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Application of Mean Centering Ratio and Multiple Wavelength Methods for Determination of Four Component Level in Multivitamin Syrup by Ultraviolet Spectrophotometry

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Abstract: The significance and logic of simultaneous determination of vitamin B1, B2, B6, and niacin levels in multivitamin syrup using the mean centering ratio (MCR) method and the multiple wavelength method (MWM), without separation. This study aims to determine the use of the divisor factor in the mean centering ratio method and use five different wavelengths to measure multiple wavelengths. The research methods are carried out using methanol-water as a solvent, triple divisor for the MCR method, and five different wavelengths for MWM. The test of validation parameters is linearity, accuracy, precision, detection, and quantitation. The determination of vitamin B1, B2, B6 and niacin levels in multivitamin syrup for MCR method are (101.44 ± 4.35)%, (100.19 ± 4.09)%, (98.75 ± 0.47)%, and (100.75 ± 1.06)% respectively and for Multiple Wavelength method are (98.05 ± 1.28)%, (99.04 ± 1.17)%, (99.02 ± 1.00)%, and (99.61 ± 1.17)% respectively. The result for validation in MCR method with the parameter: linearity, accuracy, precision, LOD, and LOQ according to USP 43 requirement. The Multiple Wavelength method has a good validation with parameter accuracy and coefficient of variation (CV). The divisor factor system of the Mean Centering Ratio method and five different wavelengths for multiple Wavelengths can be used for the determination of vitamin B1, B2, B6, and niacin levels in multivitamin syrup and fulfilled the validation of the parameters.

Keywords: multivitamin syrup, determination, mean centering ratio, multiple wavelength, spectrophotometric.

紫外分光光度法測定複合維生素糖漿中四組分含量的平均中心比和多波長法的應用

摘要：使用平均中心比法和多波長法在不分離的情況下同時測定複合維生素糖漿中維生素乙1、乙2、乙6 和菸酸水平的意義和邏輯。本研究旨在確定平均中心比方法中除數因子的使用，並使用五種不同的波長來測量多個波長。研究方法是使用甲醇-水作為溶劑、平均中心比方法的三重除數和多波長法的五種不同波長進行的。驗證參數的測試是線性、準確度、精密度、檢測和定量。平均中心比法測定複合維生素糖漿中維生素乙1、乙2、乙6和菸酸含量分別為(101.44±4.35)%、(100.19±4.09)%、(98.75±0.47)%、丹(100.75±1.06)%和丹(100.75±1.06)%。多波長法分別為(98.05±1.28)%、(99.04±1.17)%、(99.02±1.00)%和(99.61±1.17)%。根據美國藥典43 要求，在平均中心比方法中使用參數進行驗證的結果：線性、準確度、精密度、檢測限和定量限。多波長方法在參數準確性和變異係數方面得到了很好的驗證。平均中心比法的除數因子系統和五個不同波長的多波長可用於測定複合維生素糖漿中的維生素乙1、乙2、乙6 和菸酸含量，並完成了參數的驗證。

关键词：多種維生素糖漿，測定，平均中心比，多波長，分光光度法。

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1. Introduction

Various types of vitamins that the body needs are essential nutrients derived from food. These nutrients are necessary for the body's vital physiological functions and contribute significantly to optimal health. The B-group (or B-complex) vitamins, specifically, assist with producing energy and the biosynthesis of many physiologically vital molecules in cells. They are water-soluble and are not stored in the body. Therefore, daily replenishment is possible through dietary intake [1].

As already mentioned, the B-complex vitamins are water-soluble, and the vitamins in this group are easily eliminated from the body via renal excretion. Typical members of this complex are thiamine (vitamin B1), riboflavin (vitamin B2), niacin, pantothenic acid, pyridoxine (vitamin B6), biotin, folic acid, and cyanocobalamin [2].

The multivitamin syrup is a pharmaceutical dosage form containing many matrices with different characteristics, which can cause interference when analyzed. Vitamin B1, B2, B6, and niacin in multivitamin syrup show an overlapped wavelength spectra in the maximum absorbance [3], [4].

There are some studies of determination of vitamin B_1, B_2, B_6, and lipoic acid levels in multivitamin capsules by HPLC. The other is the determination of syrup containing vitamins B1, B2, B3, and B6 by HPLC isocratic elution [5].

One of the main challenges in chemical analysis by spectrophotometry is determining two or more compounds in a sample without being separated, making it difficult to resolve overlapping spectra of multi-component mixture preparations [6].In the years, the use of UV spectrophotometry was generally used to analyze single components, but in recent years it has been used for multi-component compounds by providing several modifications of the method as an application to analyze multi-components

The modification of the UV spectrophotometry method for multi-component analysis without being separated and using the matrix calculation principle in the regression equation

The development of methods performed by a research method is the multiple wavelengths with absorption observation at some point of wavelengths [6], [7].

On the other hand, the mean centering ratio (MCR) is used to precisely remove the contribution of absorbing reagent from the data matrix. Therefore the absorbance of reagent(s) is exactly eliminated [8], [9]. The mean centering ratios have presented by Afkhami and Bahram is applied for simultaneous analysis of binary or ternary mixture. The MCR method has the advantage of eliminating derivative steps, and therefore the signal-to-noise is no degraded [10], [11].

This study aims to provide a new approach from a simple, fast, and low-cost MCR method by utilizing the operational wavelength of several overlapping wavelengths as the quaternion mixture resolution.

1.1. Hypothesis

It is suspected that the levels of each component of Vitamin B1, Vitamin B2, Vitamin B6, and niacin in multivitamin syrup can be determined simultaneously by:

1. The divisor factor system for determining vectors on the mean centering ratio method.
2. Measurements at five different wavelengths in overlapping absorption spectrum systems at multiple wavelengths methods

2. Materials and Methods

2.1. Apparatus

This study used a Shimadzu model 1800 double beam UV-Visible spectrophotometer and a personal computer with UV-Probe system software.

2.2. Material and Reagents

The study used pharmaceutical grade of vitamin B_1, B_2, B_6, and niacin, distilled water, analytical methanol, and syrup containing vitamin B_1, B_2, B_6, and niacin by PT Universal throughout.

2.3. Preparation of Standard Solution

Each of accurately weighed of standard B1, B6, and niacin (25 mg) was transferred to 25 ml volumetric flasks and dissolved in methanol-water (1:1) until the designed mark to provide a solution of 1000 μg/ml for B1, B6, and niacin (a standard solution I). Then, 2,5 ml of each solution was taken and inserted into a 25 ml volumetric flask to get a solution of 100 μg/ml (standard solution II).

An accurately weighed standard B2 (6 mg) was transferred to a 100 ml volumetric flask and dissolved in methanol-water (1:1) until the designed mark to give a solution of 60 μg/ml (a standard solution I). Then 25 ml of this solution was poured into a 50 ml volumetric flask to get a solution of 30 μg/ml (standard solution II) [3], [4].

2.4. Determination of Maximum Absorption Spectrum

The absorption spectrum of each prepared standard solution was measured at a wavelength of 200-400 nm, and Beer's law is applied in the determination [3].

2.5. The Mean Centering Ratio of Vitamin B_1, Vitamin B_2, Vitamin B_6, Niacin Absorbance with MCR method

The standard solution of Vitamin B1, Vitamin B2, Vitamin B6, Niacin absorbance spectrum was manipulated with UV Probe 2.42 software by dividing it with the first divisor to get the first ratio spectra as data sets. Data sets were printed and exported to Ms.

Excel for mean centering with the help of Matlab R2010b.

This vector was then divided by the mean-centered ratio of αB2/αB6 (second divisor) to get the second ratio spectra. The second vector is then divided by the mean-centered of Vitamin B1, Vitamin B2, Vitamin B6, Niacin third divisor) to get the third ratio spectra and was mean-centered to obtain the mean-centered ratio spectra [12], [13]. The divisors' concentration used in the study can be seen in Table 1.

Table 1 Data of triple divisor and the concentration

Vitamin	1st	2nd	3rd	C (µg/mL)
B1	α_{B6}	$MC\ [\frac{\alpha B2}{\alpha B6}]$	$MC\ \frac{MC\ [\frac{\alpha N}{\alpha B6}]}{MC\ [\frac{\alpha B2}{\alpha B6}]}$	21,6864
B2	α_{B1}	$MC\ [\frac{\alpha B6}{\alpha B1}]$	$MC\ \frac{MC\ [\frac{\alpha N}{\alpha B1}]}{MC\ [\frac{\alpha B6}{\alpha B1}]}$	17,4
B6	α_{N}	$MC\ [\frac{\alpha B1}{\alpha N}]$	$MC\ \frac{MC\ [\frac{\alpha B2}{\alpha N}]}{MC\ [\frac{\alpha B1}{\alpha N}]}$	18,4
N	α_{B1}	$MC\ [\frac{\alpha B6}{\alpha B1}]$	$MC\ \frac{MC\ [\frac{\alpha B2}{\alpha B1}]}{MC\ [\frac{\alpha B6}{\alpha B1}]}$	16

2.6. Construction of Calibration Curve

The calibration curve in the MCR method is slightly different from the conventional UV spectrophotometry method. In conventional UV spectrophotometry, the Y-axis represents the absorbance of the spectrum. However, in the MCR method, Y-axis stands as the amplitude gained from the mean-centered of third ratio spectra. Concentrations of each substance plot the amplitude to obtain the regression equation [14].

2.7. Determination of Vitamin B_1, B_2, B_6, and Niacin in Multivitamin Syrup with the MCR Method

The sample solution was prepared with the standard addition method. 5 ml of the syrup contains 3 mg of vitamin B1, 1.25 mg of vitamin B2, 1 mg of vitamin B6, and 10 mg of niacin. Then take some of the syrup equivalents to 10 mg of niacin, and then count the equality of vitamin B1, B2, B6. Put each of the equivalently counted vitamins into a 50 ml volumetric flask and diluted with methanol-water (1:1) until the designated mark. Pipetted 5 ml of solution was taken and moved into a 25 ml volumetric flask and diluted. Then 4 ml of the solution was pipetted and moved into a 25 ml volumetric flask. Then added each standard solution two of 2.85 ml vitamin B1, 5.2 ml of vitamin B2, and 2.4 ml of vitamin B6 as the addition. Then made up until the designed mark with methanol-water (1:1).

Then, the predetermined divisor manipulated the final prepared solution and got the mean-centered value (amplitude). Then the regression equation can calculate using the concentration versus amplitude.

2.8. Determination with Multiple Wavelength Method; Construction of Wavelength Used in Analysis

Vitamin B1 solution is prepared in 13.6544 µg/ml, vitamin B2 in 7.05 µg/ml, vitamin B6 in 10.5 µg/ml, and niacin in 7 µg/ml. These solutions' absorbance is measured at 200-400 nm. Overlaid the spectrums and determined five points of wavelength to be used.

2.9. Determination of Quaternion Mixture of Vitamin B1, B2, B6, and Niacin

The absorbance of the solutions of vitamin B1, B2, B6, and niacin in several concentrations are measured in the five predetermined wavelengths. The absorptivity value is determined by using a regression equation in the form of $y = ax + b$, y is the absorption value, a is the regression coefficient which showed the absorption, xis the concentration, while b is the constant [7].

2.10. Determination of Vitamin B1, B2, B6, and Niacin Levels in Multivitamin Syrup

Weighed carefully of the syrup equivalents to 10 mg of niacin, transferred it into a 50 ml volumetric flask, and diluted with methanol-water until the designated mark. Piped five ml a syrup sample, and transfer into a 25 ml volumetric flask, and diluted. Then 4 ml of the solution is pipetted and moved into a 25 ml volumetric flask. And added a standard solution for each 2.85 ml of vitamin B1, 5.2 ml of vitamin B2, and 2.4 ml of vitamin B6 as the addition. Then made up until the designed mark with methanol-water. Measure the absorption at five predetermined wavelengths [7].

2.11. Calculation of Vitamin B1, B2, B6, and Niacin Levels in Multivitamin Syrup

The calculation of each component's level on the mixture is conducted from the sample's absorbance and the absorbance of each component at five wavelengths by using the matrix equation [7]:

$$[c] = [[a] \times [a^1]]^{-1} \times [a] \times Ac]$$

where:
[c] = Concentration of the mixture
[a] = the absorption matrix of constituent compounds
$[a^1]$ = the transpose matrix of constituent compounds
$[[a]\ X\ [a^1]]^{-1}$ = the inverse value of absorption matrix multiply by the transpose matrix
[Ac] = the sample absorption matrix

3. Results and Discussion

3.1. The Maximum Absorption Spectrum Measurement

The maximum absorption spectrum is measured in the absorption region at a 200-400 nm wavelength and obtained vitamin B1 at 231,6 nm, vitamin B2 at 268,6 nm, vitamin B6 at 222,8 nm, and niacin 214,4 nm (Fig.

1 - Fig. 4). The overlaid maximum absorption spectrum can be seen in Fig. 5.

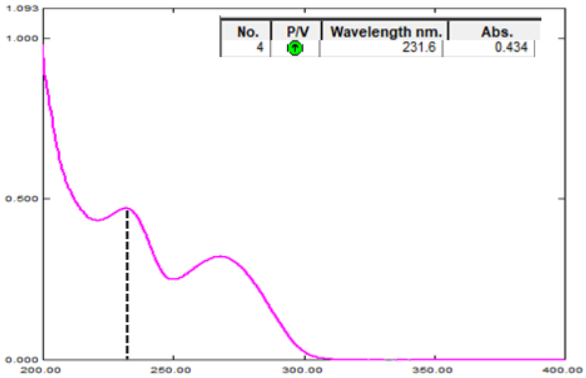

Fig. 1 Maximum absorption spectrum of vitamin B1

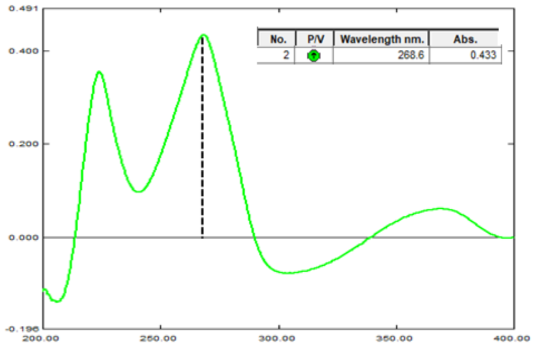

Fig. 2 Maximum absorption spectrum of vitamin B2

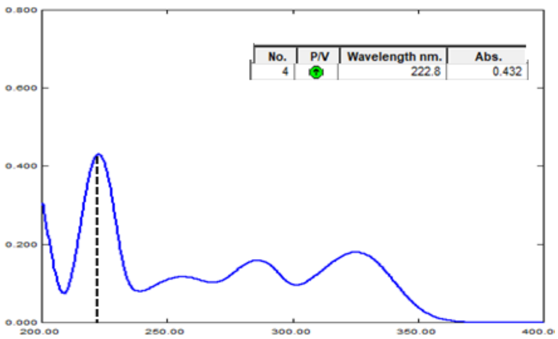

Fig. 3 Maximum absorption spectrum of vitamin B6

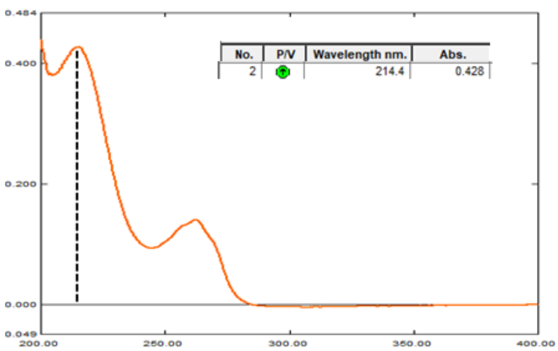

Fig. 4 Maximum absorption spectrum of niacin

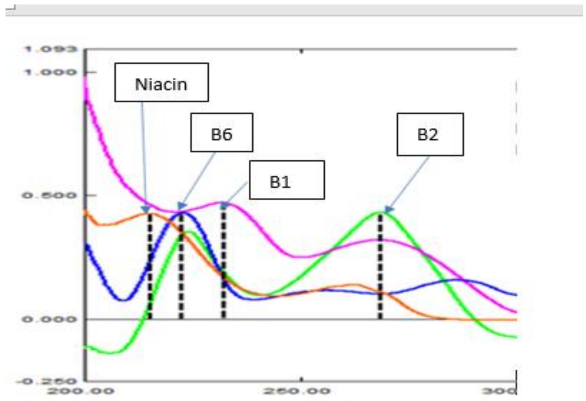

Fig. 5 Overlaid spectrum

3.2. The Selectivity of Wavelength and Divisor for the MCR Method

In MCR spectrophotometric method, the selectivity of wavelength affects the results of calculation of the levels of the active compounds in a pharmaceutical preparation [4]. The above results indicate that the wavelength selected for this study is 200-280 nm. Because these wavelength ranges of the compound absorption are still significant, some compounds have zero absorption in the range of 281-400 nm. Therefore, it can be abandoned or excluded to prevent disturbance in the process.

The divisor and the concentration used in a divisor are other important factors in determining the level of substances. The selection of divisors and the concentration was based on the calculation to give the best non-overlaid spectrum, as seen in Fig. 6.

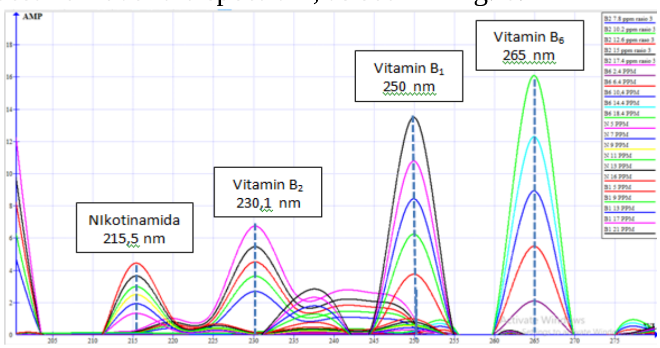

Fig. 6 Mean-centered third ratio spectra of vitamin B_1, B_2, B_6, and niacin

3.3. The Calibration Curve with the MCR Method

In the MCR method, the calibration curve has used a graph of concentration versus amplitude (mean centering results). Regression equation obtained for vitamin B1 is y = 0.611100x + 0.161522, vitamin B2 is y = 0.3646448x – 0.038194, vitamin B6 is y = 0.805607x – 0.030023, and niacin is y = 0.260926x – 0.012840.

The correlation coefficient (r) of vitamin B1 is 0.9994, vitamin B2 is 0.9997, vitamin B6 is 0.9998, and niacin is 0.9998. The value of the correlation coefficient, which is greater than 0.97 (>0.97), shows that there is a linear correlation between concentration and amplitude [14].

3.4. Result of Validation Test

The validation test with parameters calculated is linearity, accuracy, precision, LOD, and LOQ, as shown in Table 2.

Table 2 Validation tests result

Measurements	B1	B2	B6	N
Linierity	0,9994	0,9997	0,9998	0,9998
Accuracy (%)	100,34	100,28	99,85	100,60
Precision (%)	1,16	1,03	0,47	0,59
LOD	0,90	0,46	0,42	0,28
LOQ	3,01	1,55	1,41	0,95

Table 2 summarizes shown that the accuracy percentage is in the range of 80-120%. This method is accurate to be used in the study, and the value of precision is smaller than 2%.
This result shows that this method has good precision. All of the measurements' results were greater than LOD and LOQ. The concentration of LOQ is the minimum concentration of a substance to obtain the quantitative amplitude [15].

3.5. Determination of Vitamin B_1, B_2, B_6, and Niacin in Multivitamin Syrup with MCR Method

The concentration of vitamins B1, B2, B6, and niacin in the sample solution to be used, the method of adding a standard solution, and dilution of the sample solution is carried out so that the measured solution produces absorption in Lambert Beer's law region, will be converted into amplitude with Matlab software because the calculation of the regression equation in the MCR method is carried out between concentration and amplitude. The results of vitamin B1, B2, B6, and niacin levels in syrup can be seen in Table 3.

Table 3 Vitamins levels in multivitamin syrup

Vitamin	Levels (%)	Content in the label (mg)	Requirements (%)
B1	101,44 ± 4,35	3	95-115
B2	100,19 ± 4,09	1,25	95-115
B6	98,75 ± 0,47	1	95-115
N	100,75 ± 1,06	10	90-110

Table 3 shows that the assay results for vitamin B1, B2, B6 levels are (95-115)% and for niacin is (90-110)% in syrup preparations according to USP 43th edition.

3.6. Determination with Multiple Wavelength Analysis

In this study, the multiple wavelength method was used to determine the concentration of each substance in the mixture. Two or more wavelengths are used with the requirement that the selected wavelength is the wavelength in the overlapping mixture [15]. Another requirement is that each component still exhibits its absorptivity at the specified wavelength [7].

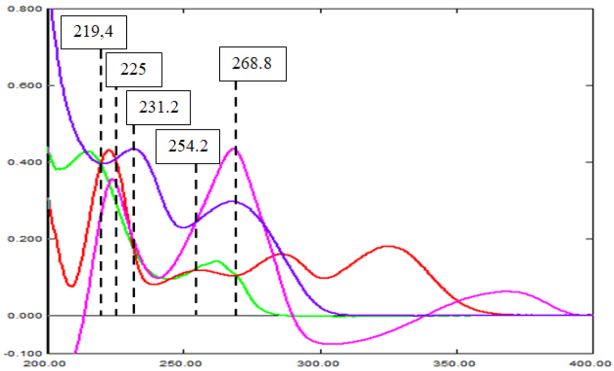

Fig. 7 Five wavelengths for the determination of vitamin B1, B2, B6, and niacin level

Fig. 7 shows that five wavelength points are chosen to be used in the assay by the principle of one maximum wavelength from one of the compounds and four points of the intersection. The other components are still giving the absorbance. The five wavelengths are 219.4 nm; 225.0 nm; 231.2 nm; 254.2 nm; and 268.8 nm.

3.7. Determination of Absorbance Spectrum in Five Wavelengths

The absorbance measurement of each at various concentrations at the five wavelengths, the absorbance value determined using the maximum absorption of each wavelength of the substance, and calculation in the linear regression equation must be analogous to Beer's law. The data of absorption value of each compound can be seen in Table 4, Table 5, Table 6, and Table 7. The data obtained is further used in determining the compound level by matrix operation.

Table 4 Data of vitamin B1 absorption

C (µg/ml)	219.4 nm	225 nm	231.2 nm	254.2 nm	268.8 nm
	Abs	Abs	Abs	Abs	Abs
0	0.0000	0.0000	0.0000	0.0000	0.0000
5.02	0.1882	0.1926	0.2067	0.1187	0.1401
9.63	0.3159	0.3432	0.3484	0.2030	0.2394
13.65	0.4582	0.4602	0.4895	0.2910	0.3342
17.67	0.5786	0.5930	0.6291	0.3689	0.4351
21.68	0.7294	0.7129	0.7606	0.4473	0.5446
a =	0.03337	0.0330	0.0351	0.0207	0.0249
b =	-0.00140	0.0055	0.00626	0.0025	-0.00123
r =	0.9997	0.9997	0.9998	0.9997	0.9998

Table 5 Data of vitamin B2 absorption

C (µg/ml)	219.4 nm	225 nm	231.2 nm	254.2 nm	268.8 nm
	Abs	Abs	Abs	Abs	Abs
0	0.0000	0.0000	0.0000	0.0000	0.0000
7.8	0.3922	0.4318	0.2399	0.4322	0.4957
10.2	0.5210	0.5673	0.3175	0.3596	0.6599
12.6	0.6496	0.7088	0.3942	0.4474	0.8133
15	0.7764	0.8258	0.4764	0.5398	0.9805
17.4	0.8934	0.9634	0.5463	0.6189	1.1207
a =	0.0516	0.0554	0.0316	0.0358	0.0648

b =	-0.0035	0.0014	0.0026	-0.0028	-0.0027
r =	0.9998	0.9998	0.9998	0.9998	0.9998

Table 6 Data of vitamin B6 absorption

C (µg/ml)	219.4 nm	225 nm	231.2 nm	254.2 nm	268.8 nm
	Abs	Abs	Abs	Abs	Abs
0	0.0000	0.0000	0.0000	0.0000	0.0000
2.4	0.1057	0.0936	0.0380	0.0250	0.0193
6.4	0.3023	0.2653	0.1089	0.0704	0.0558
10.4	0.5066	0.4387	0.1759	0.1125	0.0931
14.4	0.6971	0.6110	0.2481	0.1579	0.1314
18.4	0.9031	0.7752	0.3170	0.1978	0.1661
a =	0.0492	0.0425	0.0173	0.0108	0.0091
b =	-0.0076	-0.0040	-0.0020	0.0001	-0.0015
r =	0.9998	0.9999	0.9998	0.9998	0.9998

Table 7 Data of niacin absorption

C (µg/ml)	219.4 nm	225 nm	231.2 nm	254.2 nm	268.8 nm
	Abs	Abs	Abs	Abs	Abs
0	0.0000	0.0000	0.0000	0.0000	0.0000
7.0	0.4279	0.3236	0.2032	0.1327	0.1163
9.0	0.5540	0.4205	0.2630	0.1708	0.1509
11.0	0.6904	0.5209	0.3278	0.2108	0.1858
13.0	0.8007	0.6179	0.3853	0.2513	0.2214
16.0	0.9844	0.7492	0.4721	0.3063	0.2723
a =	0.0617	0.0472	0.0296	0.0192	0.0170
b =	0.0000	-0.0016	-0.0014	-0.0008	-0.0013
r =	0.9998	0.9998	0.9998	0.9999	0.9999

3.8. Level, Accuracy, and Precision of Vitamin B1, B2, B6, and Niacin in Multivitamin Syrup

The preparation of the sample solution is done with the addition of the standard solution because the level of vitamin B1, B2, B6 in the sample is too low to reach the Beer's law requirement. Then the sample is measured in the five predetermined wavelengths.

The calculation of the accuracy, coefficient variation, and level of each component in multivitamin syrup can be seen in Table 8 and Table 9.

Table 8 Accuracy and coefficient variation of multivitamin syrup

Parameter	Accuracy (%)	Coefficient Variation (%)
Vitamin B1	98.05	0.63
Vitamin B2	99.04	0.57
Vitamin B6	99.02	0.49
Niacin	99.61	0.57

Table 9 Vitamin B1, B2, B6, and niacin levels in multivitamin syrup

Vitamin	Level (%)	Level in syrup (mg)	Requirement (%)
B1	98,05 ± 1,28	3	95-115
B2	99,04 ± 1,17	1,25	95-115
B6	99,02 ± 1,00	1	95-115
Niacin	99,61 ± 1,17	10	90-110

Based on the table above, it is known that the vitamin B1, B2, B6, and niacin levels in multivitamin syrup have met the requirement by USP 27thEdition [15].

4. Conclusion

The use of the divisor factor in the Mean Centering Ratio method and the system of five different wavelengths in Multiple Wavelength can be used to determine simultaneously the components of a multivitamin containing vitamins B1, B2, B6, and niacin and their respective levels by the requirements of USP 43 edition.

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