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Original Article

Simultaneous Analysis for Determining Ascorbic Acid and Niacinamide Levels in Facial Serum Using UV-Visible Spectrophotometer with Chemometric Approach

Analisis Simultan untuk Menentukan Kadar Asam Askorbat dan Niacinamide dalam Serum Wajah Menggunakan Spektrofotometer UV-Visible dengan Pendekatan Kemometrik

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Abstract

Ascorbic acid and niacinamide are active brightening ingredients in facial serum skin care products. These two active substances are used together in one product. This research aims to develop a method for analyzing ascorbic acid and niacinamide simultaneously using a chemometric UV-Vis spectrophotometer. The research was carried out by testing specificity using a UV-visible spectrophotometer, and development was carried out by building a Partial Least Square (PLS) model using 20 training sets, which were cross-validated through leave-one-out cross-validation to obtain 5 test sets. Next, internal and external validation was carried out on model and method validation tests focusing on linearity, precision, limit of detection (LOD), and limit of quantification (LOQ). Tests were carried out at wavelengths of 244 and 262 nm. The model and method validation tests' internal and external validation results meet USP requirements. The method for measuring ascorbic acid and niacinamide levels using a UV-Vis chemical spectrophotometer meets the appropriate method validation parameters. The results of concentration measurements in facial serum on the market showed that the sample contained 18.97% ascorbic acid and 13.74% niacinamide. Based on these results, the analysis method for determining the levels of AA and NAC using a UV-Vis spectrophotometer chemometrically has met the validity requirements according to USP.

Abstrak

Asam askorbat dan nikotinamida adalah bahan aktif pencerah yang terdapat pada produk perawatan kulit serum wajah. Kedua zat aktif ini digunakan secara bersamaan dalam satu produk. Tujuan penelitian ini adalah mengembangkan metode analisis asam askorbat dan nikotinamida secara simultan menggunakan spektrofotometer UV-Vis secara kemometrik. Penelitian dilakukan dengan uji spesifisitas menggunakan spektrofotometer UV-visibel, dan pengembangan dilakukan dengan membangun model *Partial Least Square* (PLS) menggunakan 20 training set, yang divalidasi silang melalui *leave one out cross validation* untuk mendapatkan 5 test set. Selanjutnya dilakukan validasi internal dan eksternal terhadap uji validasi model dan metode dengan fokus pada linearitas, presisi, batas deteksi (LOD) dan batas kuantifikasi (LOQ). Pengujian dilakukan pada panjang gelombang 244 dan 262 nm. Hasil validasi internal dan eksternal uji validasi model dan metode memenuhi syarat USP. Metode pengukuran kadar asam askorbat dan nikotinamida menggunakan spektrofotometer UV-Vis memenuhi parameter validasi metode sesuai. Hasil pengukuran konsentrasi dalam serum wajah di pasaran, sampel mengandung asam askorbat sejumlah 18,97% dan nikotinamida 13,74%. Berdasarkan hasil tersebut, metode analisis untuk penentuan kadar AA dan NAC menggunakan spektrofotometer UV-Vis secara kemometrik telah memenuhi persyaratan sahih menurut USP.

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INTRODUCTION

Ascorbic acid (AA) and niacinamide (NAC) are active ingredients in cosmetics that have a skin-brightening effect. NAC, or vitamin B3, works physiologically and is useful in skin care, such as reducing inflammation, treating acne, and evening out skin tone.[1] While L-ascorbic acid is an antioxidant. Ascorbic acid is soluble in water and acts as a free radical scavenger.[2] These two active ingredients are often found in facial lotions and serum preparations.

The advantage of serum preparations is they contain much more active ingredients than other cosmetic preparations, so serum provides a faster and better effect in overcoming skin problems.[3] However, high serum concentrations do not only provide a direct therapeutic effect but can also provide unexpected irritation effects. The recommended concentration of NAC for use is 2-5%. Also, if the concentration of NAC is above 10%, the risk of skin irritation can increase.[4] AA for topical use also has a recommended concentration of above 8%, with a maximum of 20% to prevent skin irritation.[5] Serums distributed on the market can contain AA and NAC simultaneously in amounts consumers are unaware of.

For quality purposes, the analysis of AA in serum has been developed using spectrophotometer UV-Vis, HPLC-MS and screen-printed carbon electrodes.[6–8] Methods for the determination of niacinamide in cosmetics using spectrophotometer UV-Vis, HPLC and HPTLC.[9,10] None of these methods provide simultaneous determination of AA and NAC, so they are not appropriate to use them for the analysis of cosmetics, including the components as combinations. For this reason, research was conducted to determine the amount of AA and NAC simultaneously in facial serum preparations available on the market.

Simultaneous analysis of a substance is carried out using multivariate calibration. In multivariate calibration, especially the UV Vis spectrophotometry method, absorbance can be measured at two or more wavelengths. Multivariate calibration is related to chemometrics in spectroscopy to determine the levels of several compounds in a mixture matrix. The chemometric method in multivariate calibration that is widely used is partial least squares (PLS). The PLS method is a method that combines the properties of the principal components and multiple linear regression. PLS regression will produce a model that will then be tested for validation.[11]

Simultaneous analysis of multiple substances using multivariate calibration is carried out to facilitate testing. However, simultaneous testing often experiences problems because the spectra that appear overlap with each other, making it difficult to determine the analysis results accurately. Therefore, the analysis is carried out using chemometric techniques using the partial least square method to overcome this problem.[12]

Method validation occurs when a newly developed analysis method addresses specific analytical challenges, revises existing standard methods to accommodate advancements, or responds to quality assurance findings indicating changes in the standard method over time.[13–16]

This study aims to develop a chemometric UV-Visible spectrophotometer analysis method that meets the method validation parameters for determining AA and NAC levels simultaneously according to USP (United States Pharmacopeia) and determining the concentration of AA and NAC in facial serum on the market.

METHOD

Tools and Material

Tools. Spectrophotometer UV-Vis (*Genesys*), balance (*Mettler toledo*), cuvette, laboratory glassware.

Material. Standard AA dan NAC (*Life Science Manufacturer*), face serum sample, methanol p.a (*Smart Lab*).

Method

Analytical Method Development

a. Preparation of Ascorbic Acid and Nicotinamide Standard Stock Solution

About 50 mg AA and 50 mg NAC were weighed carefully and diluted using methanol in a 100 mL dark volumetric flask. The AA and NAC standard stock solution's concentration was 500 µg/mL.

b. Determination of Maximum Wavelength (Specificity Test)

The specificity test was carried out by determining the maximum wavelength of each AA and NAC standard, combination of AA and NAC, serum samples, and spiked samples. All samples were analyzed on a UV-Vis spectrophotometer in the 200-400 nm range. The resulting spectrum was overlaid to see the specificity.[15]

c. Preparation of Training Set and Test Set

A total of 20 series of mixed AA and NAC training set solutions were used with a concentration

range of 1-20 µg/mL. The calibration model selected as much as ¼ of the number of training sets with the composition of the standard compound concentration model that produced the best linearity. This calibration model will be used as a test set for linearity testing.[13,17]

d. PLS Calibration Modeling

The chemometric model used in this study is the Principal Least Square (PLS) method. The PLS method was created using Minitab and Excel software based on the training set absorbance data. Furthermore, the training set evaluation was carried out by calculating the root mean squares error of calibration (RMSEC) and coefficient of determination (R2) values to see the accuracy of the model creation. In creating the calibration model, the concentration and absorbance data of the compound from the training set were selected as the basis for forming the test set. The concentration chosen was the one that showed the smallest error value and acceptable coefficient of determination value. The calibration model was validated internally using the training set data, and externally using the test set. The leave one out cross validation method was used as internal validation using Minitab software. Internal validation parameters include the root mean squares error of cross validation (RMSECV), predicted residual error sum of square (PRESS), and coefficient of determination (R2) to see the ability of the chemometric model to predict the analysis results. Then the test set is validated externally by calculating the root mean squares error of prediction (RMSEP), PRESS, and R2 values and the method is validated by calculating the average of % recovery and % RSD.[11,12]

RESULT AND DISCUSSION

Specificity Test

The specificity test on the UV-Vis spectrophotometer was done by determining the maximum wavelength of the compound being tested. The maximum wavelength is the specific wavelength of a compound and has the highest absorbance.[14] The specificity test measures the wavelength of AA, NAC, AA + NAC solutions, serum sample solutions, and spike solutions (standard solutions + samples) with the same 10 µg/ml concentration. In **Figure 1**, it can be seen that the AA standard has a maximum wavelength of 244 nm, the NAC standard has a maximum wavelength of 215 nm and 262 nm, while the mixed standard of AA and NAC has a maximum wavelength of 215 nm and 256 nm. The mixed standard of AA and NAC has a wavelength shift, which differs from the AA and NAC alone. The wavelength shift causes overlapping spectra that will cause inaccurate test results if analyzed simultaneously using the UV spectrophotometer method.[18]

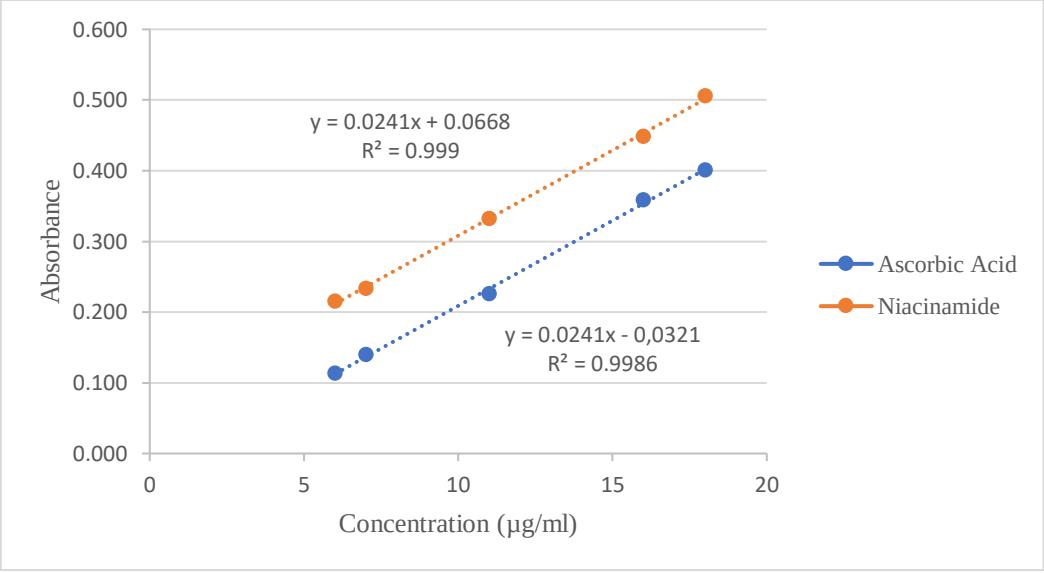

Figure 1. Calibration curve of AA and NAC

The matrix used in the specificity test is the sample solution and spike solution, then the test results of the two solutions are compared. The sample solution is serum containing AA and NAC that has been prepared while the spike is a sample solution added to the standard mixture of AA and NAC. The maximum wavelength produced by the sample solution is at 215 nm and 256 nm. Meanwhile, the spike solution produces a maximum wavelength of 215 nm and 256 nm. The absence of differences in the maximum wavelength obtained indicates that the test results are not affected by the presence of excipients in the sample. NAC has a maximum wavelength of 262 nm and AA has a maximum wavelength of 244 nm using methanol solvent.[19,20] However, based on the results of the method development, the mixture of AA and NAC produces a maximum wavelength of 215 nm and 256 nm. The shift in wavelength is due to AA ionizing at pH> 3.0 and undergoing photodegradation to dehydroascorbic acid. Ionized AA solution loses absorption at a wavelength of 244 nm. AA degradation is also affected by the presence of NAC because NAC increases the pH of the solution.[21,22]

To overcome the problem of analytes reacting with each other, the Partial Least Square (PLS) method is used with a chemometric model. In modeling with PLS, a linear relationship is built between the absorbance that most accurately describes the concentration of the compound being tested. Therefore, a training set is needed to create a calibration model that can always provide valid results and a test set to build a prediction model so that the samples are truly independent.[12] The composition of the training set consists of 1-20 µg/mL.

A good training set describes a high correlation between the actual value and the predicted value, producing a coefficient of determination (R^2) value close to 1. The closer to 1, the better the built training set because it indicates that the actual value is not much different from the predicted value. Next, the RMSEC (Root Mean Square Error of Calibration) value is calculated, showing the difference's root between the predicted value and the actual value per data point.[23] The R2 and RMSEC values of the training set can be seen in **Table 1**.

The RMSEC value and R^2 value obtained are very good, the RMSEC value is less than 1 while the R2 value is close to 1. The number of training set samples must be sufficient to build a stable and reliable calibration model. The calibration model will later be used as a test set used to check the accuracy of the model. The selection of the calibration model is done using cross validation.

Table 1. Actual values and predicted values on the training set

AA (µg/ml)		NAC (µg/ml)	
Actual	**Prediction**	**Actual**	**Prediction**
1	1.9	1	1,8
2	2.4	2	1,6
3	3.0	3	2,7
4	3.9	4	4,0
5	5.1	5	5,2
6	5.5	6	5,5
7	6.3	7	6,8
8	7.6	8	7,6
9	8.6	9	8,6
10	10.2	10	9,3
11	10.7	11	11,9
12	11.7	12	12,5
13	12.9	13	13,7
14	14.5	14	14,6
15	14.8	15	15,2
16	16.4	16	16,4
17	17.7	17	16,2
18	18.1	18	18,0
19	19.4	19	18,5
20	19.4	20	20,0
R^2= 0.9945		R^2= 0.9925	
RMSEC = 0.430		RMSEC = 0.503	

The PLS calibration model is then validated internally using cross validation techniques and with external validation.[12] Internal validation and external validation are carried out to prevent over-fitting. The existence of over-fitting, namely when the training set provides a good model, but the model cannot be applied to other data from the same material or test sample.

The cross validation used is leave one out cross validation which simulates the validation of the test series by entering one data into the test set for making the calibration model and the rest goes into the training set for model evaluation. Based on the results of cross validation, the optimum model is produced from 5 components or as much as ¼ of the total data.[24,25] The composition of the calibration model consists of 6,7,11,16,18 µg/mL.

The training set data that no longer contains test set data (6, 7, 11, 16, 18 µg/ml) is calculated for the PRESS (predicted residual error sum of square) and RMSECV (root mean squared error cross validation) values. The PRESS value, which is one of the indicators of the goodness of the model that describes the predictive ability, is calculated from the square of the difference between the actual and predicted values. The smaller the PRESS value means the smaller the prediction error so that the model is better. After that, the PRESS value is divided by the

number of data and rooted to get the RMSECV value which describes the cros validation error. The R2 value is also calculated because it shows a good correlation between the actual and predicted.[12] **Table 2** shows the R2, RMSECV, PRESS values from the crossvalidation results.

Table 2. Internal Validation Parameter

Parameter	AA	NAC
Linearity (R^2)	0.9954	0.9933
RMSECV	0.408	0.497
PRESS	2.499	3.709
a	0.0004	0.1689
b	0.9999	0.9836

Linearity Test

The linearity test was carried out using five concentration series, namely concentrations of 6 µg/ml, 7 µg/ml, 11 µg/ml, 16 µg/ml, and 18 µg/ml, and replication was carried out three times for each concentration. The results obtained were concentrations that were directly proportional to the resulting absorbance. The linear regression equation of AA obtained was y = 0.0241x – 0.0321 with a correlation coefficient of 0.9986, while the linear regression equation of NAC obtained was y = 0.0241x + 0.0668 with a correlation coefficient of 0.999.

External Validation

PRESS and RMSEP (Root Mean Square Error of Prediction) values are calculated. The smaller the difference, the smaller the error of the test set. In addition to the PRESS and RMSEP values, the R^2 value is also an external validation parameter on the test set. The results of the external validation parameters of the test set can be seen in **Table 3**.

Table 3. Actual and Calculation Value of Test Set

Concentration (µg/mL)			
AA		NAC	
Actual	Calculation	Actual	Calculation
6	5.7	6	6.1
7	6.8	7	6.9
11	10.5	11	11
16	16.3	16	15.8
18	18.1	18	18.2
R^2= 0.9986		R^2= 0.999	
RMSEP= 0.295		RMSEP= 0.149	
PRESS= 0.434		PRESS= 0.111	

Accuracy Test

The accuracy method was carried out by measuring the AA and NAC standards at concentrations of 8.8; 11, and 13.2 µg/mL. Accuracy indicates the closeness of the calculation results to the experimental results, while precision indicates how close the differences in the results are when the measurements are repeated.[26] The results obtained respectively for AA were 101.06%, 99.63%, and 97.17% (**Table 4**). At a concentration of 120% the accuracy of the AA and NAC decreased because when the concentration increased, the ions in the solution increased so that they inhibited each other's movement, thereby slowing down the conductivity resulting in a slight decrease in absorbance[22]. Meanwhile, the accuracy of NAC obtained was respectively 98.33%, 97.76%, and 96.36% (**Table 4**) with an acceptance limit of 95% - 105%.[15]

Table 4. Accuracy Result on AA & NAC

Concentration	Average *%Recovery* AA± SD	Average *%Recovery* NAC± SD
80% (8.8 µg/mL)	101.06 ± 0.002	98.33 ± 0.002
100% (11 µg/mL)	99.63 ± 0.002	97.76 ± 0.002
120% (13.2 µg/mL)	97.17 ± 0.002	96.36 ± 0.002

Determination of intraday precision of AA and NAC standards using the UV-Vis spectrophotometer method was carried out with the same concentration with six replications.[27] Precision testing aims to show the accuracy of the method being carried out. The calculation results of % RSD with a concentration equivalent to 11 µg/mL were 0.6% for AA and 0.7% for NAC (**Table 5**), with the acceptance limit of % RSD being <2%.[15]

Determination of interday precision of AA and NAC standards using the UV-Vis spectrophotometer method was carried out with the same concentration for 3 days with the same concentration. Calculation of interday precision obtained RSD of 1.3% for AA and 1.6% for NAC (**Table 5**) with the acceptance limit of % RSD is <2%.[15]

Table 5. Intraday Precision Result

Active compound	RSD Intraday (%)	RSD Interday (%)
AA	0.6	1.3
NAC	0.7	1.6

Determination of the limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) value of AA at a concentration of 0.72 µg/mL and the LOD of NAC at a concentration of 0.51 µg/mL on a UV-Vis spectrophotometer. This means that the absorbance of these concentrations can still be read, but there is a calculation bias. Meanwhile, the limit of quantification (LOQ) of AA was obtained at 2.41 µg/mL and the LOQ of NAC was obtained at 1.69

µg/mL. These concentrations are the smallest concentrations for testing that do not cause calculation bias.

Determination of Facial Serum Levels

The levels of one facial serum containing AA and NAC were determined. In addition, the levels were checked on other serums that also contained AA derivatives. The purpose of determining the levels of serum containing AA derivatives was to compare the analysis results between a mixture of L-AA and NAC with a mixture of AA and NAC derivatives. The result can be seen in **Table 6**.

Table 6. AA and NAC Levels on Facial Serum

Sample	AA (%)	NAC (%)
Serum 1	18.97	13.74
Serum 2	6.85	4.37
Serum 3	5.38	3.16
Serum 4	10.34	3.53

The AA level has met the recommended level requirements, which is a maximum of 20%, but the NAC level exceeds the maximum level of 10% in topical preparations 4.5. Serum 1, the serum used in the specificity test, contains 18.97% (w/v) AA and 13.74% (w/v) NAC. This facial serum will likely irritate because the NAC level exceeds the recommended level. However, further research is needed because irritation reactions do not occur in everyone.[28]

In the facial serum samples that have been tested, two samples, namely serum 1 and serum 4, contain the active substance L-AA, two samples, namely serum 2 and serum 4, contain 3-O-ethyl-l-AA, and one sample, namely serum 3, contains ascorbyl glucoside. Standard L-AA is used because AA derivatives also react with NAC, so the resulting UV spectrum is similar to a mixture of L-AA and NAC. The maximum wavelength of the mixture of 3-O-ethyl-l-AA or ascorbyl glucoside with NAC is 209 and 261 nm.

Serum 2-4 contains AA derivatives with a maximum wavelength different from L-AA, so the calculation of the levels was carried out only L-AA; no calculation of AA derivatives was carried out, so the claim of levels cannot be determined. This also shows that there is a possibility that the derivatives return to pure form because they are detected at a wavelength of 244 nm. Therefore, further analysis is needed regarding the reaction that occurs between AA derivatives and NAC and analysis of their levels.

CONCLUSION

The analysis method for determining the levels of AA and NAC using a UV-Vis spectrophotometer chemometrically has met the validity requirements according to USP. The results of determining the levels of AA and NAC that were carried out on one facial serum sample showed that the AA levels obtained were 18.97% (w/v), and NAC was 13.74% (w/v). Based on these results, the sample met the recommended levels in topical preparations, which is a maximum of 20% for AA, but did not meet the NAC levels, which is a maximum of 10%.

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CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Matts PJ, Oblong JE, Bissett DL. A review of the range of effects of niacinamide in human skin. *IFSCC Mag*. 2002;5(4):285-289.
2. Kembuan MV, Wangko S, Tanudjaja GN. Peran Vitamin C Terhadap Pigmentasi Kulit. *J Biomedik*. 2013;4(3):13-17. doi:10.35790/jbm.4.3.2012.1215
3. Draelos ZD. *Cosmetic Dermatology Products and Procedures*. John Wiley & Sons; 2016.
4. Panel CIRE. Final report of the safety assessment of niacinamide and niacin. *Int J Toxicol*. 2005;24:1-31.
5. Al Abadie M, Tukmatchy H, Al Abadie M, Sharaf El Din L. Vitamin C in Therapeutic Dermatology. *Int J Clin Exp Dermatology Int J Clin Expl Dermatol*. 2021;6(2):1-03. www.opastonline.com
6. Pizzo JS, Cruz VHM, Rodrigues CA, et al. Rapid determination of L-ascorbic acid content in vitamin C serums by ultra-high-performance liquid chromatography-tandem mass spectrometry. *Int J Cosmet Sci*. 2022;44(1):131-141. doi:10.1111/ics.12762
7. Alonso-Lomillo MA, Domínguez-Renedo O, Saldaña-Botín A, Arcos-Martínez MJ. Determination of ascorbic acid in serum samples by screen-printed carbon electrodes modified with gold nanoparticles. *Talanta*. 2017;174(May):733-737. doi:10.1016/j.talanta.2017.07.015
8. Normaidah N, Rahmawanty D, Hadi S, et al. Determinasi Vitamin C dalam Sediaan Losion Pemutih dan Serum Pencerah Wajah secara Spektrofotometer UV. *Pharm J Farm Indones (Pharmaceutical J Indones*. 2022;19(1):10.

doi:10.30595/pharmacy.v19i1.10381
9. Kabita B, Simhachalam R, BK G. Chromatographic Estimation of Niacinamide in Anti Aging Dermatological Product. *Asian J Pharm Anal*. 2019;9(2):62-66. doi:10.5958/2231-5675.2019.00013.9
10. Muszalska I, Kiaszewicz K, Ksoń D, Sobczak A. Determination of nicotinamide (vitamin B3) in cosmetic products using differential spectrophotometry and liquid chromatography (HPLC). *J Anal Chem*. 2013;68(11):1007-1013. doi:10.1134/S1061934813110087
11. Supriyadi E, Mariani S, Sugiman. Perbandingan Metode Partial Least Square (PLS) dan Principal Component Regression (PCR) untuk Mengatasi Multikolinearitas pada Model Regresi Linear Berganda. *Unnes J Math*. 2017;6(2):117-128.
12. Rohman A, Irnawati, Riswanto FDO. *Analisis Farmasi Dengan Spektroskopi UV-VIS Dan Kemometrika*. Gadjah Mada University Press; 2022.
13. Berrar D. Cross Validation. *Encycl Bioinforma Comput Biol*. 2018;1:542-545. doi:10.1016/B978-0-12-809633-8.20349-X
14. Gandjar IG, Rohman A. *Kimia Farmasi Analisis*. Pustaka Pelajar; 2007.
15. United States Pharmacopeia Convention (USP). *Validation of Compendial Procedure <1225> United States Pharmacopeia 37–National Formulary 32*. U.S. Pharmacopeial Convention; 2014.
16. United States Pharmacopeia Convention (USP). *Ultraviolet-Visible Spectroscopy <857> United States Pharmacopeia National Formulary 40*. U.S. Pharmacopeial Convention; 2017.
17. Refaeilzadeh P, Tang L, Liu H. Cross Validation. *Encycl database Syst*. 2009;5:532-538.
18. Anisah SU, Darmawati A, Prawita A. Validasi Metode Spektrofotometri UV untuk Penetapan Kadar Lopinavir dan Ritonavir Secara Simultan. *Berk Ilm Kim Farm*. 2021;8(2):48-54. doi:10.20473/bikfar.v8i2.31760
19. Florey K. *Analytical Profiles of Drug Substances*. Vol 17. Volume 11. Academic Press; 1983. doi:10.1177/106002808301700735
20. Florey K. *Analytical Profiles of Drug Substances*. Vol 32. Volume 20. Academic Press; 1991. doi:10.1016/0041-0101(94)90092-2
21. Ahmad I, Mobeen MF, Sheraz MA, et al. Photochemical interaction of ascorbic acid and nicotinamide in aqueous solution: A kinetic study. *J Photochem Photobiol B Biol*. 2018;182(February):115-121. doi:10.1016/j.jphotobiol.2018.04.011
22. Kim Y, Kim MG. HPLC-UV method for the simultaneous determinations of ascorbic acid and dehydroascorbic acid in human plasma. *Transl Clin Pharmacol*. 2016;24(1):37-42. doi:10.12793/tcp.2016.24.1.37
23. Rohman A, Silawati D, Sudjadi, Riyanto S. Simultaneous determination of sulfamethoxazole and trimethoprim using UV spectroscopy in combination with multivariate calibration. *J Med Sci*. 2015;15(4):178-184. doi:10.3923/jms.2015.178.184
24. Brereton RG, Jansen J, Lopes J, Marini F, Pomerantsev A, Rodionova O. Chemometrics in analytical chemistry — part II: modeling , validation , and applications. Published online 2018:6691-6704.
25. Rutledge DN, Roger JM, Lesnoff M. Different Methods for Determining the Dimensionality of Multivariate Models. *Front Anal Sci*. 2021;1(October):1-15. doi:10.3389/frans.2021.754447
26. Ulfiati R, Purnami R, Karina RM. Faktor yang Mempengaruhi Presisi dan Akurasi Data Hasil Uji dalam Menentukan Kompetensi Laboratorium (The Factor that Affect the Precision and Accuracy of Test Result Data within Determine the Laboratory Compentency Level). *Lembaran Publ Miny dan Gas Bumi*. 2017;51(1):49-63. http://www.journal.lemigas.esdm.go.id
27. Harmita. Petunjuk Pelaksanaan Validasi dan Cara Penggunaannya. *Maj Ilmu Kefarmasian*. 2004;1(3):117.
28. Berson DS, Osborne R, Oblong JE, Hakozaki T, Johnson MB, Bissett DL. Niacinamide: A Topical Vitamin with Wide-Ranging Skin Appearance Benefits. *Cosmeceuticals Cosmet Pract*. Published online 2013:103-112. doi:10.1002/9781118384824.ch10