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Simultaneous Determination of Amoxicillin and Clavulanate Potassium in Dry Syrup by Derivative Spectrophotometry

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Abstract: The aim of this study was to test the validation of derivative spectrophotometric method in simultaneous determination the content amoxicillin and clavulanate potassium in dry syrup by derivative spectrophotometric method with zero crossing technique, in buffer phosphate pH 4,4-methanol (91:9) mixture.
The research results were obtained the amoxicillin and clavulanate potassium content at the second derivative with $\Delta\lambda$ = 2 nm at the wavelength of 239.00 nm and 313.20 nm respectively. The samples Clavamox® dry syrup were (102.73 ± 8.95)% and Claneksi® (103.52 ± 8.88)% and clavulanate potassium content of the sample Clavamox® in dry syrup (97.64 ± 4.12)% and Claneksi® (95.75 ± 5.64)%. Based on the results of analysis determine the sample content of amoxicillin and clavulanate potassium compound in dry syrup supply amoxicillin fulfilled the requirements in *United States Pharmacopoeia* (USP) 30th edition (2007) and clavulanate potassium fulfilled the requirement in *United States Pharmacopoeia* (USP) 30th edition (2007). The results of validation test on the Clavamox® dry syrup, the percent recovery for the amoxicillin is 100.43%, relative standard deviation RSD = 0.98% and for clavulanate potassium, the percent recovery = 100.58%, RSD = 1.46%.
Keywords: Amoxicillin; Clavulanate Potassium; Derivative Spectrophotometry; Zero Crossing; Second Derivat; Dry syrup; Validation.

Introduction

Amoxicillin is a penicillin derivative antibiotics used to treat infections of the respiratory tract, gastrointestinal tract and urinary tract. Clavulanate potassium is a form of a salt of clavulanic acid. Clavulanic acid has antimicrobial working very weak, but can inhibit penicillinase of streptococci and β-lactamase as gram-negative microbes to bind to the active center of the enzyme. Therefore, these compounds are used in combination along with β-lactam antibiotics are not stable against β-lactamase[1,2]. Structur formula of amoxicillin and clavulanate potassium can be seen in Figure 1 and Figure 2.

According to the United States Pharmacopoeia (USP) 30th[3] for amoxicillin and clavulanate potassium suspension oral is not less than 90.0% and not more than 120.0% of the amount listed on the label.

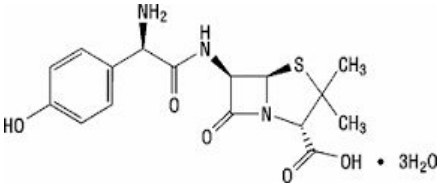

Figure 1. Structural Formula of Amoxicillin

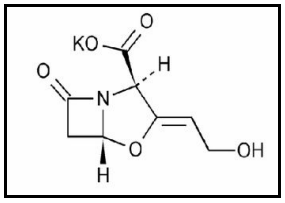

Figure 2. Structural Formula of Clavulanate Potassium

Determination the result of a mixture of amoxicillin and optimization clavulanate potassium by using high performance liquid chromatography and detected by ultraviolet spectrophotometer at a wavelength of 220 nm is obtained by comparison of the mobile phase pH 4.4 phosphate buffer-methanol (91:9). Levels of amoxicillin and clavulanate potassium mixture has also been determined the result of simultaneous determination of amoxicillin and clavulanate potassium in combined tablets by non-derivative and derivative ultraviolet spectrophotometric techniques, using aqueous solvent obtained in first derivatives at each wavelength 244.9 nm and 272 nm. Level of amoxicillin has been determined the result comparative study of RP-HPLC and UV spectrophotometric techniques for the simultaneous determination of amoxicillin and cloxacillin in capsules, using water as solvent obtained in first derivatives at each wavelength 258.0 nm[4,5,6].

Previously, UV-VIS Spectrophotometry was used preferably for quantitative estimations of concentrations of known substances at constant wavelength, because the fundamental spectra are mostly flat and are less characteristic than IR spectra, for example. However, higher-order derivatives now allow for an enhancement of the sensitiveness by a factor of 10-100 or more as well as a characterization of the substances by providing fingerprints, even in complex mixtures. This is very important for ultramicroanalysis. Therefore, the bulk of papers concerning differentiation technique deals with UV-VIS spectra. It is also the reason why in this book the field of UV-VIS spectra is treated in detail[7].

A first-order derivative is the rate of change of absorbance with respect to wavelength. A first order derivative starts and finishes at zero. It also passes through zero at the same wavelength as λ_{max} of the absorbance band. Either side of this point are positive and negative bands with maximum and minimum at the same wavelengths as the inflection points in the absorbance band. This bipolar function is characteristic of all odd-order derivatives. The most characteristic feature of a second-order derivative is a negative band with minimum at the same wavelength as the maximum on the zero-order band. It also shows two additional positive *satellite* bands either side of the main band. A fourth-order derivative shows a positive band. The first and second derivatives may be generated using this technique. It is popular for dedicated spectrophotometer designs used in, for example, environmental monitoring. First-derivative spectra may also be generated by a dual wavelength spectrophotometer. The derivative spectrum is generated by scanning with each monochromator separated by a small constant wavelength difference[8].

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision and accuracy[9].

Accordingly, in this research will be conducted as the determination of amoxicillin and clavulanate potassium in dry syrup by derivative spectrofotometric method with zero-crossing method.

Instrumental

Apparatus

Tools used in this study is UV-Visible spectrophotometer equipped with software Probe 2.42 UV (UV-1800 Shimadzu), analytical balance (Boeco), cuvette, filter paper, rubber ball, spatula, tools-glassware and equipment-other tools required in sample preparation.

Reagent

Materials used were methanol, NaH_2PO_4, distilled water, amoxicillin trihydrate (BPFI) 98.64%, potassium clavulanate (Phiexia Company) stock 99.70%, Clavamox® and Claneksi® dry syrup.

Sampling

Sampling was done by purposive, which is determined on the basis of the consideration that the samples drawn have characteristics similar to those studied. The samples used are Clavamox® dry syrup (PT. Kalbe) and Claneksi® dry syrup (PT.Sanbe), each of which contains 125 mg of amoxicillin and clavulanate potassium 31.25 mg.

Preparation of the Stock Solution Amoxicillin

About 50 mg of amoxicillin were accurately weighed, then diluted with a solvent mixture of phosphate buffer pH 4.4-methanol (91:9) in a 50 mL flask and paid back with the same solvent to obtain a solution with a concentration of 1000 µg/mL solution raw I (SS I). From this solution pipette 10 mL, was put into a 100 mL flask, diluted with a solvent mixture of phosphate buffer pH 4.4-methanol (91:9) to mark the line, shaken until homogeneous in order to obtain a solution with a concentration of 100 µg/mL solution of the parent Raw II (SS II).

Preparation of the Stock Solution Clavulanate Potassium

About 50 mg of clavulanate potassium were accurately weighed, then diluted with a solvent mixture of phosphate buffer pH 4.4-methanol (91:9) in a 50 mL flask and paid back with the same solvent to obtain a solution with a concentration of 1000 µg/mL (SS I). From this solution pipette 10 mL, was put into a 100 mL flask, diluted with a mixture of phosphate buffer pH 4.4-methanol (91:9) to mark the line, shaken until homogeneous in order to obtain a solution with a concentration of 100 µg/mL (SS II).

Preparation Maximum Absorption Spectrum Amoxicillin

Taken as much as 2.0 mL of amoxicillin concentration 100 µg/mL was then inserted into a 10 mL flask and then diluted with buffer phosphate pH 4,4-methanol (91:9) mixture solvent until the line mark, then shaken until to obtain a homogeneous amoxicillin solution with a concentration of 20 µg/mL. Absorbance was measured at a wavelength of 200-400 nm.

Preparation Maximum Absorption Spectrum Clavulanate Potassium

Taken as much as 1.65 mL of clavulanate potassium concentration 100 µg/mL was then inserted into the 10 mL flask to be diluted with buffer phosphate pH 4,4-methanol (91:9) mixture solvent until the line mark, then shaken until homogeneous to obtain a solution with a concentration of 16.5 µg/mL . Absorbance was measured at a wavelength of 200-400 nm.

Preparation Derivative Absorption Spectrum Amoxicillin

Taken by 1.0 mL; 1.5 mL; 2.0 mL; 2.5 mL; 3.0 mL and 3.5 mL of stock solution amoxicillin concentration 100 µg/mL (SS II), then each put in a 10 mL flask to be diluted with the solvent buffer phosphate pH 4,4-methanol (91:9) mixture. Then shaken until homogeneous to obtain a solution with a concentration of 10 µg/mL; 15 µg/mL; 20 µg/mL; 25 µg/mL; 30 µg/mL and 35 µg/mL Then made the absorption spectrum, then the absorption spectrum is transformed into a first derivative absorption spectrum and the second derivative at a wavelength of 200-400 nm with $\Delta\lambda$=2nm.

Preparation Derivative Absorption Spectrum Clavulanate Potassium

Taken by 0.85 mL; 1.25 mL; 1.65 mL; 2.05 mL; 2.45 and 2.65 mL of stock solution clavulanate potassium concentration 100 µg/mL (SS II), then each put in a 10 mL flask to be diluted with the solvent buffer phosphate pH 4,4-methanol (91:9) mixture. Then shaken until homogeneous to obtain a solution with a concentration of 0.85 µg/mL; 1.25 µg/mL; 1.65 µg/mL; 2.05 µg/mL; 2.45 µg/mL and 2.85 µg/mL. Then made

the absorption spectrum, then the absorption spectrum is transformed into a first derivative absorption spectrum and the second derivative at a wavelength of 200-400 nm with Δλ=2nm.

Determination of Zero-Crossing

Determination of the zero-crossing overlapping absorption spectrum obtained by each derived in different concentration of the solution. Zero-crossing each substance shown by the wavelength that has a zero uptake at various concentrations.

Determination of Wavelength Analysis

Created amoxicillin solution with a concentration of 35 µg/mL, clavulanate potassium solution with a concentration of 8.5 µg/mL, and a mixed solution of amoxicillin 35 µg/mL and clavulanate potassium 8.5 µg/mL. Each solution is then measured absorbance at a wavelength of 200-400 nm. Then absorption spectrum is transformed into the first and second derivatives of each single substance from a mixture of amoxicillin and clavulanate potassium. The second derivative absorption spectrum from a single substance solution and a mixture of both overlay. Were chosen to be the wavelength analysis is that at a particular wavelength, the absorption single one of the compounds zero while single absorption partner compound and a mixture of both is almost the same or exactly the same. Because at these wavelength can selectively measure the uptake of one of the compounds without being bothered by the uptake of compounds partner.

Preparation and Determination Linearity Calibration Curves Amoxicillin and Clavulanate Potassium

Created amoxicillin stock solution with a concentration of 10 µg/mL; 15 µg/mL; 20 µg/mL; 25 µg/mL; 30 µg/mL and 35 µg/mL, then the second derivative absorption measured (Δλ = 2 nm) in wavelength analysis has been determined. Then do the analysis of the relationship between concentration and absorbance values thus obtained linear regression equation y = ax+b. And based on the absorption at a wavelength analysis, also conducted the calculation of the Limit of Detection (LOD) and the Limit of Quantitaion (LOQ). To determined the LOD and the LOQ can be used formula.

$$SD = \sqrt{\frac{\sum (Y - Yi)^2}{n - 2}}$$

$$LOD = \frac{3 \times SD}{slope}$$

$$LOQ = \frac{10 \times SD}{slope}$$

Description:
SD = Standard Deviation
LOD = Limit of Detection
LOQ = Limit of Quantitation

Determination of Amoxicillin and Clavulanate Potassium levels in Dry Syrup

One bottle of dry syrup powder weighed. Then weighed carefully the amount of powder equivalent to 50 mg of amoxicillin and then the weight of which weighed analyte equivalent of 50 mg of amoxicillin clavulanate potassium is calculated equality contained therein (powder weighing as much as six times repetition), put in a 50 mL flask, added phosphate buffer pH 4.4-methanol (91:9) to line sign while shaken. The solution is then homogenized with an ultrasonic stirrer for 15 minutes. The solution is then filtered, approximately 10 mL of the first filtrate discarded. The filtrate subsequently accommodated. Then from this filtrate solution, 0.35 mL pipette and put into a flask and diluted with 10 mL of phosphate buffer pH 4.4-methanol (91:9) to mark the line (concentration of 35 µg/mL for amoxycillin and concentrations of 8,5 µg/mL for clavulanate potassium). The solution is measured at the second derivative absorbance at a wavelength analysis has been determined to amoxicillin and clavulanate potassium. Furthermore, the absorbance was measured at a wavelength of 200-400 nm, then the absorption spectrum is transformed into a second derivative

absorption spectrum Δλ 2 nm in wavelength analysis of amoxicillin and clavulanate potassium respectively 239.00 nm and 313.20 nm.

Validation Test

Accuracy Test

Accuracy test was conducted by the addition of raw materials is to make three samples with the analyte concentration of a specific range of 80%, 100%, 120%. Where in each specific range is used 70% and 30% of raw samples to be added and then mix the sample and standard absorbance was measured at a wavelength of 200-400 nm, then the absorption spectrum is transformed into a second derivative absorption spectrum Δλ 2 nm in wavelength analysis of amoxicillin and clavulanate potassium respectively 239.00 nm and 313.20 nm. Percentage recovery can be calculated by the formula[10].

$$\% \text{ Recovery} = \frac{C_F - C_A}{C_A^*} \times 100\ \%$$

Description:
CF = concentration of the substance after the addition of raw materials
CA = concentration of the substance before adding the raw materials
C^*A = number of raw added

Precision Test

Precision is measured as relative standard deviation or coefficient of variation. Precision measured indicates the degree of fit between the individual test results when a method is repeated for a homogeneous sample. Relative standard deviation value which meets the requirements showed a precision method performed.

Based on the results of recovery prescribed amoxicillin and clavulanate potassium standard deviation amoxicillin and clavulanate potassium of the formula:

$$SD = \sqrt{\frac{\Sigma\left(X - \bar{X}\right)^2}{n - 1}}$$

Description:
X = The number of substances in the sample
$\bar{X}$ = Number of substances sample average
n = Number of repetitions

Standart Deviation (SD) obtained based on the value, calculated relative standard deviation of amoxicillin and clavulanate potassium by the formula:

$$RSD = \frac{SD}{\bar{X}} \times 100\%$$

Description:
X = Number of substances sample average
SD = Standard deviation
RSD = Relative Standard Deviation

Results and Discussion

Results Determination of the Maximum Absorption Curves

Determination of maximum absorption spectra performed at a wavelength of 200-400 nm. Measurement of the concentration of amoxicillin in the 35 µg/mL, where as for clavulanate potassium at a concentration of 8.5 µg/mL and. Based on the research results, obtained the maximum wavelength amoxicillin and clavulanate potassium at 239.00 nm and 313.20 nm respectively.

Results Determination of Zero-Crossing at First Derivatives

Absorption spectrum of amoxicillin concentration of 35 µg/mL and clavulanate potassium concentration of 8.5 µg/mL was transformed into a first derivative absorption spectrum with $\Delta\lambda = 2$ nm. Results of the determination of the zero crossing in the first derivative of the absorption spectrum obtained by overlap first derivatives on each substance. Zero crossing in the first derivative spectrum of each wavelength is shown by agents who have zero absorption. Overlapping absorption spectrum amoxicillin and clavulanate potassium in the first derivatives can be seen in Figure 3.

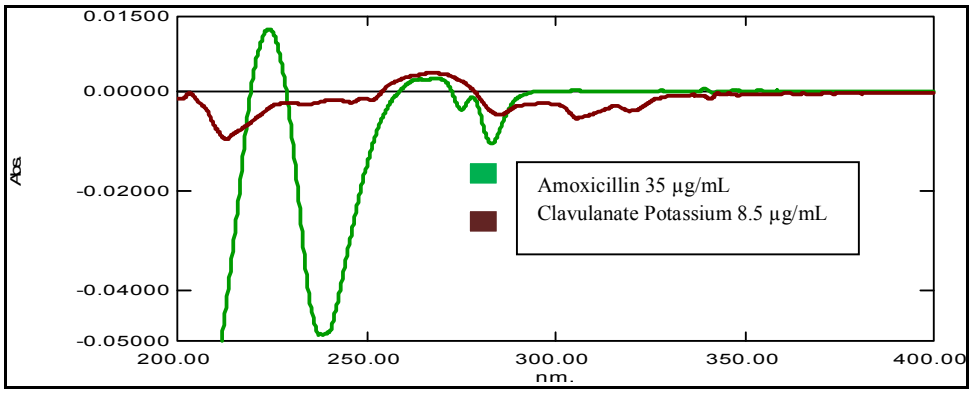

Figure 3. Overlapping absorption spectrum of amoxicillin and clavulanate potassium at first derivative at wavelength 239.00 nm and 313.20 nm respectively.

Determination of Absorption Zero crossing the Second Derivatives

Results of the second derivative absorption spectrum determination is made by first making the absorption spectrum of amoxicillin solution with a concentration of 35 µg/mL and clavulanate potassium with a concentration of 8.5 µg/mL at a wavelength of 200-400 nm. Absorption spectra have been obtained is transformed into a second derivative absorption spectrum with $\Delta\lambda = 2$ nm.

The second derivative absorption spectrum of respectively of these substances overlay. The results indicate zero crossing at a wavelength of 239.00 nm, 246.80 nm, and 249.60 nm to amoxicillin, whereas for clavulanate potassium was obtained at a wavelength of 263.40 nm, 313.20 nm and 320.60 nm. Wavelength and absorbance analysis can be seen in Table 1 and overlapping absorption spectrum of amoxicillin and clavulanate potassium on the second derivative in Figure 4.

Table 1. Wavelength Analisys and Absorbance at Second Derivative

Wavelength (nm)	Absorbance		
	Amoxicillin 35.0 µg/mL	Clavulanate Potassium 8,5 µg/mL	Amoxicillin and Clavulanate Potassium
239,00	**0,0004**	**0,0000**	**0,0003**
246,80	0,0005	0,0000	0,0004
249,60	0,0003	0,0000	0,0005
263,40	0,0000	0,0004	0,0004
313,20	**0,0000**	**0,0005**	**0,0005**
320,60	0,0000	0,0001	0,0001

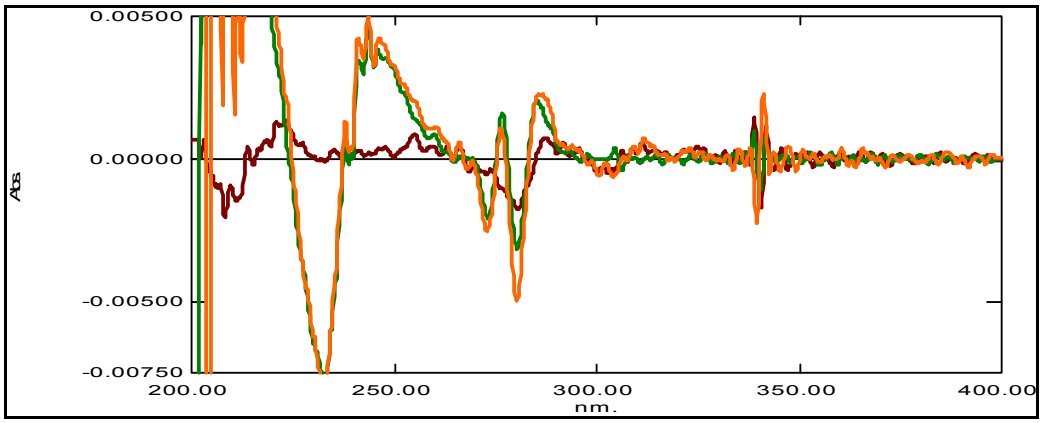

Figure 4. Overlapping absorption spectrum of amoxicillin and clavulanate potassium on the second derivative at wavelength 239.00 nm and 313.20 nm respectively.

Determination of Wavelength Analysis

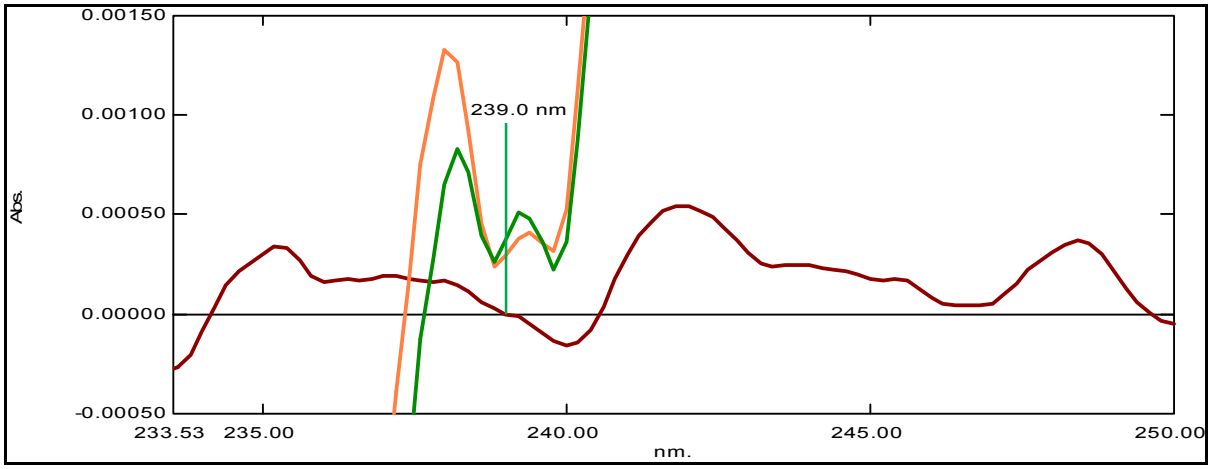

Figure 5. Wavelength analysis of amoxicillin λ = 239.00 nm

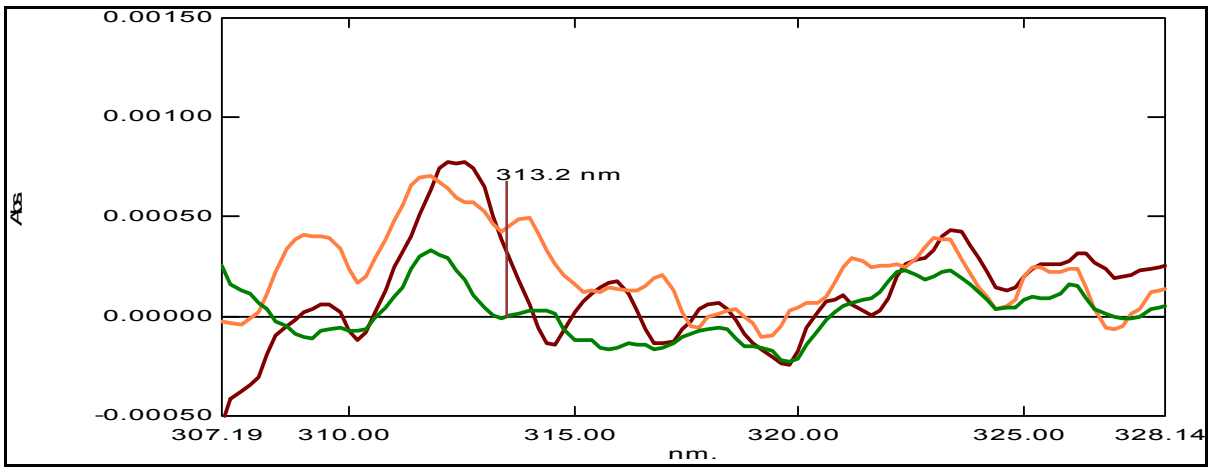

Figure 6. Wavelength analysis of clavulanate potassium λ = 313.20 nm

Determination of the wavelength of the analysis done by making a solution of amoxicillin 35 µg/mL, a solution of clavulanate potassium 8.5 µg/mL and mixed solution of amoxicillin 35 µg/mL and clavulanate potassium 8.5 µg/mL. Then made the absorption spectrum of the first and second derivatives, then overlaid. To

determine the wavelengths of the absorption spectrum analysis on each derivative is done by observing the absorption wavelength shows zero partner compounds and other compounds uptake and absorption thereof has a value equal or nearly equal. Amoxicillin wavelength spectrum analysis, and the wavelength spectrum analysis each clavulanate potassium can be seen in Figures 5 and 6.

Based on the above image, obtained by the wavelength used for the determination of the mixture of amoxicillin and clavulanate potassium uptake is on the second derivative, is 239.00 nm for amoxicillin, and 313.20 nm for clavulanate potassium. It is known based wavelength selection for each derivative analysis. The wavelength of the analysis is obtained by determining the zero crossing for amoxicillin and clavulanate potassium. At first derivative absorption, wavelength analysis for amoxicillin can be found. However, the wavelength analysis for clavulanate potassium was not found, so the assay mixture of amoxicillin and clavulanate potassium performed on the second derivative.

Determination Results Linearity Calibration Curves

The linearity of the calibration curve showed a linear relationship between the absorbance with concentration. Amoxicillin regression equation, $Y = (7X + 0.25).10^{-6}$ with a correlation coefficient, r = 0.9995 and clavulanate potassium, $Y = (21X + 1.35).10^{-6}$ with a correlation coefficient, r = 0.9997. R values > 0.995 showed a linear correlation relationship between X and Y[11]. The calibration curve amoxicillin and clavulanate potassium for each wavelength of 239.00 nm and 313.20 nm can be seen in Figures 5 and 6.

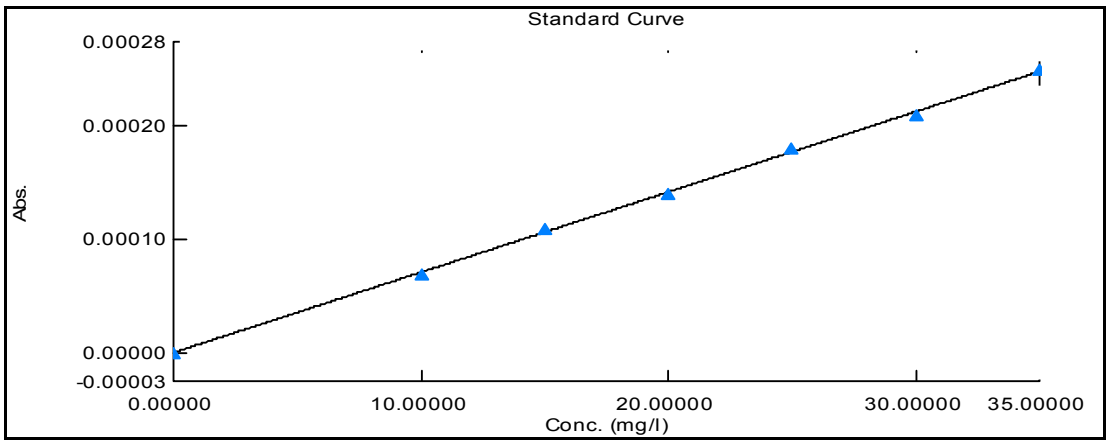

Figure 5. Calibration curve amoxicillin at a wavelength of 239.00 nm

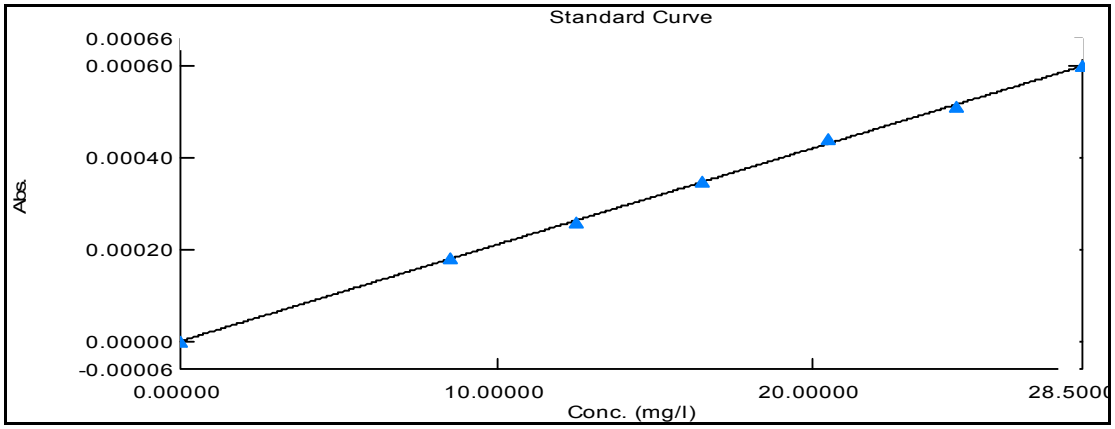

Figure 6. The calibration curve of clavulanate potassium at a wavelength of 313.20 nm

Determination Results of Amoxicillin and Clavulanate Potassium Levels in Dry Syrup

Determination is done by using Clavamox® and Claneksi® in dry syrup containing amoxicillin 125 mg and 31.25 mg potassium clavulanate. Measurement of amoxicillin and clavulanate potassium raw on both

substances each amoxicillin and clavulanate potassium 35µg/mL and 8.5 µg/mL, which is adapted to the content ratio of the two substances in the preparation, namely 125:31.25 or 4:1.

The prepared sample is then measured at a wavelength of 200-400 nm. Furthermore, the results of the absorption spectrum is transformed into a second derivative absorption spectrum with $\Delta\lambda = 2$ nm. Can be determined based on the absorbance spectrum of amoxicillin and clavulanate potassium at a wavelength analysis has been obtained previously, is wavelengths 239.00 nm and 313.20 nm. Levels of amoxicillin and clavulanate potassium in Clavamox® and Claneksi® can be seen in Table 2.

Table 2. Levels of amoxicillin and clavulanate potassium in Clavamox® and Claneksi

No	Drug	Clavamox®	Claneksi®	Label claim (mg)	Requirements (%)
1.	Amoxicillin	(102.73 ± 8.95)%	(97.64 ± 4.12)%	125	90-120
		(121.27 – 135.55) mg	(118.76 – 125.34) mg		
2.	Clavulanate Potassium	(103.52 ± 8.88)%	(95.75 ± 5.65)%	31.25	90-120
		(30.57 – 34.12) mg	(28.80 – 31.04) mg		

Amoxicillin and clavulanate potassium levels obtained in the above table shows that the dry syrup preparation Clavamox® and Claneksi® on the market meet the requirement in the Farmakope Indonesia Edisi V[12], which is not less than 90% and not more than 120% of the amount listed on the label.

Test Results Validation

Validation parameters tested were accuracy, precision, limits of detection and quantitation limits. Accuracy is expressed in percent recovery were determined using standard addition method. Precision test done using parameters Relative Standard Deviation (RSD)[10].

Accuracy Test

Accuracy test with parameter percent recovery is done by using Clavamox® in dry syrup with standard addition method, which is made by adding a certain amount of standard solution. Then the solution is measured in accordance absorbance wavelength analysis, which is 239.00 nm and 313.20 nm. Results recovery of amoxicillin and clavulanate potassium by standard addition method standard Clavamox® in dry syrup can be seen in Table 3.

Table 3. Test Results Recovery Amoxicillin and Clavulanate Potassium

Specific ranges (%)	Amoxicillin recovery (%)	Clavulanate Potassium recovery (%)
80	102.28	99.65
	101.43	98.96
	100.59	98.00
100	101.07	101.98
	100.40	101.35
	99.66	100.50
120	100.05	101.98
	99.49	101.35
	98.93	100.50
Rata-rata *% recovery*	100.43	100.58
Standard Deviation (SD)	0.98	1.46
Relative Standard Deviation (RSD) (%)	0.98	1.46

Based on the results obtained in Table 4 shows that the average percent recovery obtained for amoxicillin is 100.43% and 100.58% for clavulanate potassium. The results obtained are eligible for the accuracy of the validation of analytical procedures because the average is between the range of 98-102%[9].

Precision Test

Precision test is done by calculating the relative standard deviation. Based on the calculation of data on levels of amoxicillin and clavulanate potassium, obtained relative standard deviation is 0.98% for amoxicillin and clavulanate potassium 1.46%. The relative standard deviation of the results of the two substances which meet the requirements of ≤2%[9].

Table 5. Validation parameters for derivative spectrophotometric

Parameters	Amoxicillin	Clavulanate Potassium
Corr.Coef (r)	0.9995	0.9997
Slope	0.000007	0.000021
Intercept	0.00000025	0.00000135
Accuration (%)	100.43 %	100.58 %
LOD (µg/mL)	1.57 µg/mL	5.26 µg/mL
LOQ (µg/mL)	0.70 µg/mL	2.30 µg/mL

Conclusion

Based on the research conducted, it can be concluded spectrophotometric method with zero crossing derivatives can be used to set the levels of amoxicillin and clavulanate potassium. Levels of amoxicillin and clavulanate potassium in dry syrup preparation Clavamox® and Claneksi® meet the requirements of an oral suspension levels according to the Farmakope Indonesia edisi V[11]. Validation test conducted on dry syrups Clavamox® showed that the spectrophotometric method of derivatives meet the requirements validation, which includes parameters of accuracy and precision.

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