methodbias}}$, $(u)^2_{\mathrm{runeffect}}$ $(=s^2_{\mathrm{BD}})$ and $(u)^2_{\mathrm{repeatability}}$ $(=s^2_{\mathrm{r}})$ in formula (1) from information obtained in precision studies and assessment of accuracy applied in a reversed phase high performance liquid chromatographic (RP-HPLC) analysis of hydro- and lipo-soluble vitamins in nutrients and multivitamin preparations. The term $(u)^2_{\mathrm{labbias}}$ is not considered here, as no interlaboratory data are available.

We started with a pilot study on two and three batches of two different types of effervescent tablets from the same producer. Each batch is analyzed in duplicate during seven different days. So, 70 analysis results for each vitamin examined are available for further precision estimations, which may lead to the proposal of an intralaboratory expanded uncertainty.

In assessment of accuracy, both proportional and constant bias were established by means of the standard addition method (SAM) and the Youden method. The results obtained were evaluated as described by Cuadros Rodriguez et al. [8].

The examined batches differ in that two have a similar composition resulting in a particular taste and aspect, whereas three other have the same nominal vitamin contents but with a different particular composition. The mean mass of an effervescent tablet for the two types of batches is about 4.5 g.

2.2. Methods of analysis and experimental set-up

Four different types of HPLC methods of analysis are applied.

A first method is in-house developed and analyzes the hydro-soluble vitamins nicotinamide (B3), pyridoxine (B6), riboflavine or riboflavine phosphate (B2) and thiamine (B1) in a same run. On the chromatograms obtained also ascorbic acid can be quantified. However, the sample treatment and isolation steps for the vitamins as described below, do not always guarantee the stability of ascorbic acid during the whole time of analysis. As a consequence a selective in-house method for stable analysis of ascorbic acid is developed.

A third separate method is applied for analysis of calcium pantothenate (B5) and a fourth one for the analysis of the lipo-soluble vitamins retinol acetate, retinol palmitate, α-tocopherol acetate and α-tocopherol succinate. Both methods are described in literature [9–11].

The four isocratic HPLC procedures are carried out on a Waters 600 multi-solvent delivery chromatographic system with four solvent reservoirs, a Waters 625 pump, a Waters 717 automatic injector, a Waters in-line degasser and a Waters 996 photodiode array detector (PDA). The chromatographic system is controlled by the WATERS MILLENIUM[32] version 3.20 software (Waters Corporation, Milford, MA 01757 USA).

Control of ''Mass uniformity'', according to the Ph.Eur.3° ed., is performed together with calculation of the mean mass of a tablet unit.

It is verified if the repeatability uncertainty components, obtained by statistical analysis of the assay measurements of each vitamin in the pilot study, might be considered as reference

values, comparable and applicable to other galenic forms of multivitamin preparations and nutrients. Therefore, all in duplicate assay results of vitamin contents, obtained for the last 2 years in all kind of nutrients (e.g. tablets, capsules containing powder mixtures, capsules with oily content, syrups, drinkable solutions, coated tablets, other effervescent tablets, ..., all with a different composition and mean mass) are compiled and subdivided in groups with a corresponding range of nominal amounts for each vitamin. This way, for a specific amount of vitamin in different galenic preparations, several in duplicate analytical results, spread in time, are suitable and sufficient to calculate the repeatability uncertainty component following the ISO5725-2 guide, which may be mutually compared, no matter what is the type of galenic form of the preparation or the amount of sample taken for analysis. A restriction, however, is that, due to an unequal qualitative and quantitative composition of the different preparations examined, no relevant and meaningful intermediate precision estimates might be calculated. An advantage, however, is that overall repeatability variances are available for the different ranges of vitamin amount from several independent samples' in duplicate analysis results. From the overall repeatability variances for each range of vitamin amount, relative repeatability standard deviations are calculated, which might be compared with the corresponding ones from the pilot study. On the other hand, intermediate precision data from the pilot study are available, which might be reference or limit values in measuring intermediate precision for other specific galenic matrices.

All reagents and chemicals were of pro analysis quality; water was treated with a Milli-Q system (Millipore, Bedford, MA, USA). The vitamin reference substances were from Fluka (Buchs, Switzerland); sodium hexane sulfonate and sodium pentane sulfonate were from Acros (Geel, Belgium). Methanol and acetonitrile for HPLC analysis were from BDH (Poole, England). *n*-Hexane for residue analysis was from Merck (Darmstadt, Germany). Vitamin solutions are protected from actinic light in suitable certified glassware.

2.3. Analysis of the hydro-soluble vitamins B1, B2, B3, B6 and C

The nominal contents of each vitamin per effervescent tablet are 0.87 mg thiamin mononitrate, 1.20 mg riboflavine phosphate, 9.0 mg nicotinamide, 1.22 mg pyridoxine hydrochloride and 30.0 mg ascorbic acid. The quantity of analyzed hydro-soluble vitamin per amount of sample, calculated from the validated linearity range of the calibration curve, ranges from 15 to 120 mg for vitamin C, from 4.5 to 36 mg for vitamin B3, from 0.63 to 5 mg for vitamin B6, from 0.65 to 5.2 mg for vitamin B2 and from 0.45 to 3.6 mg for vitamin B1.

Twenty tablets are crushed, the powder is homogenized and two equal amounts of sample corresponding to two times the mean mass (about 9 g) are taken for analysis in a 50.0 ml volumetric flask. To both samples 10 ml of dimethylsulfoxide (DMSO) is added, followed by adding carefully small fractions of a 0.0125 M solution of sodium hexanesulfonate in a 0.1% acetic acid solution. The mixtures are sonicated for 10 min and centrifuged. A dilution of 4.0 ml of supernatant to 10.0 ml with the HPLC mobile phase is suitable for injected on the HPLC system.

About 18.0 mg thiamine mononitrate, 25.0 mg riboflavine (phosphate), 180.0 mg nicotinamide, 25.0 mg pyridoxine hydrochloride and 600.0 mg ascorbic acid reference standards are weighed in a 50.0 ml volumetric flask. The mixture is dissolved in about 30 ml 0.0125 M sodium hexanesulfonate in 0.1% (v/v) acetic acid solution and sonicated for 30 min in an ultra-sonic bath. Of a tenfold dilution of this solution with the HPLC mobile phase 2.0, 3.0, 4.0 and 6.0 ml are transferred into a 10.0 ml volumetric flask together with 0.8 ml of DMSO and further diluted with the same mobile phase. These reference solutions are injected for construction of the calibration line. The third reference solution is injected three times to check the linearity lack-of-fit of the calibration curve.

The analytical column is a 250×4.6 mm 5 μm Alltima C8 (Alltech Europe, Lokeren, Belgium) column. The mobile phase is composed by mixing two solvent solutions in a 45% A–55% B (v/v) ratio (analysis of riboflavine phosphate) or in a

30% A–70% B (v/v) ratio (analysis of riboflavine). The flow of the mobile phase is 1.0 ml/min and the injection volume is 20 μl [12].

The composition of solvent A is 0.205% (w/v) sodium hexanesulfonate in 1.0% (v/v) acetic acid solution. Solvent B contains 1.88 g triethanolamine, 120 ml of methanol, 6.0 ml glacial acetic acid and 0.390 g sodium hexane sulfonate dissolved to 500 ml with water. Phosphoric acid is added in drops to reach a pH of 3.1.

The chromatograms are recorded at specific detection wavelengths for each vitamin and at a general detection wavelength suitable for all vitamins. These specific detection wavelengths are 245 nm for thiamin, 260 nm for nicotinamide, 290 nm for pyridoxine and 350 nm for riboflavine. The general detection wavelength is 270 nm. Ascorbic acid is measured at 270 nm. The peak areas are integrated at the specific and general wavelength and are further used for assay calculations. System suitability of the chromatographic system is checked by resolution control of the vitamin peaks on the chromatogram. Performance verification of the HPLC equipment is checked from quality criteria imposed on the linearity of the calibration curve, constructed from four reference standard solutions, the injection of one is repeated three times, spread over the whole measuring program.

2.4. Analysis of calcium pantothenate

The nominal content of calcium pantothenate in the effervescent tablets is 2.72 mg. The quantity of analyzed calcium pantothenate may range from 2 to 7.5 mg per amount of sample, calculated from the linearity range of the calibration curve.

From the homogenized tablet powder, two equal amounts of sample, corresponding to two times the mean mass (about 9 g) are taken for analysis in a 100.0 ml volumetric flask. About 20 ml of water is slowly added and the mixture is sonicated for 10 min in an ultra-son bath. The mixture is further diluted with water and centrifuged for 10 min at 2000 rpm. The clear supernatant is injected for HPLC analysis.

About 25.0 mg of calcium pantothenate reference standard is weighed in a 50.0 ml volumetric flask, dissolved and diluted in water. From this solution 2.0, 3.0, 5.0 and 7.0 ml are diluted with water to 50.0 ml. These reference solutions are injected on the HPLC system for construction of the calibration line. The third reference solution is injected three times.

The analytical column is a 250×4.6 mm 5 μm Alltima C8 (Alltech) column. The mobile phase is composed by 97% sodium dihydrogeniumphosphate (0.25 M)–3% acetonitrile (v/v). The pH is adjusted to 2.5 by adding a few drops of phosphoric acid. The flow of the mobile phase is 1.5 ml/min and the injection volume is 20 μl.

The chromatograms are recorded at 205 nm. The integrated peak areas are further used for assay calculations.

A system suitability solution with calcium pantothenate and a saccharine checks the resolution of both compounds on the chromatogram.

2.5. Analysis of the lipo-soluble vitamins A-acetate and E-acetate

The nominal contents of retinol acetate and α-tocopherol acetate in the effervescent tablets are 1500 I.U. (= 520 μg) and 5.0 mg, respectively. The quantity of analyzed retinol acetate may range from 750 to 6150 U.I. and of α-tocopherol acetate from 2 to 17 mg per amount of sample, calculated from the linearity range of the calibration curve.

Two equal amounts of homogenized sample powder, corresponding to the mean mass of one tablet, are weighed in 200 ml centrifuge tube and 25 ml of a phosphoric acid solution (0.1 M) and 25 ml of ethanol are carefully added (carbon dioxide!). This mixture is shaken until complete escape of the carbon dioxide, 50.0 ml of *n*-hexane is added, the mixture shaken for 25 min and the *n*-hexane phase allowed to separate from the aqueous phase. Next 10.0 ml of the clear *n*-hexane phase is transferred in an Erlenmeyer flask and evaporated under nitrogen at 50 °C. The residue is dissolved in 2.0 ml of isopropanol and injected for HPLC analysis.

About 125 mg retinol acetate reference standard is weighed in a volumetric flask of 25.0 ml and dissolved in ethanol. From a tenfold dilution of this solution with the same solvent, 0.5, 1.0, 2.0 and 4.0 ml are transferred in centrifuge tubes.

About 50.0 mg of α-tocopherol acetate are weighted in a volumetric flask of 50.0 ml and dissolved in ethanol; 2.5, 5.0, 10.0 and 20.0 ml of this solution are transferred in the centrifuge tubes with the retinol acetate standard solutions.

To each centrifuge tube 25 ml of a 0.1 M phosphoric acid solution, 25 ml of ethanol and 50.0 ml of *n*-hexane are added. The mixtures are shaken for 25 min after which the *n*-hexane phase is allowed to separate from the aqueous phase. Next 10.0 ml of the clear *n*-hexane phases are transferred in an Erlenmeyer flask and evaporated under nitrogen at 50 °C. The reference standard residues are dissolved in 2.0 ml of isopropanol and injected on the HPLC system for construction of the calibration line. The second reference solution is injected three times.

The analytical column is a 150 × 4.6 mm 5 μm spheric 120 Å YMC-pack ODS-A C18 HPLC column (Waters), the temperature of which is maintained at 30 °C in a thermostatized water bath. The mobile phase is acetonitrile and the flow rate is 1.8 ml/min. The injection volume is 20 μl. The chromatograms are recorded at 330 nm as specific detection wavelength for retinol acetate and at 280 nm as detection wavelength for α-tocopherol acetate.

The peak areas are integrated at both wavelengths and are further used for assay calculations.

System suitability is checked by resolution control of vitamin peaks on the chromatogram.

2.6. Selective HPLC analysis of ascorbic acid

An important restriction encountered with the general method for hydro-soluble vitamins, is that only a limited type of galenic forms of nutrients guarantee stability of ascorbic acid present during analysis. So a selective HPLC method for ascorbic acid assays was developed.

Two amounts of homogenized sample (or the homogenized content of capsules), containing, respectively, about 15 and 30 mg of ascorbic acid are weighed in a 100.0 ml volumetric flask. About 10 ml of acetone is added and the mixture is well homogenized. Then about 20 ml of an anti-oxidant solution is added and shaken with the sample content.

This antioxidant solution contains 15 g of metaphosphoric acid and 40 ml of glacial acetic acid, further diluted to 500 ml with water. Both sample preparations are filtered and the second sample, containing 30 mg of ascorbic acid, is twofold diluted with the antioxidant solution. Both final sample solutions contain about 0.15 mg/ml.

About 60.0 mg of ascorbic acid reference standard is weighed in a 100.0 ml volumetric flask, 10 ml of acetone is added and further diluted with the above mentioned solution with metaphosphoric acid and glacial acetic acid. From this stock-solution 2.0, 3.0, 4.0 and 6.0 ml are diluted in 20.0 ml volumetric flasks with the same solution.

The analytical column is a 250 × 4.6 mm 5 μm Alltima C18 (Alltech) column. A column temperature of 40 °C is maintained during chromatography. A mobile phase gradient, composed by two mobile phase solutions A and B, is applied during the chromatography. Mobile phase A contains 1.0 g of sodium pentane sulfonate and 6.8 ml of phosphoric acid per liter of water; mobile phase B contains 1.0 g of sodium pentane sulfonate and 6.8 ml of phosphoric acid in 200 ml of water, diluted to 1 l with acetonitrile.

From 0 to 7 min a linear gradient, going from 97% mobile phase A and 3% mobile phase B (v/v) to 81% mobile phase A to 19% mobile phase B (v/v), is applied. From 7 to 10 min, a linear gradient, returning to the starting mobile phase conditions is applied. The flow rate is 1.0 ml/min and the injection volume is 10 μl.

The chromatograms are recorded at 245 nm. The integrated peak areas are further used for assay calculations. System suitability is controlled by a peak resolution of minimal 5.0 between the ascorbic acid and acetone peak on the chromatogram.

2.7. Assessment of accuracy

In the assessment of accuracy proportional and constant bias are calculated. In our case blank samples are not available and the analyte is already present in the sample. By means of the SAM, proportional bias, due to response variations that are dependent of the concentration of

the analyzed compound, are expressed as recovery and can only be calculated from spiked samples, where different amounts of analyte are added to the sample. In the case where the instrumental response is plotted against the concentration added, the slope of the SAM curve is an estimate of the product of the sensitivity of the analytical procedure, which corresponds to the slope of a standard curve, and the method recovery. A proportional bias is often due to matrix interferences.

Constant bias must be calculated using the Youden method [13]. This method analyzes various amounts of sample under repeatability conditions. A concentration for each amount of sample is found. By applying linear regression to sample data a slope and intercept are found. The Youden plot can be defined as a sample concentration curve. The Youden method provides a good estimate of constant bias, whenever the matrix effect is the same for all amounts of sample. The intercept of the Youden plot is an estimate of the total Youden blank (TYB).

Assays are carried out on one batch of the effervescent tablets, conserved for several weeks under stressed conditions (35 °C; 85% relative humidity), with the restriction that for construction of the standard calibration curve (SC) each reference standard concentration was injected once. On the other hand, two more calibration points were added: one below the lowest calibration point and one above the highest calibration point.

As to the preparation of the samples, one sample was analyzed as such and four other samples were spiked with 1.0, 2.0, 3.0 and 4.0 ml of reference stock solution.

With the Youden calibration (YC) six increasing amounts of sample, ranging from 25 to 200% of the usual amount of sample are analyzed. The analytical results were expressed as instrumental responses. Each sample is analyzed once.

The standard addition methodology and YC calculations statistical significance tests are performed using the statistical protocol and methodology, described by Cuadros Rodriguez et al. [8], implemented in an EXCEL® (Microsoft Corporation) calculation spread-sheet.

2.8. Precision studies

In this study precision is assumed to be approximately the same across the concentration ranges in which the analytical procedures are validated. By

Table 1
Statistical testing of equality of slopes from standard AC and SC and comparison of assay results for hydro-soluble vitamins, obtained by both calibrations

Vitamin	s(p)	t(b)	b(p)	SC	AC	t(c)
Vit. B1 (245 nm) mg/tablet: 0.83 mg	5125	1.337	47 926	13.02 μg/ml 0.82 mg	12.45 μg/ml 0.80 mg	8.272
Vit. B2-ph (350 nm) mg/tablet: 1.185 mg	1897	0.176	12 012	19.27 μg/ml 1.21 mg	18.72 μg/ml 1.18 mg	5.271
Vit. B3 (260 nm) mg/tablet: 8.99 mg	39 048	1.642	36 391	141.69 μg/ml 8.92 mg	136.89 μg/ml 8.82 mg	6.758
Vit. B6 (290 nm) mg/tablet: 1.31 mg	21 241	1.399	47 203	20.69 μg/ml 1.30 mg	20.66 μg/ml 1.30 mg	0.074
Significance level (%)	1			5		
Critical value $t(b)$	3.499			2.365		
Degrees of freedom	$(n\mathrm{SC}+n\mathrm{AC}-4)=7$			7		
Critical value $t(c)$	3.355			2.306		
Degrees of freedom	$(n\mathrm{SC}+n\mathrm{AC}-3)=8$			8		

s(p), pooled standard error of the estimate AC and SC; t(b): t-test statistic for similarity of slope; b(p): pooled slope of AC and SC; t(c), t-test statistic for similarity of concentrations, calculated from the AC and SC.

means of the in duplicate assay results of the five different batches of the examined effervescent tablets over 7 different days, analyzed with the described HPLC analysis procedures, the different types of precision, such as repeatability variance, between-day intermediate variance and within-day variance can be calculated and compared for the two series of analyzed nutrient batches. Combination of these variance components leads to calculation of the standard uncertainty and the expanded uncertainty. There are two analogous ways to calculate these variance components and de facto the precision uncertainty: ANOVA and the algorithms described in the international standard ISO 5725-2 [14], where between lab results are replaced by between day results.

The ANOVA calculations are performed using Statgraphics [15] and the variance calculations according to the ISO 5725-2 standard, using an EXCEL (Microsoft) spread-sheet.

Using the ISO 5725-2, a general repeatability variance is calculated from the in duplicate assay results of vitamin contents, subdivided in groups with a corresponding range of nominal amounts for each vitamin obtained for the last 2 years in all kind of nutrients (e.g. tablets, capsules containing powder mixtures, capsules with oily content, syrups, drinkable solutions, coated tablets, other effervescent tablets, ...) all with a different composition and mean mass. This way, for a particular range of a vitamin amount, present in a nutrient, repeatability uncertainty components might be calculated and mutually compared, no matter which is the type of galenic form of the preparation or the amount of sample taken for analysis.

3. Results and discussion

3.1. Results of the standard addition method (SAM) and the Youden calibration (YC)

The results of the SAM and YC experiments for the hydro-soluble vitamins B1, B2, B3 and B6, applied on one batch of the examined effervescent tablets, are presented in Table 1. In this table the different possible assay calculation results (using the addition calibration (AC), the SC and the YC) as described in a statistical procedure by Cuadros Rodriguez et al. [8] are compiled. In this statistical procedure the following calibration parameters are considered: (1) regression standard deviation of the SC; (2) check of similarity of slopes by means of a Student t-test; if the slopes are statistically similar, a pooled slope is calculated; (3) estimation of the intercept of the YC, which is the Youden blank (TYB); a difference between the intercepts of the SC and the YC indicates a constant bias component due to a sample matrix effect. As both intercepts are obtained from different independent variables, this difference is only checked if the value of YC-intercept is included in the SC-intercept confidence interval; (4) check of accuracy, carried out in two ways: (a) from the SC and the AC and (b) by checking the average value of all recoveries, obtained for each addition, with unity by means of a Student t-test.

In Table 1, statistical testing results for similarity of slopes from the standard AC and the SC curves for these vitamins is given as well as the significance test for the intercept of the YC line.

In Table 2, the recoveries of the four added amounts are compiled for each vitamin, as well as analogous results for vitamin E-acetate.

From these results obtained, we may conclude that no corrections for Youden blanks are required and consequently that no significant constant bias for this type of matrix could be detected.

The t-test for similarity of slopes $b(\mathrm{SC})$ and $b(\mathrm{AC})$, the null-hypothesis $b(\mathrm{SC})-b(\mathrm{AC})=0$ is accepted at the $\alpha=0.05$ significance level; it may be concluded that no significant proportional bias is detected for this type of matrix examined.

3.2. Precision components in the pilot study according to the ISO 5725-2 standard

The in duplicate analysis results of each vitamin for both examined nutrient series of two and three batches, measured over 7 days, together with the estimated precision components as the between-day intermediate variance (s^2_{BD}), the repeatability variance (s^2_{r}) and the total variance (s^2_{R}), according to the ISO-5725-2 standard and necessary to calculate the measurement uncertainty, are given in Tables 3–10. This ISO-standard also provides

Table 2
Recovery results of vitamins B1, B2, B3, B6 and E-acetate by means of SAM

Vit. B1–245 nm		Vit. B2-phosph–350 nm		Vit. B3–260 nm		Vit. B6–290 nm	
Added (μg/ml)	Found (μg/ml)	Added (μg/ml)	Found (μg/ml)	Added (μg/ml)	Found (μg/ml)	Added (μg/ml)	Found (μg/ml)
3.62	3.43	5.14	4.946	36.24	35.35	5.06	4.67
7.24	7.17	10.28	10.40	72.48	73.16	10.12	10.25
10.86	10.95	15.42	15.47	108.72	108.72	15.18	15.46
14.48	14.57	20.56	20.53	144.96	143.62	20.24	20.50
Mean recovery (%)							
99.6		99.4		99.4		99.2	
$s(RC)$—*standard deviation of recovery* (%)							
2.97		2.17		1.44		4.58	
$t(RC)$—*t statistic for recovery*							
0.6356		0.5611		0.8459		0.3534	
$t(RC)$—*critical value at 5% significance level* (*3 d.f.*)							
3.1824							

Vit. E-acetate	
Added (mg/ml)	Found (mg/ml)
0.21	0.22
0.43	0.41
0.85	0.84
Mean recovery (%)	
100.11	
$t(RC)$—*t statistic for recovery*	
0.0591	
$t(RC)$—*critical value at 5% significance level* (*2 d.f.*)	
4.3027	

two approaches to decide if there are individual values present that appear to be inconsistent with all other values: numerical outlier tests and a graphical consistency technique.

The Cochran's test and the Grubb's test are the numerical tests to identify stragglers or outliers: if the test statistic is less than or equal to its 5% critical value, the item tested is accepted as correct. If the test statistic is greater than its 5% critical value and less than or equal to its 1% critical value, the item tested is called a straggler. If the test statistic is greater than its 1% critical value, the item is called a statistical outlier. The Cochran's test is here a test of the within-day variability and is applied first. Cochran's criterion tests only the highest value in a set of standard deviations and is, therefore, a one-side outlier test. The Grubb's test is here primarily a test of between-day variability and can also be used where Cochran's test has raised suspicions as to whether the high within-day variation is attributable to only one of the test results. By means of the Grubb's test, one as well as two outlying observations may be detected.

In the graphical consistency technique Mandel's h and k statistics [16] are applied. It can be established that h is the deviation of each cell-average from the grand average of a certain level, divided by the standard deviation between cell-averages:

$$(h)_{ij} = \frac{(y)_{ij} - (C)_j}{(s_L)_j}$$

where y_{ij} is the cell-average for cell (i,j), $C_j = (1/p)\Sigma\, y_{ij}$ (p = number of cells) and

Table 3
Calcium pantothenate: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
3.8 mg/effervescent tablet					
1	3.74	3.69	3.66	3.60	3.61
1	3.73	3.69	3.61	3.63	3.57
2	3.83	3.83	3.77	3.75	3.65
2	3.83	3.81	3.76	3.65	3.66
3	3.76	3.82	3.70	3.55	3.66
3	3.75	3.78	3.71	3.56	3.70
4	3.99	3.94	3.75	3.82	3.82
4	3.75	4.00	3.76	3.79	3.83
5	3.65	3.67	3.82	3.71	3.83
5	3.65	3.73	3.80	3.70	3.85
6	3.98	3.98	4.05	3.77	3.90
6	4.01	4.02	3.90	3.75	3.93
7	3.83	3.69	3.59	3.68	3.72
7	3.89	3.78	3.68	3.68	3.80
Cell averages					
1	3.735	3.690	3.635	3.615	3.590
2	3.830	3.820	3.765	3.700	3.655
3	3.755	3.800	3.705	3.555	3.680
4	3.870	3.970	3.755	3.805	3.825
5	3.650	3.700	3.810	3.705	3.840
6	3.995	4.000	3.975	3.760	3.915
7	3.860	3.735	3.635	3.680	3.760
Grubbs' test[a]					
Gp (single high)	1.624	1.469	1.879	1.385	1.406
Gp (single low)	1.464	1.012	1.015	1.589	1.399
Gp (double high)	0.367	0.146	0.190	0.393	0.419
Gp (double low)	0.404	0.559	0.519	0.249	0.389
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
Cell ranges					
	$W(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	0.010	0.000	0.050	0.030	0.040
2	0.000	0.020	0.010	0.100	0.010
3	0.010	0.040	0.010	0.010	0.040
4	0.240	0.060	0.010	0.030	0.010
5	0.000	0.060	0.020	0.010	0.020
6	0.030	0.040	0.150	0.020	0.030
7	0.060	0.090	0.090	0.000	0.080
Sum(w^2)	0.062	0.019	0.034	0.012	0.011
Cochran's test					
Test value	0.925	0.429	0.666	0.806	0.577
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727
	Outlier	No outliers	No outliers	Straggler	No outliers

Table 3 (*Continued*)

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
Computation of precision components					
T1	26.695	26.715	26.280	25.820	26.265
T2	101.8782	102.0496	98.7455	95.2813	98.6306
T3	0.0623	0.0189	0.0338	0.0124	0.0111
s_r^2	0.0044500	0.0013500	0.0024143	0.0008857	0.0007929
s_{BD}^2	0.010255952	0.014947619	0.012596429	0.006621429	0.013027381
s_R^2	0.0147060	0.0162976	0.0150107	0.0075071	0.0138202
m	3.81357	3.81643	3.75429	3.68857	3.75214
s_r	0.06671	0.03674	0.04914	0.02976	0.02816
s_R	0.12127	0.12766	0.12252	0.08664	0.11756
% s_r	1.7492	0.9627	1.3088	0.8068	0.7504
% s_R	3.1799	3.3451	3.2634	2.3490	3.1331
r	0.187	0.103	0.138	0.0833	0.079
R	0.339	0.357	0.343	0.243	0.329
Mandel's h consistency statistic					
1	−0.703	−1.012	−1.015	−0.875	−1.399
2	0.147	0.029	0.091	0.136	−0.838
3	−0.524	−0.131	−0.419	−1.589	−0.623
4	0.505	1.229	0.006	1.385	0.629
5	−1.464	−0.931	0.474	0.195	0.758
6	1.624	1.469	1.879*	0.850	1.406
7	0.416	−0.651	−1.015	−0.102	0.068
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	0.106	0.000	0.720	0.713	1.004
2	0.000	0.385	0.144	2.376**	0.251
3	0.106	0.770	0.144	0.238	1.004
4	2.544**	1.155	0.144	0.713	0.251
5	0.000	1.155	0.288	0.238	0.502
6	0.318	0.770	2.159*	0.475	0.753
7	0.636	1.732	1.295	0.000	2.009*
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	0.223	0.250	0.235	0.168	0.232

$$(s_L)_j^2 = \frac{\sum(y_{ij} - C_j)^2}{p - 1}$$

The quantity k is obtained by dividing the standard deviation among replicates in a cell by the repeatability standard deviation:

$$k_{ij} = \frac{s_{ij}}{\tilde{s}_j}$$

where s_{ij} is the standard deviation among replicates in cell (i,j) and

$$\tilde{s}_j = \left[\frac{\sum_i s_{ij}^2}{p}\right]^{1/2}$$

The h and k values for each cell in order of day are plotted in groups for each examined batch sample. Various patterns can appear in the h plots. Both positive and negative h values at the different

Table 4
Nicotinamide: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
9.5 mg/effervescent tablet					
1	10.05	9.35	9.29	9.50	9.21
1	10.06	9.38	9.19	9.40	9.16
2	9.57	9.40	9.80	9.39	9.34
2	9.64	9.46	9.60	9.54	9.36
3	9.61	9.69	9.47	8.75	8.84
3	9.41	9.77	9.33	8.87	9.04
4	9.76	10.18	9.01	9.27	9.13
4	9.66	10.17	8.94	9.33	9.17
5	9.66	9.36	9.04	9.33	9.47
5	9.72	9.37	9.02	9.33	9.42
6	10.02	9.69	9.54	9.02	9.16
6	9.96	9.48	9.34	9.01	8.99
7	9.86	9.32	9.07	9.16	9.24
7	9.87	9.29	8.95	9.18	9.47
Cell averages					
1	10.055	9.365	9.240	9.450	9.185
2	9.605	9.430	9.700	9.465	9.350
3	9.510	9.730	9.400	8.810	8.940
4	9.710	10.175	8.975	9.300	9.150
5	9.690	9.365	9.030	9.330	9.445
6	9.990	9.585	9.440	9.015	9.075
7	9.865	9.305	9.010	9.170	9.355
Grubbs' test[a]					
Gp (single high)	1.390	1.987	1.633	1.023	1.293
Gp (single low)	1.316	0.847	1.036	1.712	1.537
Gp (double high)	0.286	0.082	0.301	0.541	0.473
Gp (double low)	0.437	0.735	0.558	0.169	0.326
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
Cell ranges					
	$W(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	0.010	0.030	0.100	0.100	0.050
2	0.070	0.060	0.200	0.150	0.020
3	0.200	0.080	0.140	0.120	0.200
4	0.100	0.010	0.070	0.060	0.040
5	0.060	0.010	0.020	0.000	0.050
6	0.060	0.21	0.200	0.010	0.170
7	0.010	0.030	0.120	0.020	0.230
Sum(w^2)	0.062	0.056	0.129	0.051	0.129
Cochran's test					
Test value	0.642	0.786	0.309	0.441	0.411
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727
	No outliers	Straggler	No outliers	No outliers	No outliers

Table 4 (*Continued*)

Day i	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
Computation of precision components					
T1	68.425	66.955	64.795	64.540	64.500
T2	669.0977	640.9901	600.2128	595.4029	594.5125
T3	0.0623	0.0561	0.1293	0.0510	0.1288
s_r^2	0.0044500	0.0040071	0.0092357	0.0036429	0.0092000
s_{BD}^2	0.038325	0.092254762	0.069138095	0.055520238	0.027245238
s_R^2	0.0427750	0.0962619	0.0783738	0.0591631	0.0364452
m	9.77500	9.56500	9.25643	9.22000	9.21429
s_r	0.06671	0.06330	0.09610	0.06036	0.09592
s_R	0.20682	0.31026	0.27995	0.24323	0.19091
% s_r	0.6824	0.6618	1.0382	0.6546	1.0410
% s_R	2.1158	3.2437	3.0244	2.6381	2.0719
r	0.187	0.177	0.269	0.169	0.268
R	0.579	0.869	0.784	0.681	0.534
Mandel's h consistency statistic					
1	1.390	−0.651	−0.060	0.960	−0.164
2	−0.844	−0.440	1.633	1.023	0.761
3	−1.316	0.537	0.529	−1.712*	−1.537
4	−0.323	1.987**	−1.036	0.334	−0.360
5	−0.422	−0.651	−0.834	0.459	1.293
6	1.068	0.065	0.676	−0.856	−0.781
7	0.447	−0.847	−0.907	−0.209	0.789
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	0.106	0.335	0.736	1.172	0.369
2	0.742	0.670	1.472	1.757	0.147
3	2.120*	0.894	1.030	1.406	1.474
4	1.060	0.112	0.515	0.703	0.295
5	0.636	0.112	0.147	0.000	0.369
6	0.636	2.346**	1.472	0.117	1.253
7	0.106	0.335	0.883	0.234	1.696
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	0.403	0.61	0.54	0.479	0.357

[a] No single outlier/straggler. No double outliers/stragglers.

levels or batches within the experiment are possible and h plots corresponding to tabulated indicator values at the 1 and 5% significance level can be given. These indicator plots serve as guides when examining patterns in the data. If 1 day stands out on the k plot as having many large values, this indicates that on that day a poorer repeatability was performed than on other days. Also here k plots corresponding to tabulated indicator values at the 1 and 5% significance level can be given which serve as guides when examining patterns in the data. The k statistic is never negative.

As to Mandel the statistic h is algebraically identical with one of Grubb's outlier statistics and k is algebraically related to Cochran's C. However, these quantities are used in a different way. Grubb's statistic applies only to an extreme value (smallest or largest) and similarly, Cochran's C

Table 5
Ascorbic acid: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
32 mg/effervescent tablet					
1	37.04	34.37	28.97	30.68	27.55
1	38.29	31.94	29.39	30.03	26.66
2	28.95	29.26	30.84	30.18	30.62
2	28.80	27.84	30.23	32.42	30.45
3	33.79	33.24	31.80	30.65	29.85
3	32.09	35.47	34.01	32.18	31.12
4	31.33	31.75	27.55	29.40	28.37
4	29.78	32.37	27.91	29.82	29.52
5	34.85	33.24	31.70	31.46	31.43
5	35.04	33.56	31.36	32.47	30.91
6	33.76	32.20	32.01	27.04	26.66
6	33.44	30.25	31.00	26.38	24.16
7	30.18	26.60	28.65	27.79	28.35
7	29.13	25.88	26.65	27.80	27.76
Cell averages					
1	37.665	33.155	29.180	30.355	27.105
2	28.875	28.550	30.535	31.300	30.535
3	32.940	34.355	32.905	31.415	30.485
4	30.555	32.060	27.730	29.610	28.945
5	34.945	33.400	31.530	31.965	31.170
6	33.600	31.225	31.505	26.710	25.410
7	29.655	26.240	27.650	27.795	28.055
Grubbs' test[a]					
Gp (single high)	1.614	1.054	1.364	1.056	1.123
Gp (single low)	1.189	1.731	1.235	1.604	1.623
Gp (double high)	0.288	0.621	0.472	0.601	0.552
Gp (double low)	0.465	0.116	0.310	0.150	0.252
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
Cell ranges					
	$W(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	1.250	2.430	0.420	0.650	0.890
2	0.150	1.420	0.610	2.240	0.170
3	1.700	2.230	2.210	1.530	1.270
4	1.550	0.620	0.360	0.420	1.150
5	0.190	0.320	0.340	1.010	0.520
6	0.320	1.950	1.010	0.660	2.500
7	1.050	0.720	2.000	0.010	0.590
Sum(w^2)	8.118	17.702	10.698	9.413	10.625
Cochran's test					
Test value	0.356	0.334	0.457	0.533	0.588
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727
	No outliers	No outliers	No outliers	No outliers	No outliers

Table 5 (*Continued*)

Day i	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
Computation of precision components					
T1	228.235	218.985	211.035	209.150	201.705
T2	7500.6015	6901.5644	6386.7790	6272.5177	5838.5355
T3	8.1185	17.7019	10.6979	9.4132	10.6249
s_r^2	0.5798929	1.2644214	0.7641357	0.6723714	0.7589214
s_{BD}^2	9.543278571	7.856370238	3.705589286	3.566228571	4.021530952
s_R^2	10.1231714	9.1207917	4.4697250	4.2386000	4.7804524
m	32.60500	31.28357	30.14786	29.87857	28.81500
s_r	0.76151	1.12446	0.87415	0.81998	0.87116
s_R	3.18169	3.02006	2.11417	2.05879	2.18642
% s_r	2.3356	3.5944	2.8995	2.7444	3.0233
% s_R	9.7583	9.6538	7.0127	6.8905	7.5878
r	2.132	3.148	2.448	2.296	2.439
R	8.909	8.456	5.919	5.765	6.122
Mandel's h consistency statistic					
1	1.614	0.642	−0.479	0.241	−0.815
2	−1.189	−0.938	0.191	0.720	0.820
3	0.107	1.054	1.364	0.778	0.796
4	−0.654	0.266	−1.196	−0.136	0.062
5	0.746	0.726	0.684	1.056	1.123
6	0.317	−0.020	0.671	−1.604	−1.623
7	−0.941	−1.731*	−1.235	−1.055	−0.362
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	1.161	1.528	0.340	0.561	0.722
2	0.139	0.893	0.493	1.932*	0.138
3	1.579	1.402	1.788	1.319	1.031
4	1.439	0.390	0.291	0.362	0.933
5	0.176	0.201	0.275	0.871	0.422
6	0.297	1.226	0.817	0.569	2.029*
7	0.975	0.453	1.618	0.009	0.479
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	6.272	5.827	4.044	3.950	4.196

[a] No single outlier/straggler. No double outliers/stragglers.

applies only to an extreme observation. Some h-values may be small, indicating proximity to the overall average, and some are large, indicating some distance from this average. It is not recommended to consider the tabulated indicator values as criteria for rejection. Within each cell the h and k values are calculated independently of each other. Any criterion for judging h and k values must be based on all of them.

In addition to these h and k graphs histographs of cell means and cell ranges can reveal the presence of distinct populations.

In Table 11 it is shown for the analysis results for calcium pantothenate in the pilot study, how ANOVA yields two sum of squares. By dividing them by the appropriate number of degrees of freedom one obtains two mean squares: (1) the repeatability variance $s^2(r)$ and (2) the sum of the

Table 6
α-Tocopherolacetate: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
5.5 mg/effervescent tablet					
1	5.78	5.27	5.63	4.84	5.36
1	5.62	5.38	5.24	5.09	5.65
2	5.69	5.50	5.37	5.36	5.16
2	5.70	5.49	5.32	5.32	5.15
3	5.64	5.71	5.25	5.05	5.17
3	5.77	5.47	5.45	5.19	5.20
4	5.64	5.47	5.58	5.34	5.30
4	5.64	5.43	5.50	5.40	5.30
5	5.67	5.53	5.28	5.08	5.41
5	5.83	5.61	5.29	5.17	5.56
6	5.41	5.17	5.14	5.15	5.27
6	5.11	4.90	5.27	4.90	5.07
7	5.62	5.03	4.92	5.12	5.22
7	5.51	5.21	4.99	5.13	5.30
Cell averages					
1	5.700	5.325	5.435	4.965	5.505
2	5.695	5.495	5.345	5.340	5.155
3	5.705	5.590	5.350	5.120	5.185
4	5.640	5.450	5.540	5.370	5.300
5	5.750	5.570	5.285	5.125	5.485
6	5.260	5.035	5.205	5.025	5.170
7	5.565	5.120	4.955	5.125	5.260
Grubbs' test					
Gp (single high)	0.796	1.009	1.276	1.440	1.438
Gp (single low)	2.123	1.528	1.862	1.246	0.951
Gp (double high)	0.790	0.566	0.512	0.158	0.122
Gp (double low)	0.036	0.157	0.188	0.475	0.621
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
	Single straggler		No single outlier/straggler No double outliers/stragglers		
Cell ranges					
	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	0.160	0.110	0.390	0.250	0.290
2	0.010	0.010	0.050	0.040	0.010
3	0.130	0.240	0.200	0.140	0.030
4	0.000	0.040	0.080	0.060	0.000
5	0.160	0.080	0.010	0.090	0.150
6	0.300	0.270	0.130	0.250	0.200
7	0.110	0.180	0.070	0.010	0.080
Sum(w^2)	0.170	0.183	0.223	0.158	0.154
Cochran's test					
Test value	0.528	0.398	0.682	0.396	0.546
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727

Table 6 (*Continued*)

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
	No outliers	No outliers	No outliers	No outliers	No outliers
Computation of precision components					
T1	39.315	37.585	37.115	36.070	37.060
T2	220.9790	202.0918	196.9976	186.0000	196.3350
T3	0.1703	0.1831	0.2229	0.1580	0.1540
s_r^2	0.0121643	0.0130786	0.0159214	0.0112857	0.0110000
s_{BD}^2	0.02209881	0.041322619	0.026804762	0.017097619	0.015961905
s_R^2	0.0342631	0.0544012	0.0427262	0.0283833	0.0269619
m	5.61643	5.36929	5.30214	5.15286	5.29429
s_r	0.11029	0.11436	0.12618	0.10623	0.10488
s_R	0.18510	0.23324	0.20670	0.16847	0.16420
% s_r	1.9637	2.1299	2.3798	2.0617	1.9810
% s_R	3.2957	4.3440	3.8985	3.2695	3.1015
r	0.309	0.320	0.353	0.297	0.294
R	0.518	0.653	0.579	0.472	0.460
Mandel's h consistency statistic					
1	0.498	−0.202	0.713	−1.246	1.438
2	0.468	0.575	0.230	1.241	−0.951
3	0.528	1.009	0.257	−0.218	−0.746
4	0.140	0.369	1.276	1.440	0.039
5	0.796	0.917	−0.092	−0.185	1.302
6	−2.123**	−1.528	−0.521	−0.848	−0.848
7	−0.306	−1.139	−1.862*	−0.185	−0.234
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	1.026	0.680	2.186*	1.664	1.955*
2	0.064	0.062	0.280	0.266	0.067
3	0.833	1.484	1.121	0.932	0.202
4	0.000	0.247	0.448	0.399	0.000
5	1.026	0.495	0.056	0.599	1.011
6	1.923*	1.669	0.729	1.664	1.348
7	0.705	1.113	0.392	0.067	0.539
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	0.336	0.438	0.373	0.302	0.293

repeatability variance $s^2(r)$ and twice the between-day intermediate variance (in duplicate analysis). From both mean square values, the between-day variance can easily be calculated. The total variance (reproducibility variance in ISO 5725-2) is the sum of these two variance estimates: $s^2(R) = s^2(r) + s^2(\mathrm{BD})$.

If two replicate analysis under repeatability conditions are performed, the standard uncertainty u for an individual becomes:

Table 7
Pyridoxine·HCl: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
1.7 mg/effervescent tablet					
1	1.41	1.54	1.48	1.61	1.36
1	1.48	1.54	1.46	1.58	1.35
2	1.56	1.53	1.50	1.54	1.51
2	1.58	1.55	1.49	1.56	1.53
3	1.52	1.58	1.48	1.43	1.39
3	1.52	1.59	1.46	1.43	1.40
4	1.50	1.53	1.36	1.56	1.50
4	1.49	1.35	1.27	1.55	1.50
5	1.52	1.47	1.33	1.48	1.48
5	1.53	1.49	1.34	1.49	1.52
6	1.54	1.50	1.39	1.44	1.42
6	1.53	1.47	1.34	1.45	1.39
7	1.59	1.48	1.36	1.49	1.48
7	1.59	1.51	1.32	1.49	1.52
Cell averages					
1	1.445	1.540	1.470	1.595	1.355
2	1.570	1.540	1.495	1.550	1.520
3	1.520	1.585	1.470	1.430	1.395
4	1.495	1.440	1.315	1.555	1.500
5	1.525	1.480	1.335	1.485	1.500
6	1.535	1.485	1.365	1.445	1.405
7	1.590	1.495	1.340	1.490	1.500
Grubbs' test[a]					
Gp (single high)	1.347	1.563	1.261	1.437	1.002
Gp (single low)	1.691	1.431	1.093	1.261	1.486
Gp (double high)	0.382	0.365	0.429	0.390	0.655
Gp (double low)	0.273	0.460	0.562	0.390	0.315
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
Cell ranges					
	$W(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	0.070	0.000	0.020	0.030	0.010
2	0.020	0.020	0.010	0.020	0.020
3	0.000	0.010	0.020	0.000	0.010
4	0.010	0.180	0.090	0.010	0.000
5	0.010	0.020	0.010	0.010	0.040
6	0.010	0.030	0.050	0.010	0.030
7	0.000	0.030	0.040	0.000	0.040
Sum(w^2)	0.006	0.035	0.013	0.002	0.005
Cochran's test					
Test value	0.875	0.923	0.614	0.563	0.340
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727
	Outlier	Outlier	No outliers	No outliers	No outliers

Table 7 (*Continued*)

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
Computation of precision components					
T1	10.680	10.565	9.790	10.550	10.175
T2	16.3083	15.9597	13.7271	15.9228	14.8165
T3	0.0056	0.0351	0.0132	0.0016	0.0047
s_r^2	0.0004000	0.0025071	0.0009429	0.0001143	0.0003357
s_{BD}^2	0.002078571	0.001091667	0.00537619	0.003683333	0.004229762
s_R^2	0.0024786	0.0035988	0.0063190	0.0037976	0.0045655
m	1.52571	1.50929	1.39857	1.50714	1.45357
s_r	0.02000	0.05007	0.03071	0.01069	0.01832
s_R	0.04979	0.05999	0.07949	0.06162	0.06757
% s_r	1.3109	3.3176	2.1955	0.7093	1.2605
% s_R	3.2631	3.9747	5.6838	4.0889	4.6484
r	0.056	0.140	0.086	0.0299	0.051
R	0.139	0.1678	0.223	0.173	0.189
Mandel's h consistency statistic					
1	−1.691	0.634	0.934	1.437	−1.486
2	0.928	0.634	1.261	0.701	1.002
3	−0.120	1.563	0.934	−1.261	−0.883
4	−0.643	−1.431	−1.093	0.782	0.700
5	−0.015	−0.605	−0.831	−0.362	0.700
6	0.195	−0.501	−0.439	−1.016	−0.732
7	1.347	−0.295	−0.766	−0.280	0.700
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	2.475**	0.000	0.461	1.984*	0.386
2	0.707	0.282	0.230	1.323	0.772
3	0.000	0.141	0.461	0.000	0.386
4	0.354	2.542**	2.073*	0.661	0.000
5	0.354	0.282	0.230	0.661	1.544
6	0.354	0.424	1.151	0.661	1.158
7	0.000	0.424	0.921	0.000	1.544
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	0.095	0.097	0.153	0.122	0.133

[a] No single outlier/straggler. No double outliers/stragglers.

$$u_x = \sqrt{\frac{s_r^2}{2} + (s)_{BD}^2}$$

The expanded uncertainty $U_x = k \times u_x$ defines an interval around the measurement result, which is expected to include the largest fraction of the distribution of measurement results. The coverage factor k is typically in the range 2–3; $k = 2$ corresponds to a confidence interval of 95%.

In Table 12 a survey of the expanded uncertainties U_x for the vitamins for each batch of the two different types of effervescent tablets as well as expanded uncertainties, calculated from pooled variances, after checking of variance homogeneity, is shown [17].

Similarly, for ascorbic acid, variance components with expanded uncertainty are calculated from duplicate analysis results, measured during 8

Table 8
Thiamine mononitrate: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
0.92 mg/effervescent tablet					
1	0.94	0.86	0.87	0.89	0.86
1	0.95	0.85	0.85	0.87	0.87
2	0.88	0.86	0.92	0.88	0.87
2	0.89	0.86	0.91	0.90	0.87
3	0.87	0.90	0.88	0.80	0.80
3	0.87	0.90	0.87	0.81	0.81
4	0.89	0.92	0.83	0.85	0.85
4	0.89	0.92	0.83	0.86	0.86
5	0.91	0.88	0.84	0.86	0.88
5	0.91	0.90	0.84	0.87	0.88
6	0.90	0.87	0.87	0.83	0.84
6	0.89	0.85	0.86	0.82	0.80
7	0.93	0.85	0.83	0.86	0.88
7	0.92	0.86	0.83	0.86	0.88
Cell averages					
1	0.945	0.855	0.860	0.880	0.865
2	0.885	0.860	0.915	0.890	0.870
3	0.870	0.900	0.875	0.805	0.805
4	0.890	0.920	0.830	0.855	0.855
5	0.910	0.890	0.840	0.865	0.880
6	0.895	0.860	0.865	0.825	0.820
7	0.925	0.855	0.830	0.860	0.880
Grubbs' test[a]					
Gp (single high)	1.644	1.641	1.845	1.194	0.890
Gp (single low)	1.282	0.848	0.970	1.647	1.636
Gp (double high)	0.216	0.213	0.201	0.499	0.630
Gp (double low)	0.515	0.665	0.561	0.158	0.085
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
Cell ranges					
	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	0.010	0.010	0.020	0.020	0.010
2	0.010	0.000	0.010	0.020	0.000
3	0.000	0.000	0.010	0.010	0.010
4	0.000	0.000	0.000	0.010	0.010
5	0.000	0.020	0.000	0.010	0.000
6	0.010	0.020	0.010	0.010	0.040
7	0.010	0.010	0.000	0.000	0.000
Sum(w^2)	0.0004	0.001	0.001	0.001	0.002
Cochran's test					
Test value	0.250	0.400	0.571	0.333	0.842
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727
	No outliers	No outliers	No outliers	No outliers	Outlier

Table 8 (*Continued*)

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
Computation of precision components					
T1	6.320	6.140	6.015	5.980	5.975
T2	5.7100	5.3898	5.1741	5.1140	5.1054
T3	0.0004	0.0010	0.0007	0.0012	0.0019
s_r^2	0.0000286	0.0000714	0.0000500	0.0000857	0.0001357
s_{BD}^2	0.000642857	0.000646429	0.000886905	0.000852381	0.000813095
s_R^2	0.0006714	0.0007179	0.0009369	0.0009381	0.0009488
M	0.90286	0.87714	0.85929	0.85429	0.85357
s_r	0.00535	0.00845	0.00707	0.00926	0.01165
s_R	0.02591	0.02679	0.03061	0.03063	0.03080
% s_r	0.5920	0.9635	0.8229	1.0837	1.3648
% s_R	2.8700	3.0546	3.5621	3.5853	3.6087
r	0.015	0.024	0.010	0.026	0.033
R	0.073	0.075	0.086	0.086	0.086
Mandel's h consistency statistic					
1	1.644	−0.848	0.024	0.859	0.385
2	−0.697	−0.656	1.845*	1.194	0.554
3	−1.282	0.875	0.520	−1.647	−1.636
4	−0.502	1.641	−0.970	0.024	0.048
5	0.279	0.492	−0.639	0.358	0.890
6	−0.307	−0.656	0.189	−0.979	−1.131
7	0.864	−0.848	−0.970	0.191	0.890
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	1.323	0.837	2.000*	1.528	0.607
2	1.323	0.000	1.000	1.528	0.000
3	0.000	0.000	1.000	0.764	0.607
4	0.000	0.000	0.000	0.764	0.607
5	0.000	1.673	0.000	0.764	0.000
6	1.323	1.673	1.000	0.764	2.428**
7	1.323	0.837	0.000	0.000	0.000
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	0.051	0.052	0.060	0.060	0.059

[a] No single outlier/straggler. No double outliers/stragglers.

different days on the same multivitamin capsule preparation by the selective HPLC method and the results are given in the same Table.

Repeatability variances for different multivitamin preparations with corresponding ranges of nominal amounts of vitamins according to the ISO 5725-2 standard.

For analysis of particular galenic forms as capsules with oily content, the applied general methods as described in the pilot study, have slightly to be modified just in order to guarantee as much as possible complete exemption and dissolution of the vitamins in the final injected solution of analysis:

Table 9
Riboflavine phosphate: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
1.33 mg/effervescent tablet					
1	1.37	1.26	1.27	1.27	1.18
1	1.36	1.27	1.25	1.23	1.22
2	1.43	1.45	1.37	1.31	1.38
2	1.46	1.50	1.32	1.28	1.38
3	1.39	1.46	1.37	1.28	1.31
3	1.39	1.44	1.35	1.27	1.32
4	1.40	1.52	1.29	1.31	1.30
4	1.37	1.48	1.26	1.30	1.32
5	1.40	1.36	1.28	1.31	1.33
5	1.42	1.36	1.29	1.30	1.34
6	1.43	1.40	1.34	1.28	1.290
6	1.43	1.37	1.32	1.28	1.260
7	1.46	1.38	1.31	1.35	1.33
7	1.45	1.39	1.30	1.34	1.38
Cell averages					
1	1.365	1.265	1.260	1.250	1.200
2	1.445	1.475	1.345	1.295	1.380
3	1.390	1.450	1.360	1.275	1.315
4	1.385	1.500	1.275	1.305	1.310
5	1.410	1.360	1.285	1.305	1.335
6	1.430	1.385	1.330	1.280	1.275
7	1.455	1.385	1.305	1.345	1.355
Grubbs' test[a]					
Gp (single high)	1.305	1.215	1.368	1.725	1.186
Gp (single low)	1.391	1.724	1.292	1.461	1.864
Gp (double high)	0.369	0.469	0.350	0.332	0.542
Gp (double low)	0.414	0.286	0.430	0.435	0.161
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
Cell ranges					
	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	0.010	0.010	0.020	0.040	0.040
2	0.030	0.050	0.050	0.030	0.000
3	0.000	0.020	0.020	0.010	0.010
4	0.030	0.040	0.030	0.010	0.020
5	0.020	0.000	0.010	0.010	0.010
6	0.000	0.030	0.020	0.000	0.030
7	0.010	0.010	0.010	0.010	0.050
Sum(w^2)	0.002	0.006	0.005	0.003	0.006
Cochran's test					
Test value	0.375	0.446	0.521	0.552	0.446
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727
	No outliers	No outliers	No outliers	No outliers	No outliers

Table 9 (*Continued*)

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
Computation of precision components					
T1	9.880	9.820	9.160	9.055	9.170
T2	13.9516	13.8144	11.9950	11.7186	12.0336
T3	0.0024	0.0056	0.0048	0.0029	0.0056
s_r^2	0.0001714	0.0004000	0.0003429	0.0002071	0.0004000
s_{BD}^2	0.001028571	0.006190476	0.001242857	0.000785714	0.003283333
s_R^2	0.0012000	0.0065905	0.0015857	0.0009929	0.0036833
M	1.41143	1.40286	1.30857	1.29357	1.31000
s_r	0.01309	0.02000	0.01852	0.01439	0.02000
s_R	0.03464	0.08118	0.03982	0.03151	0.06069
% s_r	0.9276	1.4257	1.4150	1.1126	1.5267
% s_R	2.4543	5.7869	3.0431	2.4359	4.6329
r	0.037	0.056	0.052	0.040	0.056
R	0.097	0.227	0.111	0.088	0.170
Mandel's h consistency statistic					
1	−1.391	−1.724*	−1.292	−1.461	−1.864*
2	1.006	0.902	0.969	0.048	1.186
3	−0.642	0.590	1.368	−0.623	0.085
4	−0.792	1.215	−0.893	0.383	0.000
5	−0.043	−0.536	−0.627	0.383	0.424
6	0.556	−0.223	0.570	−0.455	−0.593
7	1.305	−0.223	−0.095	1.725*	0.762
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	0.540	0.354	0.764	1.965*	1.414
2	1.620	1.768	1.909*	1.474	0.000
3	0.000	0.707	0.764	0.491	0.354
4	1.620	1.414	1.146	0.491	0.707
5	1.080	0.000	0.382	0.491	0.354
6	0.000	1.061	0.764	0.000	1.061
7	0.540	0.354	0.382	0.491	1.768
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	0.067	0.160	0.075	0.060	0.118

[a] No single outlier/straggler. No double outliers/stragglers.

1) For analysis of hydro-soluble vitamins, 10 ml of acetone is added to the weighed oily content and the mixture is shaken until homogeneous dispersion of the sample mass. Then the sodium hexane sulfonate solution in 0.1% acetic acid and 1 ml of glacial acetic acid are added.
2) For analysis of calcium pantothenate, about 10 ml of acetone is added to the weighed oily content and the mixture shaken until homogeneous dispersion of the sample mass. Then 10 ml of water is added, the mixture sonicated for 40 min and further diluted to 50.0 ml with water.
3) For analysis of lipo-soluble vitamins, 50.0 ml of *n*-hexane is added at first. Then the mixture is thoroughly shaken to allow complete dissolution of the oily sample. Afterwards, the

Table 10
Retinol acetate: assay results and ISO 5725-2 calculations of uncertainty

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
519 μg/effervescent tablet					
1	574.23	528.49	485.89	468.23	493.39
1	606.82	537.29	446.66	446.74	527.54
2	629.60	572.14	535.46	580.56	560.53
2	524.96	564.16	504.92	570.90	499.98
3	563.59	622.74	490.70	511.90	521.96
3	522.08	571.57	463.18	530.25	498.41
4	559.93	561.05	475.29	453.90	502.43
4	556.53	516.72	470.74	467.19	507.21
5	546.12	514.62	463.71	448.24	552.03
5	545.29	491.59	514.60	473.52	494.72
6	608.65	602.86	590.00	426.04	498.62
6	597.92	548.31	486.87	499.99	505.49
7	592.72	553.39	482.65	461.10	420.04
7	510.23	462.71	482.62	455.80	547.67
Cell averages					
1	590.525	532.894	466.277	457.488	510.466
2	577.277	568.146	520.194	575.727	530.257
3	542.832	597.156	476.940	521.078	510.184
4	558.229	538.883	473.013	460.548	504.823
5	545.704	503.101	489.156	460.879	523.375
6	603.286	575.583	538.437	463.015	502.051
7	551.477	508.051	482.635	458.453	483.857
Grubbs' test[a]					
Gp (single high)	1.538	1.441	1.721	1.969	1.393
Gp (single low)	1.028	1.222	0.976	0.606	1.690
Gp (double high)	0.226	0.367	0.072	0.001	0.350
Gp (double low)	0.563	0.379	0.658	0.835	0.329
Straggler (sin $l+h$)	2.020	2.020	2.020	2.020	2.020
Outlier (sin $l+h$)	2.139	2.139	2.139	2.139	2.139
Straggler (dbl $l+h$)	0.0708	0.0708	0.0708	0.0708	0.0708
Outlier (dbl $l+h$)	0.0308	0.0308	0.0308	0.0308	0.0308
Cell ranges					
	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$	$w(ij)$
1	32.593	8.799	39.229	21.494	34.147
2	104.637	7.979	30.538	9.660	60.547
3	41.513	51.177	27.528	18.348	23.556
4	3.405	44.326	4.553	13.290	4.778
5	0.837	23.030	50.893	25.286	57.315
6	10.733	54.547	103.132	73.958	6.868
7	82.490	90.676	0.035	5.301	127.6
Sum(w^2)	20 666.686	16 452.861	16 476.397	7205.752	25 031.804
Cochran's test					
Test value	0.530	0.500	0.646	0.759	0.651
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.838	0.838	0.838	0.838	0.838
Critical value 5%	0.727	0.727	0.727	0.727	0.727
	No outliers	No outliers	No outliers	Straggler	No outliers

Table 10 (*Continued*)

Day *i*	Matrix 1		Matrix 2		
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3
Computation of precision components					
T1	3969.331	3823.814	3446.651	3397.189	3565.013
T2	2 254 129.53	2 096 278.11	1 701 352.92	1 661 355.80	1 816 975.94
T3	20 666.6865	16 452.8608	16 476.3975	7205.7517	25 031.8037
s_r^2	1476.19189	1175.20434	1176.88553	514.69655	1787.98598
s_{BD}^2	0	659.8902662	127.3928514	1852.134553	0
s_R^2	1476.19189	1835.09461	1304.27838	2366.83110	1787.98598
m	567.04729	546.25912	492.37876	485.31270	509.28753
s_r	38.42124	34.28125	34.30577	22.68692	42.28458
s_R	38.42124	42.83800	36.11479	48.65009	42.28458
% s_r	6.7757	6.2756	6.9674	4.6747	8.3027
% s_R	6.7757	7.8421	7.3348	10.0245	8.3027
r	107.579	95.987	96.056	63.523	118.397
R	107.579	119.946	101.121	136.220	118.397
Mandel's h consistency statistic					
1	0.996	−0.378	−0.976	−0.606	0.078
2	0.434	0.620	1.040	1.969*	1.393
3	−1.028	1.441	−0.577	0.779	0.060
4	−0.374	−0.209	−0.724	−0.539	−0.297
5	−0.906	−1.222	−0.120	−0.532	0.936
6	1.538	0.830	1.721*	−0.485	−0.481
7	−0.661	−1.082	−0.364	−0.585	−1.690
h (1%)	1.980	1.980	1.980	1.980	1.980
	−1.980	−1.980	−1.980	−1.980	−1.980
h (5%)	1.710	1.710	1.710	1.710	1.710
	−1.710	−1.710	−1.710	−1.710	−1.710
Mandel's k consistency statistic					
1	0.600	0.181	0.809	0.670	0.571
2	1.926*	0.165	0.629	0.301	1.012
3	0.764	1.056	0.567	0.572	0.394
4	0.063	0.914	0.094	0.414	0.080
5	0.015	0.475	1.049	0.788	0.958
6	0.198	1.125	2.126*	2.305**	0.115
7	1.518	1.870*	0.001	0.165	2.134*
k (1%)	2.2	2.2	2.2	2.2	2.2
k (5%)	1.87	1.87	1.87	1.87	1.87
U_x	54.336	70.640	53.510	91.858	59.799

[a] No single outlier/straggler. No double outliers/stragglers.

phosphoric acid solution and the ethanol are added.

The results of the repeatability variances, for the in duplicate assay results of vitamin contents, subdivided in groups with a corresponding range of nominal amounts for each vitamin, are given in Tables 13 and 14 Tables 15–19.

The Cochran's tests or Mandel's *k* statistics do not reveal important precision outliers so that for different ranges of amounts of vitamins per unit, general repeatability values *r* and repeatability variances, covering all types of multi-vitamin preparations, are available.

As to the ISO 5725 standard the repeatability $r = 2.8s_r$, where s_r is the repeatability standard

Table 11
Uncertainty calculation from ANOVA-table (mg calcium pantothenate by day) (Matrix 1, batch 1)

Source	Sum of squares	DF	Mean square	F-ratio	P-value
Between days	0.149771	6	0.0249619	5.61	0.0197
Within days	0.03115	7	0.00445		
Total (corr.)	0.180921	13			

Within days mean square = repeatability variance $s^2(r)$ in ISO 5725-2 = 0.00445. Between days mean square = $s^2(r)+n$ (= 2; duplicate analysis) × s^2(between days) = 0.0249619. Intermediate variance s^2(BD) (= between days) = (0.0249619 − 0.00445)/2 = 0.0102559. Total variance $s^2(R) = s^2$ (between days) + $s^2(r)$ = 0.014706. $U(x)$ = "expanded" uncertainty = $k \times \sqrt{(s^2(r)/2 + s^2(\mathrm{BD})} =$ 2 × sqrt(0.00445/2 + 0.0102559) = 0.22344.

deviation These general repeatability values r, measured for different ranges of amounts of vitamins per unit, might be suitable for evaluation of the found in duplicate analysis result differences, measured in forthcoming samples.

4. Conclusion

We consider here uncertainty estimations using information from in-house validation results of the analytical HPLC methods of multivitamin preparations. This implies that no real estimate of complete reproducibility is known, but that only an estimate of the intermediate between-day precision is available. The estimated bias is an overall bias, which is a combination of the lab bias and the method bias. To separate the method bias from the lab bias inter-laboratory studies are required. It is clear that in our situation only within-laboratory uncertainty is considered, which implies calculation of intermediate precision, accounting for the repeatability and the run effect. To have an idea of our within-laboratory uncertainty, in duplicate vitamin analysis on different batches of two different types of effervescent tablets were performed over a period of 7 days. We are aware of the limitations of this pilot study, which gives no comparable information of within-laboratory uncertainty of the vitamin analysis methods, applied to other types of galenic forms. Many other multivitamin preparations are composed with completely different excipients and often contain vegetable ingredients, different trace elements or several other nutritional supplements. An additional complication is that the multivitamin preparations, presented for analysis, have disperse mean masses, ranging from 100 mg to about 5 g and that they may contain amounts of vitamins in different or similar ranges.

To overcome partially this important limitation we rely on repeatability variances, calculated from in duplicate analysis results on many different types of multivitamin preparations. For each vitamin, the analysis results obtained are classified in the most occurring ranges of nominal amounts per unit. These repeatability values (s_r^2, s_r, r), obtained for a certain range of amount of vitamin, are generally comparable to these, calculated from the pilot study and may serve to evaluate in duplicate analysis results on forthcoming analysis of vitamins in multivitamin preparations.

Table 12
Survey of uncertainties, calculated for each vitamin examined

	Matrix 1		Matrix 2			Pooled
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3	
Retinol acetate: 519 μg/effervescent tablet						
U_x	54.336	70.64	53.510	91.858	59.799	67.56
s_r	38.42124	34.28125	34.30577	22.68692	42.28458	35.02
s_R	38.42124	42.83800	36.11479	48.65009	42.28458	41.88
% s_r	6.7757	6.2756	6.9674	4.6747	8.3027	6.73
% s_R	6.7757	7.8421	7.3348	10.0245	8.3027	8.05
r	107.579	95.987	96.056	63.523	118.397	98.05
R	107.579	119.946	101.121	136.220	118.397	117.27
Riboflavine phosphate: 1.33 mg/effervescent tablet						
U_x	0.067	0.160	0.075	0.060	0.118	0.103
s_r	0.01309	0.02000	0.01852	0.01439	0.02000	0.0174
s_R	0.03464	0.08118	0.03982	0.03151	0.06069	0.0530
% s_r	0.9276	1.4257	1.4150	1.1126	1.5267	1.30
% s_R	2.4543	5.7869	3.0431	2.4359	4.6329	3.94
r	0.037	0.056	0.052	0.040	0.0560	0.049
R	0.097	0.227	0.111	0.088	0.170	0.148
Thiamine mononitrate: 0.92 mg/effervescent tablet						
U_x	0.051	0.052	0.060	0.060	0.059	0.0567
s_r	0.00535	0.00845	0.00707	0.00926	0.01165	0.0086
s_R	0.02591	0.02679	0.03061	0.03063	0.03080	0.029
% s_r	0.5920	0.9635	0.8229	1.0837	1.3648	0.99
% s_R	2.8700	3.0546	3.5621	3.5853	3.6087	3.34
r	0.015	0.024	0.0120	0.026	0.033	0.024
R	0.073	0.075	0.086	0.086	0.086	0.081
Pyridoxine·HCl: 1.7 mg/effervescent tablet						
U_x	0.095	0.097	0.153	0.12	0.13	0.122
s_r	0.02000	0.05007	0.03071	0.01069	0.01832	0.029
s_R	0.04979	0.05999	0.07949	0.06162	0.06757	0.064
% s_r	1.3109	3.3176	2.1955	0.7093	1.2605	1.98
% s_R	3.2631	3.9747	5.6838	4.0889	4.6484	4.36
r	0.056	0.140	0.086	0.0299	0.051	0.082
R	0.139	0.168	0.223	0.173	0.189	0.180
α-Tocopherolacetate: 5.5 mg/effervescent tablet						
U_x	0.33574	0.43755	0.37291	0.30160	0.29300	0.352
s_r	0.11029	0.11436	0.12618	0.10623	0.10488	0.112
s_R	0.18510	0.23324	0.20670	0.16847	0.16420	0.193
% s_r	1.9637	2.1299	2.3798	2.0617	1.9810	2.11
% s_R	3.2957	4.3440	3.8985	3.2695	3.1015	3.61
r	0.309	0.320	0.353	0.297	0.294	0.315
R	0.518	0.653	0.579	0.472	0.460	0.541
Ascorbic acid: 32 mg/effervescent tablet						
U_x	6.27159	5.82703	4.04359	3.95091	4.19571	4.96
s_r	0.76151	1.12446	0.87415	0.81998	0.87116	0.899
s_R	3.18169	3.02006	2.11417	2.05879	2.18642	2.559
% s_r	2.3356	3.5944	2.8995	2.7444	3.0233	2.94
% s_R	9.7583	9.6538	7.0127	6.8905	7.5878	8.38
r	2.132	3.148	2.448	2.296	2.439	2.52
R	8.909	8.456	5.919	5.765	6.122	7.16

Table 12 (*Continued*)

	Matrix 1		Matrix 2			Pooled
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 3	
U_x	1.67	Selective method 30 mg/capsule				
s_r	0.89100					
s_R	1.04552					
% s_r	3.0251					
% s_R	3.5498					
r	2.495					
R	2.927					
Nicotinamide: 9.5 mg/effervescent tablet						
U_x	0.403	0.61	0.543	0.479	0.357	0.488
s_r	0.06671	0.06330	0.09610	0.06036	0.09592	0.078
s_R	0.20682	0.31026	0.27995	0.24323	0.19091	0.2502
% s_r	0.6824	0.6618	1.0382	0.6546	1.0410	0.83
% s_R	2.1158	3.2437	3.0244	2.6381	2.0719	2.66
r	0.187	0.177	0.269	0.169	0.268	0.22
R	0.579	0.869	0.784	0.681	0.534	0.70
Calcium pantothenate: 3.8 mg/effervescent tablet						
U_x	0.223	0.250	0.2350	0.168	0.232	0.22
s_r	0.06671	0.03674	0.04914	0.02976	0.02816	0.0445
s_R	0.12127	0.12766	0.12252	0.08664	0.11756	0.116
% s_r	1.7492	0.9627	1.3088	0.8068	0.7504	1.18
% s_R	3.1799	3.3451	3.2634	2.3490	3.1331	3.08
r	0.187	0.103	0.138	0.083	0.079	0.12
R	0.339	0.357	0.343	0.243	0.329	0.32

Table 13
Repatability calculations for different samples, divided in different ranges of amount per unit: Ca-pantothenate

Sample *i*	4 mg/unit	5–6 mg/unit	7–8 mg/unit	9–10 mg/unit	17–20 mg/unit
1	4.66 capsules	5.82 capsules	7.78 tablets	8.52 tablets	19.75 tablets
1	4.75 ~1 g	5.80 ~0.4 g	7.82 ~1.25 g	8.56 ~0.4 g	19.70 ~0.3 g
2	4.70 tablets	5.75 capsules	8.17 tablets	10.52 capsules	18.27 capsules
2	4.64 ~1.73 g	5.70 ~0.4 g	8.05 ~1.25 g	10.44 ~0.25 g	18.21 ~0.33 g
3	4.76 tablets	5.78 capsules	6.41 tablets	9.39 capsules	20.87 tablets
3	4.63 ~1.75 g	5.72 ~0.4 g	6.62 ~0.33 g	9.52 ~0.5 g	20.82 ~0.25 g
4	4.10 capsules	5.52 tablets	7.36 effervescent tablet	11.61 capsules	20.680 tablets
4	4.09 ~0.5 g	5.62 ~2.1 g	7.39 ~1.2 g	11.34 ~0.44 g	20.630 ~0.25 g
5	4.89 tablets	6.04 tablets	6.39 capsules	12.07 capsules	16.97 tablets
5	4.82 ~0.8 g	6.22 ~1.1 g	6.49 ~1.2 g	12.26 ~0.45 g	17.12 ~0.6 g
6	4.150 capsules	5.51 tablets	6.920 capsules	12.28 tablets	18.96 tablets
6	4.130 ~0.67 g	5.54 ~1.1 g	6.710 ~0.4 g	11.99 ~2.2 g	19.26 ~0.2 g
7	3.95 dragees	5.19 capsules	6.33 capsules	11.28 tablets	
7	3.82 ~9.1 g	5.04 ~0.5 g	6.12 ~0.22 g	11.07 ~0.4 g	
8	3.92 effervescent tablet		7.03 tablets		
8	3.95 ~9.5 g		7.17 ~0.5 g		
9			8.17 capsules		
9			8.12 ~0.5 g		
10			7.46 tablets		
10			7.47 ~1.0 g		
11			7.57 capsules		
11			7.68 0.16 g		
12			8.03 capsules		
12			7.93 ~0.33 g		
Cell averages					
1	4.705	5.810	7.800	8.540	19.725
2	4.670	5.725	8.110	10.480	18.240
3	4.695	5.750	6.515	9.455	20.845
4	4.095	5.570	7.375	11.475	20.655
5	4.855	6.130	6.440	12.165	17.045
6	4.140	5.525	6.815	12.135	19.110
7	3.885	5.115	6.225	11.175	
8	3.935		7.100		
9			8.145		
10			7.465		
11			7.625		
12			7.980		
Cell ranges					
1	0.090	0.020	0.040	0.040	0.050
2	0.060	0.050	0.120	0.080	0.060
3	0.130	0.060	0.210	0.130	0.050
4	0.010	0.100	0.030	0.270	0.050
5	0.070	0.180	0.100	0.190	0.150
6	0.020	0.030	0.210	0.290	0.300
7	0.130	0.150	0.210	0.210	
8	0.030		0.140		
9			0.050		
10			0.010		
11			0.110		
12			0.100		
Sum(w^2)	0.052	0.072	0.204	0.262	0.124

Table 13 (*Continued*)

Sample *i*	4 mg/unit	5–6 mg/unit	7–8 mg/unit	9–10 mg/unit	17–20 mg/unit
Cochran's test					
Test value	0.326	0.448	0.217	0.321	0.728
Results (*n*)/cell	2	2	2	2	2
Critical value 1%	0.797	0.838	0.653	0.838	0.883
Critical value 5%	0.68	0.727	0.541	0.727	0.781
	No outliers	No outliers	No outliers	No outliers	No outliers
Computation of repeatability					
T1	34.980	39.625	87.595	75.425	115.620
T2	154.0461	224.8849	644.4147	823.9607	2238.6404
T3	0.0518	0.0723	0.2035	0.2621	0.1236
s_r^2	0.0032375	0.0051643	0.0084792	0.0187214	0.0103000
s_r	0.05690	0.07186	0.09208	0.13683	0.10149
% s_r	1.3013	1.2695	1.2615	1.2698	0.5267
r	0.159	0.201	0.258	0.383	0.284
Mandel's k consistency statistic					
1	1.118	0.197	0.307	0.207	0.348
2	0.746	0.492	0.921	0.413	0.418
3	1.616	0.590	1.613	0.672	0.348
4	0.124	0.984	0.230	1.395	0.348
5	0.870	1.771	0.768	0.982	1.045
6	0.249	0.295	1.613	1.499	2.090*
7	1.616	1.476	1.613	1.085	
8	0.373		1.075		
9			0.384		
10			0.077		
11			0.845		
12			0.768		
k (1%)	2.25	2.2	2.36	2.2	2.14
k (5%)	1.88	1.87	1.92	1.87	1.85

Table 14
Repatability calculations for different samples, divided in different ranges of amount per unit: nicotinamide

Sample *i*	7–9 mg/unit	7–9 mg/unit	15–18 mg/unit	16–18 mg/unit	CIT95003
1	8.88 effervescent tablet	6.98 capsules	16.39 capsules	19.32 capsules	9.21
1	8.69 6.8 g	7.00 0.5 g	16.17 1.25 g	19.97 1.32 g	9.16
2	9.27 effervescent tablet	7.86 tablets	18.95 tablets	19.12 capsules	9.34
2	9.43 6.8 g	7.73 2.6 g	19.03 0.7 g	19.84 0.3 g	9.36
3	9.48 effervescent tablet	8.88 effervescent tablet	16.87 capsules	16.27 capsules	8.84
3	9.51 9.0 g	8.69 6.8 g	17.04 1.2 g	16.62 1.37 g	9.04
4	9.61 effervescent tablet	9.27 effervescent tablet	18.97 tablets	17.94 tablets	9.13
4	9.68 9.0 g	9.43 6.8 g	19.01 1.0 g	17.64 1.5 g	9.17
5	9.54 effervescent tablet	9.48 effervescent tablet	19.50 capsules	19.93 effervescent tablet	9.47
5	9.52 9.0 g	9.51 9.0 g	19.72 1.25 g	19.47 4.3 g	9.42
6	9.50 effervescent tablet	9.98 solution	17.43 effervescent tablet	18.14 tablets	9.16
6	9.48 9.0 g	9.95 2.0 ml	17.43 4.3 g	18.32 1.26 g	8.99
7	9.46 effervescent tablet	9.61 effervescent tablet	15.55 effervescent tablet	19.87 tablets	9.24
7	9.52 9.0 g	9.68 9.0 g	15.33 5.0 g	19.83 1.2 g	9.47
8		9.54 effervescent tablet	15.37 effervescent tablet	17.25 tablets	
8		9.52 9.0 g	15.42 5.0 g	17.41 1.1 g	
9		8.98 solution	19.93 effervescent tablet	18.16 tablets	
9		8.96 2.0 ml	19.47 4.3 g	18.35 1.1 g	
10		9.28 solution	20.93 tablets	17.63 capsules	
10		9.25 2.0 ml	20.58 1.5 g	17.67 1.0 g	
11		9.50 effervescent tablet	18.22	19.81 tablets	
11		9.48 9.0 g	18.42	19.59 0.65 g	
12		9.46 effervescent tablet	19.87 tablets	17.80 capsules	
12		9.52 9.0 g	19.83 1.2 g	17.89 1.0 g	
13			18.21 tablets	20.59 tablets	
13			18.41 1.2 g	20.95 0.6 g	
14			20.02 effervescent tablet	18.48 tablets	
14			19.91 2.4 g	18.28 0.7 g	
15				18.71 effervescent tablet	
15				19.06 3.0 g	
16				19.72 tablets	
16				19.63 0.75 g	
Cell averages					
1	8.785	6.990	16.280	19.645	9.185
2	9.350	7.795	18.990	19.480	9.350
3	9.495	8.785	16.955	16.445	8.940
4	9.645	9.350	18.990	17.790	9.150
5	9.530	9.495	19.610	19.700	9.445
6	9.490	9.965	17.430	18.230	9.075
7	9.490	9.645	15.440	19.850	9.355
8		9.530	15.395	17.330	

Table 14 (*Continued*)

Sample *i*	7–9 mg/unit	7–9 mg/unit	15–18 mg/unit	16–18 mg/unit	CIT95003
9		8.970	19.700	18.255	
10		9.265	20.755	17.650	
11		9.490	18.320	19.700	
12		9.490	19.850	17.845	
13			18.310	20.770	
14			19.965	18.380	
15				18.885	
16				19.675	
Cell ranges					
1	0.190	0.020	0.220	0.650	0.050
2	0.160	0.130	0.080	0.720	0.020
3	0.030	0.190	0.170	0.350	0.200
4	0.070	0.160	0.040	0.300	0.040
5	0.020	0.030	0.220	0.460	0.050
6	0.020	0.030	0.000	0.180	0.170
7	0.060	0.070	0.220	0.040	0.230
8		0.020	0.050	0.160	
9		0.020	0.460	0.190	
10		0.030	0.350	0.040	
11		0.020	0.200	0.220	
12		0.060	0.040	0.090	
13			0.200	0.360	
14			0.110	0.200	
15				0.350	
16				0.090	
Sum(w^2)	0.072	0.091	0.612	1.819	0.129
Cochran's test					
Test value	0.502	0.395	0.346	0.285	0.411
Results (*n*)/cell	2	2	2	2	2
Critical value 1%	0.838	0.653	0.599	0.553	0.838
Critical value 5%	0.727	0.541	0.492	0.452	0.727
Day *i*/level *j*	No outliers	No outliers	No outliers	No outliers	No outliers
Computation of repeatability					
T1	65.785	108.770	255.990	299.630	64.500
T2	618.7209	993.9454	4719.8695	5631.3606	594.5125
T3	0.0719	0.0914	0.6124	1.8190	0.1288
s_r^2	0.0051357	0.0038083	0.0218714	0.0568437	0.0092000
s_r	0.07166	0.06171	0.14789	0.23842	0.09592
% s_r	0.7626	0.6808	0.8088	1.2731	1.0410
r	0.201	0.173	0.414	0.667	0.268
Mandel's k consistency statistic					
1	1.875*	0.229	1.052	1.928	0.369
2	1.579	1.490	0.383	2.135*	0.147
3	0.296	2.177*	0.813	1.038	1.474
4	0.691	1.833	0.191	0.890	0.295
5	0.197	0.344	1.052	1.364	0.369
6	0.197	0.344	0.000	0.534	1.253
7	0.592	0.802	1.052	0.119	1.696
8		0.229	0.239	0.475	
9		0.229	2.199*	0.564	

Table 14 (*Continued*)

Sample *i*	7–9 mg/unit	7–9 mg/unit	15–18 mg/unit	16–18 mg/unit	CIT95003
10		0.344	1.673	0.119	
11		0.229	0.956	0.652	
12		0.687	0.191	0.267	
13			0.956	1.068	
14			0.526	0.593	
15				1.038	
16				0.267	
k (1%)	2.2	2.36	2.39	2.42	2.2
k (5%)	1.87	1.92	1.92	1.93	1.87

Table 15
Repatability calculations for different samples, divided in different ranges of amount per unit: pyridoxine·HCl

Sample *i*	2–2.5 mg/unit	3–3.5 mg/unit	5–6 mg/unit	1–1.5 mg/unit	Matrix 2 (3)
1	2.06	3.32	5.41 capsules	1.39 effervescent tablet	1.36 effervescent tablet
1	2.01	3.19	5.50 0.2 g	1.37 9.0 g	1.35 9.0 g
2	2.46	3.08	6.18 tablets	1.05 solution	1.51
2	2.31	3.08	6.26 0.6 g	1.04 2.0 ml	1.53
3	2.27	3.50	6.00 tablets	1.08	1.39
3	2.20	3.37	6.09 0.26 g	1.06	1.40
4	2.13	3.01	6.72 tablets	1.21 effervescent tablet	1.50
4	2.20	2.93	6.84 0.7 g	1.23 9.0 g	1.50
5	2.36	3.14	5.41 tablets	1.70	1.48
5	2.34	3.03	5.50 0.2 g	1.69	1.52
6	2.16	3.43	6.30 tablets	1.84	1.42
6	2.14	3.57	6.26 0.26 g	1.85	1.39
7	2.93 tablets	3.55	5.99 tablets	1.93	1.48
7	2.90 1.25 g	3.55	6.08 0.26 g	1.90	1.52
8	2.93 tablets	3.37	6.29 capsules	1.33 tablets	
8	2.89 1.25 g	3.40	6.34 0.2 g	1.29 1.11 g	
9	2.01	3.39	6.20 capsules	1.24 effervescent tablet	
9	2.14	3.39	6.28 0.6 g	1.25 9.0 g	
10	2.21	3.54	5.44 capsules		
10	2.27	3.55	5.52 0.2 g		
11	2.07				
11	2.11				
12	2.41				
12	2.45				
13	2.59 capsules				
13	2.59 0.25 g				
14	2.67 effervescent tablet				
14	2.66 4.31 g				
15	2.81 tablets				
15	2.67 2.1 g				
16	2.51 capsules				
16	2.40 1.38 g				
Cell averages					
1	2.035	3.255	5.455	1.380	1.355
2	2.385	3.080	6.220	1.045	1.520
3	2.235	3.435	6.045	1.070	1.395
4	2.165	2.970	6.780	1.220	1.500
5	2.350	3.085	5.455	1.695	1.500
6	2.150	3.500	6.280	1.845	1.405
7	2.915	3.550	6.035	1.915	1.500
8	2.910	3.385	6.315	1.310	
9	2.075	3.390	6.240	1.245	
10	2.240	3.545	5.478		
11	2.090				
12	2.430				
13	2.590				
14	2.665				
15	2.740				
16	2.455				
Cell ranges					
1	0.050	0.130	0.090	0.020	0.010

Table 15 (*Continued*)

Sample *i*	2–2.5 mg/unit	3–3.5 mg/unit	5–6 mg/unit	1–1.5 mg/unit	Matrix 2 (3)
2	0.150	0.000	0.080	0.010	0.020
3	0.070	0.130	0.090	0.020	0.010
4	0.070	0.080	0.120	0.020	0.000
5	0.020	0.110	0.090	0.010	0.040
6	0.020	0.140	0.040	0.010	0.030
7	0.030	0.000	0.090	0.030	0.040
8	0.040	0.030	0.050	0.040	
9	0.130	0.000	0.080	0.010	
10	0.060	0.010	0.084		
11	0.040				
12	0.040				
13	0.000				
14	0.010				
15	0.140				
16	0.110				
Sum(w^2)	0.094	0.073	0.071	0.004	0.005
Cochran's test					
Test value	0.240	0.269	0.204	0.390	0.340
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.553	0.718	0.718	0.754	0.838
Critical value 5%	0.452	0.602	0.602	0.638	0.727
	No outliers	No outliers	No outliers	No outliers	No outliers
Computation of repeatability					
T1	38.430	33.195	60.303	12.725	10.175
T2	93.5634	110.5886	365.3978	18.8401	14.8165
T3	0.0936	0.0729	0.0708	0.0041	0.0047
s_r^2	0.0029250	0.0036450	0.0035378	0.0002278	0.0003357
s_r	0.05408	0.06037	0.05948	0.01509	0.01832
% s_r	2.2517	1.8188	0.9863	1.0674	1.2605
r	0.151	0.169	0.166	0.042	0.051
Mandel's k consistency statistic					
1	0.654	1.523	1.070	0.937	0.386
2	1.961*	0.000	0.951	0.469	0.772
3	0.915	1.523	1.070	0.937	0.386
4	0.915	0.937	1.427	0.937	0.000
5	0.261	1.288	1.070	0.469	1.544
6	0.261	1.640	0.476	0.469	1.158
7	0.392	0.000	1.070	1.406	1.544
8	0.523	0.351	0.594	1.874	
9	1.700	0.000	0.951	0.469	
10	0.784	0.117	0.999		
11	0.523				
12	0.523				
13	0.000				
14	0.131				
15	1.830				
16	1.438				
k (1%)	2.42	2.32	2.32	2.29	2.2
k (5%)	1.93	1.9	1.9	1.9	1.87

Table 16
Repatability calculations for different samples, divided in different ranges of amount per unit: riboflavine

Sample *i*	1.6 mg/unit	2.4 mg/unit	5.0 mg/uni	Matrix 2 (2)	Matrix 2 (3)
1	1.69 tablets	2.35 tablets	5.09 capsules	1.27	1.18
1	1.53 9.0 g	2.42 1.25 g	5.34 0.2 g	1.23	1.22
2	1.62 capsules	2.57 capsules	5.67 capsules	1.31	1.38
2	1.74 0.32 g	2.59 0.25 g	5.69 0.2 g	1.28	1.38
3	1.77 tablets	2.29 tablets	5.54 capsules	1.28	1.31
3	1.78 9.1 g	2.27 1.2 g	5.44 0.2 g	1.27	1.32
4	1.38 tablets	2.53 capsules	5.06 capsules	1.31	1.30
4	1.36 1.11 g	2.55 0.25 g	5.30 0.2 g	1.30	1.32
5	1.59	2.33 tablets	5.91 capsules	1.31	1.33
5	1.60	2.41 1.25 g	5.73 0.6 g	1.30	1.34
6	1.82	2.53 capsules	5.25 tablets	1.28	1.29
6	1.86	2.46 0.25 g	5.54 0.26 g	1.28	1.26
7	1.67	2.31	5.86 tablets	1.35	1.33
7	1.60	2.33	5.69 0.6 g	1.34	1.38
8	1.72	2.22	5.25 tablets		
8	1.64	2.22	5.55 0.26 g		
9	1.99	2.23			
9	1.96	2.14			
10	1.74	2.28			
10	1.75	2.34			
11	1.75	2.34			
11	1.79	2.40			
12	1.66	2.61			
12	1.66	2.56			
13	1.76	2.62			
13	1.76	2.66			
14	1.83				
14	1.83				
15	1.33				
15	1.51				
16	1.94				
16	1.96				
17	1.49				
17	1.54				
Cell averages					
1	1.610	2.385	5.215	1.250	1.200
2	1.680	2.580	5.680	1.295	1.380
3	1.775	2.280	5.490	1.275	1.315
4	1.370	2.540	5.180	1.305	1.310
5	1.595	2.370	5.820	1.305	1.335
6	1.840	2.495	5.395	1.280	1.275
7	1.635	2.320	5.775	1.345	1.355
8	1.680	2.220	5.400		
9	1.975	2.185			
10	1.745	2.310			
11	1.770	2.370			
12	1.660	2.585			
13	1.760	2.640			
14	1.830				
15	1.420				
16	1.950				
17	1.515				
Cell ranges					
1	0.160	0.070	0.250	0.040	0.040
2	0.120	0.020	0.020	0.030	0.000
3	0.010	0.020	0.100	0.010	0.010
4	0.020	0.020	0.240	0.010	0.020
5	0.010	0.080	0.180	0.010	0.010

Table 16 (*Continued*)

Sample *i*	1.6 mg/unit	2.4 mg/unit	5.0 mg/uni	Matrix 2 (2)	Matrix 2 (3)
6	0.040	0.070	0.290	0.000	0.030
7	0.070	0.020	0.170	0.010	0.050
8	0.080	0.000	0.300		
9	0.030	0.090			
10	0.010	0.060			
11	0.040	0.060			
12	0.000	0.050			
13	0.000	0.040			
14	0.000				
15	0.180				
16	0.020				
17	0.050				
Sum(w^2)	0.091	0.037	0.366	0.003	0.006
Cochran's test					
Test value	0.354	0.218	0.246	0.552	0.446
Results (n)/cell	2	2	2	2	2
Critical value 1%	0.532	0.624	0.797	0.838	0.838
Critical value 5%	0.434	0.515	0.68	0.727	0.727
	No outliers	No outliers	No outliers	No outliers	No outliers
Computation of repeatability					
T1	28.810	31.280	43.955	9.055	9.170
T2	49.2621	75.5264	241.9202	11.7186	12.0336
T3	0.0914	0.0372	0.3659	0.0029	0.0056
s_r^2	0.0026882	0.0014308	0.0228688	0.0002071	0.0004000
s_r	0.05185	0.03783	0.15122	0.01439	0.02000
% s_r	3.0594	1.5720	2.7523	1.1126	1.5267
r	0.145	0.106	0.42	0.0403	0.056
Mandel's k consistency statistic					
1	2.182*	1.309	1.169	1.965*	1.414
2	1.637	0.374	0.094	1.474	0.000
3	0.136	0.374	0.468	0.491	0.354
4	0.273	0.374	1.122	0.491	0.707
5	0.136	1.496	0.842	0.491	0.354
6	0.546	1.309	1.356	0.000	1.061
7	0.955	0.374	0.795	0.491	1.768
8	1.091	0.000	1.403		
9	0.409	1.682			
10	0.136	1.122			
11	0.546	1.122			
12	0.000	0.935			
13	0.000	0.748			
14	0.000				
15	2.455**				
16	0.273				
17	0.682				
k (1%)	2.44	2.38	2.25	2.2	2.2
k (5%)	1.93	1.92	1.88	1.87	1.87

Table 17
Repatability calculations for different samples, divided in different ranges of amount per unit: thiamine mononitrate

Sample *i*	1.5–2 mg/unit	2–2.5 mg/unit	0.5–1 mg/unit	5–7 mg/unit
1	1.75 capsules	2.68 capsules	1.07 tablets	6.45 capsules
1	1.67 0.6 g	2.71 0.6 g	1.07 0.6 g	6.48 0.6 g
2	1.51 capsules	2.44 capsules	0.49 tablets	6.44 tablets
2	1.46 1.1 g	2.37 1.0 g	0.50 0.5 g	6.45 0.6 g
3	1.66 capsules	2.61 tablets	0.86 tablets	5.35 capsules
3	1.56 1.4 g	2.70 0.5 g	0.87 1.0 g	5.36 0.4 g
4	1.59 effervescent tablet	2.06 tablets	0.53 capsules	5.15 tablets
4	1.63 3.0 g	2.04 0.75 g	0.55 0.5 g	5.23 0.27 g
5	1.87 tablets	2.05 capsules	0.85 effervescent tablet	5.16 tablets
5	1.84 1.0 g	2.03 0.7 g	0.83 6.8 g	5.24 0.35 g
6	1.90 tablets	2.68 capsules	0.99 capsules	
6	1.90 0.65 g	2.71 0.5 g	0.98 0.6 g	
7	1.67 capsules	2.34 syrup	1.12 effervescent tablet	
7	1.68 0.7 g	2.33 8.0 g	1.09 9.0 g	
8	1.65 tablets	2.48 tablets	0.79 effervescent tablet	
8	1.66 0.7 g	2.46 1.2 g	0.81 9.1 g	
9	1.54 capsules	2.00 tablets	1.11 effervescent tablet	
9	1.59 0.3 g	2.01 1.5 g	1.08 9.0 g	
10	1.87 syrup	2.26 effervescent tablet	0.80 effervescent tablet	
10	1.89 7.5 g	2.18	0.81 9.0 g	
11	1.59 effervescent tablet	1.97 capsules		
11	1.59 5.0 g	1.93 1.2 g		
12		2.03 capsules		
12		2.04 0.26 g		
13		2.01 capsules		
13		2.02 2.6 g		
14		2.45 tablets		
14		2.45 1.1 g		
15		2.15 solution		
15		2.10 2.0 ml		
16		1.91 capsules		
16		1.93 1.2 g		
17		2.23 capsules		
17		2.24 1.25 g		
Cell averages				
1	1.710	2.695	1.070	6.465
2	1.485	2.405	0.495	6.445
3	1.610	2.655	0.865	5.355
4	1.610	2.050	0.540	5.190
5	1.855	2.040	0.840	5.200
6	1.900	2.695	0.985	
7	1.675	2.335	1.105	
8	1.655	2.470	0.800	
9	1.565	2.005	1.095	
10	1.880	2.220	0.805	
11	1.590	1.950		
12		2.035		
13		2.015		
14		2.450		
15		2.125		
16		1.920		
17		2.235		
Cell ranges				
1	0.080	0.030	0.000	0.030
2	0.050	0.070	0.010	0.010
3	0.100	0.090	0.010	0.010
4	0.040	0.020	0.020	0.080
5	0.030	0.020	0.020	0.080

Table 17 (*Continued*)

Sample *i*	1.5–2 mg/unit	2–2.5 mg/unit	0.5–1 mg/unit	5–7 mg/unit
6	0.000	0.030	0.010	
7	0.010	0.010	0.030	
8	0.010	0.020	0.020	
9	0.050	0.010	0.030	
10	0.020	0.080	0.010	
11	0.000	0.040		
12		0.010		
13		0.010		
14		0.000		
15		0.050		
16		0.020		
17		0.010		
Sum(w^2)	0.025	0.027	0.003	0.014
Cochran's test				
Test value	0.408	0.296	0.265	0.460
Results (n)/cell	2	2	2	2
Critical value 1%	0.684	0.532	0.718	0.928
Critical value 5%	0.57	0.434	0.602	0.841
	No outliers	No outliers	No outliers	No outliers
Computation of repeatability				
T1	18.535	38.300	8.600	28.655
T2	31.4209	87.4285	7.8137	165.9864
T3	0.0245	0.0274	0.0034	0.0139
s_r^2	0.0011136	0.0008059	0.0001700	0.0013900
s_r	0.03337	0.02839	0.01304	0.03728
% s_r	1.9805	1.2600	1.5161	0.6505
r	0.093	0.079	0.036	0.104
Mandel's k consistency statistic				
1	1.695	0.747	0.000	0.569
2	1.059	1.744	0.542	0.190
3	2.119*	2.242*	0.542	0.190
4	0.848	0.498	1.085	1.517
5	0.636	0.498	1.085	1.517
6	0.000	0.747	0.542	
7	0.212	0.249	1.627	
8	0.212	0.498	1.085	
9	1.059	0.249	1.627	
10	0.424	1.993*	0.542	
11	0.000	0.996		
12		0.249		
13		0.249		
14		0.000		
15		1.245		
16		0.498		
17		0.249		
k (1%)	2.34	2.44	2.32	2.05
k (5%)	1.91	1.93	1.9	1.81

Table 18
A repatability calculations for different samples, divided in different ranges of amount per unit: ascorbic acid (selective method)

Day	30 mg/unit	Sample number	Level	
			60 mg/unit	90 mg/unit
Original data				
1	31.21	1	57.97	101.15
1	29.83	1	56.69	97.71
2	30.35	2	67.45	95.94
2	28.92	2	65.60	94.05
3	31.27	3	62.66	88.03
3	29.82	3	63.24	85.03
4	29.13	4	62.77	94.13
4	28.08	4	61.05	90.93
5	28.89	5	56.99	92.33
5	28.11	5	56.03	92.28
6	30.36	6	61.99	103.09
6	28.89	6	61.16	98.27
7	30.34	7	57.02	99.21
7	28.95	7	55.98	99.16
8	29.01	8	56.96	
8	28.09	8	56.08	
9	62.03			
9	61.23			
10	61.95			
10	61.09			
11	66.06			
11	66.30			
12	58.37			
12	57.07			
13	57.57			
13	56.31			
Cell averages				
1	30.520	1	57.330	99.430
2	29.635	2	66.525	94.995
3	30.545	3	62.950	86.530
4	28.605	4	61.910	92.530
5	28.500	5	56.510	92.305
6	29.625	6	61.575	100.68
7	29.645	7	56.500	99.185
8	28.550	8	56.520	
		9	61.630	
		10	61.520	
		11	66.180	
		12	57.720	
		13	56.940	
Grubbs' test (30 mg/unit)[a]				
Gp (single high)	1.309			
Gp (single low)	1.142			
Gp (double high)	0.362			
Gp (double low)	0.528			
Straggler (sin $l+h$)	2.126			
Outlier (sin $l+h$)	2.274			
Straggler (dbl $l+h$)	0.1101			
Outlier (dbl $l+h$)	0.0563			

Table 18 (*Continued*)

Day	30 mg/unit	Sample number	Level	
			60 mg/unit	90 mg/unit
Cell ranges				
1	1.380	1	1.280	3.440
2	1.430	2	1.850	1.890
3	1.450	3	0.580	3.000
4	1.050	4	1.720	3.200
5	0.780	5	0.960	0.050
6	1.470	6	0.830	4.820
7	1.390	7	1.040	0.050
8	0.920	8	0.880	
9		9	0.800	
10		10	0.860	
11		11	0.240	
12		12	1.300	
13		13	1.260	
Sum(w^2)	12.702		16.537	57.883
Cochran's test				
Test value	0.170		0.207	0.401
Result (n)/cell	2		2	2
Critical value 1%	0.797		0.624	0.838
Critical value 5%	0.68		0.515	0.727
	No outliers	No outliers	No outliers	
Computation of precision components				
T1	235.625		783.810	665.655
T2	6944.7658		47 415.9980	63 453.9564
T3	12.7021		16.5370	57.8831
s_r^2	0.7938813	0.6360385	4.1345071	
s_D^2	0.2992375			
s_R^2	1.0931187			
m	29.45313	60.29308	95.09357	
s_r	0.89100		0.79752	2.03335
s_R	1.04552			
% s_r	3.0251		1.3227	2.1383
% s_R	3.5498			
r	2.495		2.23306	5.69338
R	2.9275			
Mandel's h consistency statistic				
1	1.279			
2	0.218			
3	1.309			
4	−1.016			
5	−1.142			
6	0.206			
7	0.230			
8	−1.082			
h (1%)	2.060			
	−2.060			
h (5%)	1.750			
	−1.750			

Table 18 (*Continued*)

Day	30 mg/unit	Sample number	Level	
			60 mg/unit	90 mg/unit
Mandel's k consistency statistic				
1	1.095	1	1.135	1.196
2	1.135	2	1.640	0.657
3	1.151	3	0.514	1.043
4	0.833	4	1.525	1.113
5	0.619	5	0.851	0.017
6	1.167	6	0.736	1.676
7	1.103	7	0.922	0.017
8	0.730	8	0.780	
		9	0.709	
		10	0.763	
		11	0.213	
		12	1.153	
		13	1.117	
k (1%)	2.25		2.38	2.2
k (5%)	1.88		1.92	1.87
U_x	1.669			

Table 19
Repeatability calculations for different samples, divided in different ranges of amount per unit: α-tocopherol acetate

Sample *i*	5 mg/unit	10 mg/unit	15 mg/unit	30 mg/unit
1	5.14 tablets	11.37 tablets	13.92 capsules	32.00 tablets
1	4.97 ∼0.4 g	11.55 ∼0.74 g	13.92 ∼1.77 g	31.27 ∼0.16 g
2	6.85 effervescent tablet	10.17 capsules	15.02 capsules	34.77 tablets
2	6.93 ∼4.5 g	10.18 ∼0.93 g	14.66 ∼0.1 g	34.23 ∼0.63 g
3	4.85 solution	11.77 capsules	16.69 capsules	31.97 capsules
3	4.97 2.00 ml	11.55 ∼0.3 g	16.82 ∼0.20 g	33.01 ∼0.14 g
4	5.33 effervescent tablet	11.55 effervescent tablet	15.32 capsules	
4	5.51 ∼4.6 g	12.16 ∼4.48 g	15.58 ∼0.25 g	
5	5.27 dragées	10.69 solution	16.95 tablets	
5	5.28 ∼0.75 g	9.75 ∼6.0 ml	17.03 ∼0.29 g	
6	5.73 gelulen	11.99 tablets	15.60 effervescent tablet	
6	5.82 ∼0.31 g	11.42 ∼0.64 g	15.48 ∼5.95 g	
7	5.14 tablets	10.23 effervescent tablet	16.30 capsules	
7	4.95 ∼0.4 g	10.24 ∼5 g	15.40 ∼0.26 g	
8		11.50 tablets	13.93 capsules	
8		11.51 ∼0.74 g	13.89 ∼1.2 g	
9		10.42 effervescent tablet	14.44 tablets	
9		10.24 ∼2.43 g	14.81 ∼1.7 g	
10		11.16 capsules	13.68 capsules	
10		11.46 ∼0.4 g	13.80 ∼1.2 g	
11		9.31 capsules	13.94 capsules	
11		9.25 ∼0.53 g	13.89 ∼1.5 g	
12		10.17 capsules	14.64 capsules	
12		10.17 ∼0.9 g	14.38 ∼1.17 g	
13		9.90 capsules		
13		10.03 ∼0.7 g		
Cell averages				
1	5.055	11.460	13.920	31.635
2	6.890	10.175	14.840	34.500
3	4.910	11.660	16.755	32.490
4	5.420	11.855	15.450	
5	5.275	10.220	16.990	
6	5.775	11.705	15.540	
7	5.045	10.235	15.850	
8		11.505	13.910	
9		10.330	14.625	
10		11.310	13.740	
11		9.280	13.915	
12		10.170	14.510	
13		9.965		
Cell ranges				
1	0.170	0.180	0.000	0.730
2	0.080	0.010	0.360	0.540
3	0.120	0.220	0.130	1.040
4	0.180	0.610	0.260	
5	0.010	0.940	0.080	
6	0.090	0.570	0.120	
7	0.190	0.010	0.900	
8		0.010	0.040	
9		0.180	0.370	
10		0.300	0.120	

Table 19 (*Continued*)

Sample *i*	5 mg/unit	10 mg/unit	15 mg/unit	30 mg/unit
11		0.060	0.050	
12		0.000	0.260	
13		0.130		
Sum(w^2)	0.126	1.805	1.268	1.906
Cochran's test				
Test value	0.286	0.490	0.639	0.567
Results (n)/cell	2	2	2	2
Critical value 1%	0.838	0.624	0.653	0.993
Critical value 5%	0.727	0.515	0.541	0.967
	No outliers	No outliers	Straggler	No outliers
Computation of repeatability				
T1	38.370	139.870	180.045	98.625
T2	213.1379	1513.4081	2715.1324	3246.6233
T3	0.1264	1.8046	1.2679	1.9061
s_r^2	0.0090286	0.0694077	0.0528292	0.3176833
s_r	0.09502	0.26345	0.22985	0.56363
% s_r	1.7335	2.4486	1.5319	1.7145
r	0.266	0.738	0.643	1.578
Mandel's k consistency statistic				
1	1.265	0.483	0.000	0.916
2	0.595	0.027	1.108	0.677
3	0.893	0.590	0.400	1.305
4	1.340	1.637	0.800	
5	0.074	2.523**	0.246	
6	0.670	1.530	0.369	
7	1.414	0.027	2.769**	
8		0.027	0.123	
9		0.483	1.138	
10		0.805	0.369	
11		0.161	0.154	
12		0.000	0.800	
13		0.349		
k (1%)	2.2	2.38	2.36	1.71
k (5%)	1.87	1.92	1.92	1.65

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