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Implementation of multiple wavelength spectrophotometry method with matrix calculation to determination of acetominophen and caffeine in commercial tablet

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ABSTRACT

A multiple wavelenght spectrophotometric method was validation for determination of binary mixtures acetominophen and caffein that available in antipyretic and analgetics. The aim of the research to determination of acetominophen and caffeine combinations in commercial tablets,using spectrophotometry ultraviolet with multiple wavelength method. The determination of commercial tablet that content 600 mg acetominophen and 50 mg caffeine can use the spectrophotometry ultraviolet with multiple wavelength method, in the solvent 0.1 N hydrochloride acid at the six wavelengths are 223 nm; 243,8 nm; 254 nm; 258 nm; 262 nm; and 271 nm then data calculation with matrix operation. The results of research was exhibited that acetominophen in tablet are 91.75% ± 0.31% and caffeine are 99.49% ± 0.38% and percent coefficient variation of acetominophen are 0.40% and caffeine are 0.36%. So it can be concluded that the spectrophotometry ultraviolet with multiple wavelength method in the present investigation was found to be simple, sensitive, accurate and precise and can be successfully applied for determine the content of acetominophen and caffeine in commercial tablet.

Keywords: Acetominophen, Caffeine, tablet, spectrophotometry ultraviolet, multiple wavelength.

INTRODUCTION

The common combination of analgesics in tablet is acetominophen and caffeine. These compound are selected based on these substances are often found in drugs and sold freely. The use of the mixture of acetominophen and caffeine as an analgesic and antipyretic is well established in pharmaceutical formulation [1]. In order to achieve better curative effect and lower toxicity, it is very important to control the content of acetominophen and caffeine in pharmaceutical tablets. Acetominophen or p aminophenol is 4-hydroxyacetanilide, sparingly soluble in water [2]. Its a metabolite of phenacetin, analgesic efficacy antipiretik without anti-inflammatory activity. Effect analgetics can be enhanced by up to 50 % caffeine. Its an effective to prevent headache and other pain like migraine headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, general pain, toothache, teething pain, period pain; however, its anti-inflammatory effects are much weaker, it has very few side effects [1]. Caffeine or 1, 3, 7 trimethylxanthine is a central nervous system (CNS) stimulant of the methylxanthine class mainly used as diuretic, stimulant to the central nervous and to the cardiovascular systems[2]. Acetominophen assay using ultra violet spectrometry in acid solution at 245 nm and caffeine assay using ultra violet spectrophotometry in acid solution at 273 nm [2] This combination is used for moderate pain in case of headaches. In order to achieve better curative effect and lower toxicity, it is very important to control the content of acetominophen and caffeine in pharmaceutical tablets. However,the presence of more than one active substances in a dosage causing the difficulty to analyze the level of each component. The chemical structure of acetominophen and caffeine was in Fig.1.

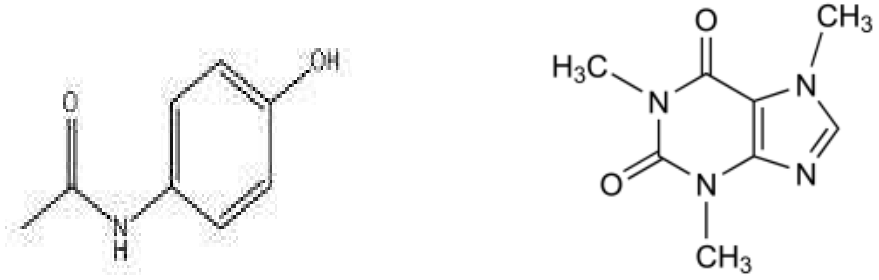

Figure 1. Chemical structure of (a) Acetominophen, (b) Caffein [2]

Several studies on the assay of acetominophen and caffein had been done before, among others, the spectrophotometric derivatives methods, the HPLC method, and ultraviolet spectrophotometry method by multicomponent [3,4,5].

One of the main challenges facing analytical chemists is the spectrophotometry to determination of two or more compounds in the same sample without preliminary separation. Resolving the overlapped spectra of multicomponent mixtures dosage forms whether binary or more as mixtures was rather a difficult task [6]. Ultraviolet spectrophotometry method can be used to analyze single component, but by doing method modification, ultraviolet spectrophotometry can be used for analyze multicomponent. The modification of ultraviolet spectrophotometry method for multicomponent analysis without having prior separation is conducted with the principle of regression equation through matrix calculation. The development of methods performed in accordance with methods of research conducted by Zainuddin (1999), is the multiple wavelength by spectrophometry method with absorption observations of binary mixtures at some point wavelength.

In multiple wavelength method, we get six analysis wavelengths by overlapping the maximum absorption spectrum of each component, then the determination is performed with the principle of regression equation through a matrix calculation. This method is not required the component separation process because the levels of acetominophen and caffeine can be determined together without separation in a short time [7].

The pharmaceutical dosage forms such as tablet must fulfill several requirements in accordance with existing standards, for example in pharmacopoeia. One is the levels requirement. According to the Indonesian pharmacopeia (2014), acetominophen and caffeine tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetominophen and caffeine [2].

In the determination of several substances mixture by multiple wavelength spectrophotometry method must fulfill the validation requirements by several parameters: accuracy and precision. The parameters are sufficient to represent to assess the validity of multiple wavelength spectrophotometry method [7].

The aim of this work is to developed a spectrophotometric method for determination of acetominophen and caffein mixture in tablet dosage form using 0.1 N HCl.

MATERIALS AND METHODS

Apparatus A Shimadzu model 1800 double beam UV-Visible spectrophotometer with spectral bandwith of 1 nm, wavelength accuracy of 0.1 nm (at 656.1 nm D2) was used to measure absorbance of all the solutions. Spectra automatically was obtained by UV-Probe system software was used in the study.

Material And Reagents Pharmaceutical grade of acetominophen and caffeine were supplied by Food and Drug Regulatory Agency of Indonesia, analytical 0.1 N HCl was used throughout these experiments. The commercial contained 600 mg of acetominophen and 50 mg of caffeine per tablet was manufactured by PT Tempo Scan Pacific.

Preparation of Standard Solution An accurately weighed standard acetominophen and caffeine powder (50 mg) were weighed each and transferred to 50 ml separate volumetric flasks and dissolved in 0.1 N HCl. The flasks volume were made up to mark with 0.1 N HCl to give solution contain concentration 1000 µg/ml of each acetominophen and caffeine (standard solution I). Then, the standard solution I was taken 5 ml and inserted into a 50 ml volumetric flasks each, to get 50 µg/ml of volumetric flasks concentration each (standard solution II).

Preparation Maximum Absorption Spectrum of Acetominophen The standard solution II was taken 0.65 ml of acetominophen, then inserted into a 10 ml flask and diluted with 0.1N HCl. Subsequently, the solution was made up to mark with 0.1N HCl to give concentration 6.5 µg/ml of acetominophen. Absorbance was measured at a wavelength of 200-400 nm.

Preparation Maximum Absorption Spectrum of Caffeine The standard solution II was taken 0.86 ml of caffeine concentration 100 µg/ml, then inserted into a 10 ml flask and diluted with 0.1N HCl. Subsequently, the solution was made up to mark with 0.1N HCl to give concentration 8.6 µg/ml of caffeine. Absorbance was measured at a wavelength of 200-400 nm.

Determination Wavelength Analysis And Absorbance Spectrum Acetominophen solution is prepared in concentration 6.5µg/ml and caffeine solution in concentration 8.6µg/ml. Then, both of these solution's absorbance was measured at 200-400 nm wavelength. Furthermore absorption spectrum of each component overlaid, Then searches six point as the wavelength to be used. Wavelength selection is taken from the absorption spectrum of components, the absorbance measured at predetermined multiple wavelengths. Both material's absorbance are determined using linear regression method operated in the concentration and absorbance data of each compound at each wavelength measurements.

Determination Binary Mixture of Aceominophen and Caffein. Carefully weighed 10.0 mg of reference material of acetaminophen (Indonesian Standard) and then inserted into the 10.0 mL flask, diluted with 0.1 N HCl until dissolved to obtain a solution with a concentration of 6.5 ug/mL and carefully weighed 10.0 mg of reference material of caffein (Indonesian Standard) and then inserted into the 10.0 mL flask, diluted with 0.1 N HCl until dissolved to obtain a solution with a concentration of 8.6 ug/ mL caffein. Furthermore, the absorbance measured at six predetermined wavelength.

The standard solution was obtained from the regression equation, y = ax + b, y is the price of absorption (A), a is the regression coefficient showed absorption, x is the concentration, while b is the constant.

Determination Levels Of Acetominophen And Caffeine In Commercial Tablet. Weighed twenty commercial tablets contain 600 mg of acetominophen and 50 mg of caffeine, then crushed in mortar until smooth and homogeneous. Furthermore, a number of weighed powder equivalent to 10 mg of acetominophen, Caffeine was calculated equality contained there in (weighing powders do as much as 6 repetitions). Subsequently put in a 50 ml flask, and dissolved with 0.1 N HCl (homogenized with sonicator for 15 minutes), and then were made up with 0.1 N HCl until the line mark, shaken until homogeneous. The solution was filtered, approximately 10 ml of the first filtrate discarded. Subsequently, the filtrate was accommodated, pipetted 1.58 ml and put in a 50 ml flask.

Caffeine standard solution (concentration = 100 µg/ml) as much as 4.03 ml (to make 8.6 ug/ml Caffeine in tablet solution) put in to 50 ml flask in which contain 1.58 ml filtrate. The mixture was made up to mark with HCl 0.1N in order to obtain solution which contain concentration 6.5µg/ml of acetominophen and concentration 8.6 µg/ml of caffeine. The solution was measured absorption at six wavelengths which have been determined. It should be weighed up to six repetitions.

Calculation Levels of Acetominophen And Caffeine In Commercial Tablet The calculation levels of each component on the mixture were tested on the base of sample absorbance (Ac) and uptake types of each component in the multiple wavelenght known from the results of measurement using matrix equation [7]:

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

Note:
[c] : the levels of the components mixture
[a] : the absorption of matrix of constituent compounds mixture
$[a^1]$: the value of matrix transpose of constituent compounds mixture
$[[a] X [a^1]]^{-1}$: the inverse value of absorption and matrix transpose of constituent compounds mixture
Ac : the value of sample

RESULTS AND DISCUSSION

Determination Of Maximum Absorption Spectrum
Determination of maximum absorption spectrum of Acetominophen and Caffeine performed at wavelength 200-400 nm. Based on the research results were obtained the maximum wavelength of(6.5 µg/ml) Acetominophen at 243.8

nm (Figure 2) and 8.6 µg/ml Caffeine at 271 nm (Figure 3). Then, yhe overlapping maximum absorption spectrum of 6.5 µg/ml Acetominophen and 8.6 µg/ml Caffeine (Figure 4).

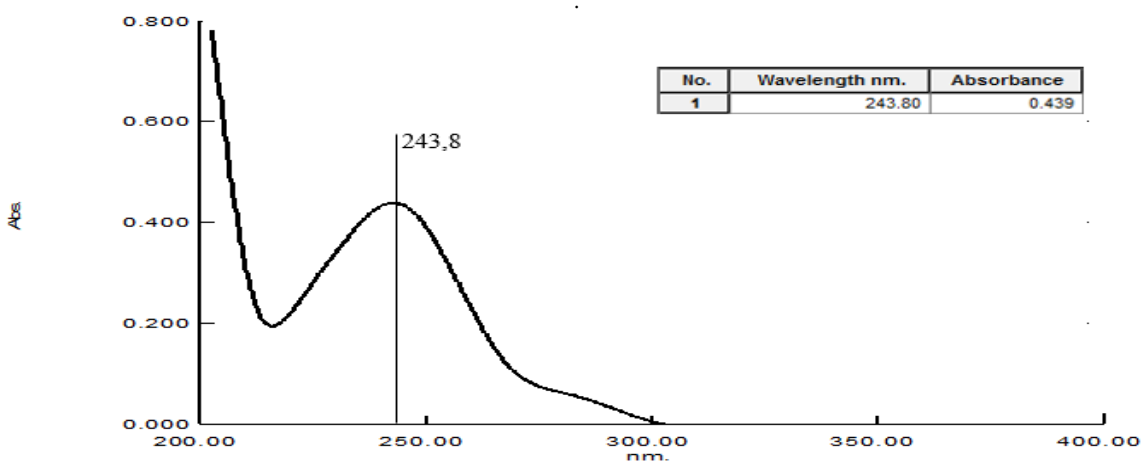

Figure 2. The maximum absorption spectrum of 6.5 ug/ml acetominophen

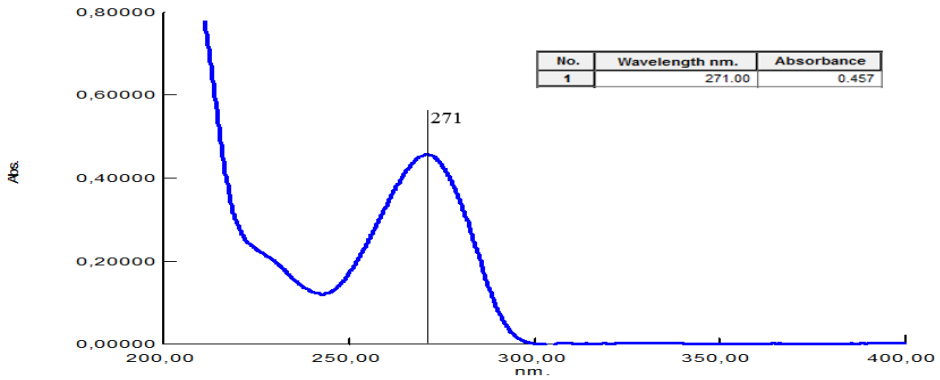

Figure 3. The maximum absorption spectrum of 8.6 ug/ml caffeine

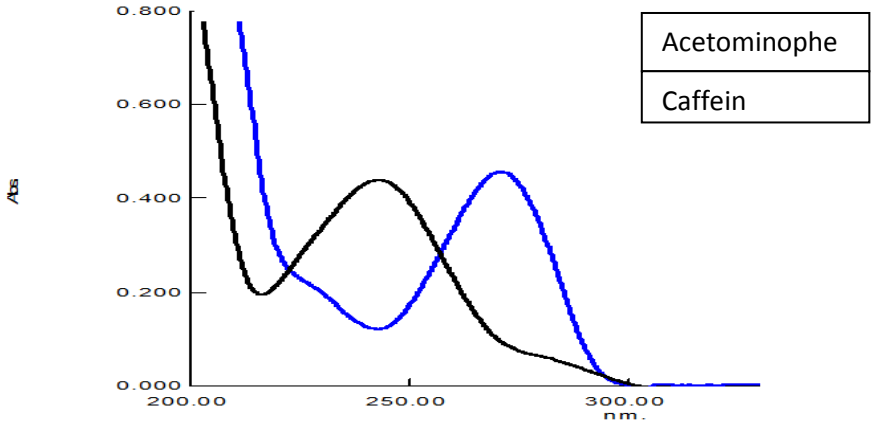

Figure 4. Overlapping maximum absorption spectrum of 6.5 ug/ml acetominophen and 8.6 ug/ml caffeine

Based on the figure 2 and figure 3 showed that the maximum wavelength of each acetominophen and caffeine is 243 nm and 271 nm, Based of Moffat (2011) that the difference in the maximum wavelength of each acetominophen and caffeine are no less and no more than 2 nm. In this case, the maximum wavelength of each component is qualified because the maximum wavelength of acetominophen in acidic condition (A^1_1 = 668a) is located at 245 nm, whereas Caffeine in acidic condition (A^1_1 = 504a) is located at 273 nm [4].

The Determination of Multilenght Wavelength Analysis

In this study, have six points wavelengths to be used in the assay of acetominophen and caffeine in tablet dosage form for a standard solution of acetominophenand caffeine made in six series of concentration to get up to 30 data is the minimal amount to get the data with the characteristics of a normally distributed population [7]. The selected spectrum from absorption spectrum is the concentration of acetominophen 6,5µg / mL and caffeine concentration of 8.6 mg / mL. six point wavelengths to be used can be seen in Figure 5 below.

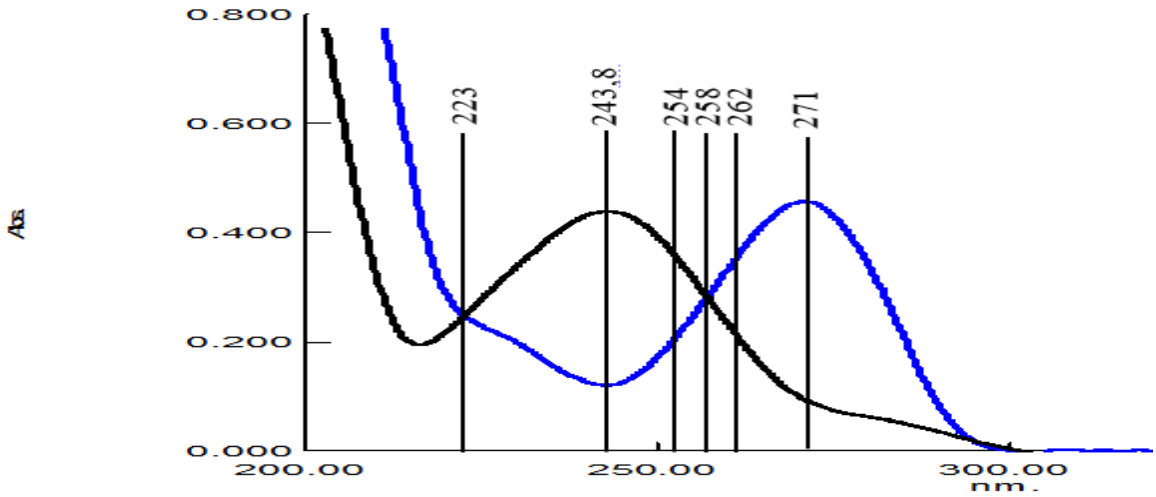

Figure 5. The six wavelengths were used in the determination levels of acetominophen and caffeine in tablets

Based on Figure 5. it can be seen whether the six wavelengths still had absorption at predetermined wavelength. The six wavelengths that be used are 223 nm, was the first intersection of Acetominophen and Caffeine and at this wavelength Acetominophen started giving absorption, toward 243.8 nm was the maximum absorption of acetominophen. Acetominophen provided an appreciable absorption whereas caffein began to decline at 254 nm, and than the second intersection of acetominophen and caffeine on 258 nm and caffeine provided an appreciable absorption whereas acetominophen began to decline at 262 nm, and then the maximum absorption of caffeine at 271 nm.

Determination Absorbance Spectrum Of Multiple Wavelength Method.

The absorbance value is a value that indicates the contributions of a substance's absorbance to the mixture of compounds' absorbance at a particular wavelength. After measuring absorbance of each solution by various concentrations at the six wavelengths (223 nm; 243,8 nm; 254 nm; 258 nm; 262 nm; and 271 nm), the determintation of absorbance value is conducted by operating the absorption data at each wavelength to the concentration of the solution in linear regression equation which analogous to the equation in Beer's law. The linear regression equation is: Y = aX + b. In the linear regression equation, Y shows the absorbance (A), a show absorbance, then the data of acetominophen and caffeine calculation absorption can be seen in Table 1 and Table 2.

The used absorbance value (a) is the absorption value of acetominophen and caffeine at Table 1 and Table 2. The selection of this absorbance value (a) is determined based on the price of (r) count. The (r) count value compared with the value of (r) table with a level of 95% is 0.8114. Based on these data, it appears that the value of (r) count of acetominophen and caffeine is greater than the value of (r) table. This means that the equation has good linearity, because the value of (r) count ranges with values $-1 \leq r \leq 1$ [10]. The obtained absorbance data is then used to establish the levels of acetominophen and caffeine in the mix with matrix calculation [9-10].

Table 1. Data of absorption calculation of acetominophen

C (µg/mL)	λ1(223 nm)	λ2(243.8 nm)	λ3(254 nm)	λ4(258 nm)	λ5(262 nm)	λ6(271 nm)
	A	A	A	A	A	A
0	0	0	0	0	0	0
3	0.12897	0.21126	0.16492	0.13570	0.10417	0.05876
4.5	0.19606	0.31613	0.24577	0.20288	0.15692	0.08293
6.5	0.27245	0.45010	0.35638	0.28804	0.22153	0.12358
7.5	0.31456	0.51523	0.41245	0.32903	0.25388	0.13927
9	0.38654	0.62891	0.49312	0.40448	0.31181	0.16801
	A = 0.0424 B = 0.0009	A = 0.0693 B = 0.0014	A = 0.0549 B = -0.0001	A = 0.0444 B = 0.0008	A = 0.0343 B = 0.0006	A = 0.0186 B = 0.0009
	r = 0.999627	r = 0.999871	r = 0.999992	r = 0.999766	r = 0.999759	r = 0.999667

Table 2. Data of absorption calculation of caffeine

C (µg/mL)	λ1(223 nm)	λ2(243.8 nm)	λ3(254 nm)	λ4(258 nm)	λ5(262 nm)	λ6(271 nm)
	A	A	A	A	A	A
0	0	0	0	0	0	0
4	0.11899	0.05576	0.11423	0.13538	0.16377	0.21051
6	0.17996	0.08450	0.16805	0.20416	0.25090	0.31732
8.6	0.25688	0.12495	0.23895	0.29681	0.35294	0.45888
10	0.30049	0.14691	0.27908	0.34697	0.42247	0.53935
12	0.36343	0.17093	0.33359	0.42044	0.49663	0.64505
	A = 0.0302	A = 0.0145	A = 0.0277	A = 0.035	A = 0.0417	A = 0.0539
	B = -0.0012	B = -0.0008	B = 0.0013	B = -0.003	B = -0.0007	B = -0.003
	r = 0.9999	r = 0.9995	r = 0.9999	r = 0.9998	r = 0.9997	r = 0.9999

Note:
c = concentration
a = the value of regression coefficient
b = constant
r = correlation coefficient

Content, Coefficient Of Variation, Acuracy and Precesion Of Acetominophen And Caffeine In Commercial Tablet

Sample solution preparation of the commercial tablet was done by standard addition method, because that measurement in this mixture berdasarkn concentration of acetaminophen with a concentration of 6.5 ug / ml resulting content is very small because of the concentration of caffeine in the mixture is small so need to add caffeine to the measurement meets the requirements of the Beer's law. According Harmita (2004), the method of addition, a number of samples analyzed plus analyte concentration is usually 80% to 120 % of the estimated analyte concentration, mixed and re-analyzed . Difference between the two results are compared with actual levels [10].

The prepared samples were measured absorbance at six wavelengths which had been obtained earlier, those were 223 nm; 243.8 nm; 254 nm; 258 nm; 262 nm; and 271 nm.

Table 3. Data of absorption spectrum of acetaminophen and caffeine mixture in commercial tablet

Replication	λ1(223 nm)	λ2(243.8 nm)	λ3(254 nm)	λ4(258 nm)	λ5(262 nm)	λ6(271 nm)
1	0.49612	0.50152	0.49744	0.49723	0.49914	0.49848
2	0.54002	0.54234	0.53725	0.53744	0.54147	0.54091
3	0.55977	0.56002	0.56008	0.55953	0.56145	0.56275
4	0.56885	0.57003	0.56549	0.56432	0.56921	0.56948
5	0.57813	0.57901	0.57648	0.57552	0.57935	0.57853
6	0.56876	0.56921	0.56825	0.56902	0.56901	0.56930

Based of Table 3 that data of absorbance of acetaminophen and caffeine mixture in commercial tablet that has been obtained is used to measure the levels of each substances by entering the data available on the matrix calculation formula. After the calculation, it will be obtained the concentration of each component with the accuracy from the results of the matrix and the coefficient of variation (CV) [7,9-13].

Matrix calculation of acetominophen and caffein, for example sample preparation number one :

$$\begin{pmatrix} C1 \\ C2 \end{pmatrix} = \left(\begin{pmatrix} 0.0424 & 0.0693 & 0.0549 & 0.0444 & 0.0343 & 0.0186 \\ 0.0302 & 0.0145 & 0.0302 & 0.0350 & 0.0417 & 0.0539 \end{pmatrix} \begin{pmatrix} 0.0424 & 0.0302 \\ 0.0693 & 0.0145 \\ 0.0549 & 0.0302 \\ 0.0444 & 0.0350 \\ 0.0343 & 0.0417 \\ 0.0186 & 0.0539 \end{pmatrix}^{1} \right)^{-1} \times \begin{pmatrix} 0.0424 & 0.0693 & 0.0549 & 0.0444 & 0.0343 & 0.0186 \\ 0.0302 & 0.0145 & 0.0302 & 0.0350 & 0.0417 & 0.0539 \end{pmatrix} \begin{pmatrix} 0.49612 \\ 0.54002 \\ 0.55977 \\ 0.56885 \\ 0.57813 \\ 0.56876 \end{pmatrix}$$

$$\begin{pmatrix} C1 \\ C2 \end{pmatrix} = \begin{pmatrix} 5.939427 \\ 8.597759 \end{pmatrix}$$

The result of the calculation of the levels and the coefficient of variation of acetominophen and caffein levels in the commercial tablet can be seen in Table 4.

Based of Table 4 can be seen that the coefficient of variation (% CV) obtained in acetominophen and caffein is 0.40% and 0.36%, respectively. It means having a good precision.

The absorbance data of sample solution has been obtained is used to measure each levels by entering the available data on the matrix calculation formula. From the calculation will be obtained each component mixture concentration, then calculated the accuracy of the matrix result and coefficient variation (%CV). Accuracy of matrix

calculation are used to determine the accuracy of an analytical method, while the coefficient of variation (% CV) is used to determine the precision of an analytical method. The accuracy of an analytical method for the drug substance with low concentrations considered good when the value range between 90-107% accuracy, while an analytical method is said to have good precision if the coefficient of variation (% CV) <2% [9,11-14]. The coefficient of variation (% CV) obtained in acetominophen and caffein is 0.40% and 0.36%. The results of the levels acetominophen and caffeine in commercial tablet can be seen in Table 5.

Table 4. Accuracy, precision and coefficient of variation of acetominophen and caffeine in commercial tablet

Sample	Acetominophen			Caffein		
	Measurable levels (µg/mL)	Theoretical levels (ug / mL)	The accuracy of the matrix (%)	Measurable levels (µg/mL)	Theoretical levels (ug / mL)	The accuracy of the matrix (%)
1	5.9677	6.5213	91.51	0.5313	0.5357	99.17
2	6.0157	6.5345	92.06	0.5293	0.5299	99.87
3	5.9385	6.6492	91.47	0.5310	0.5356	99.13
4	5.9209	6.4737	91.46	0.5285	0.5328	99.20
5	5.9889	6.5009	92.01	0.5324	0.5338	99.72
6	5.9746	6.4941	92.00	0.5338	0.5344	99.87
Mean			91.75	Mean		99.38
% CV			0.40	% CV		0.36
Accuracy			good	Accuracy		good
Precision			good	Precision		good

Table 5. Levels of acetominophen and caffeine in commercial tablet

No.	Drug	Commercial Tablet	Accuracy	Coefficient Variation (%CV)
1.	Acetominophen	91.75% ± 0.39% (550.26 mg – 554.94 mg)	91.71% – 92,49%	0.40%
2.	Caffeine	99,49% ± 0.34% (48.88 mg – 49.22 mg)	97,76% – 98,44%	0.36%

Based from Table 5 can be seen that acetominophen and caffein levels in preparation Tablet meets the requirements according to Indonesian Pharmacopoeia [2], namely for the preparation of acetominophen and caffein mixture in tablets that is not less than 90.0% and not more than 110.0% of the amount listed on the label [2].

CONCLUSION

Based on the result of this researched, it can be concluded:

1. The multiple wavelengths by spectrophotometry with matrix operation method was implementation for the simultaneous determination of acetaminophen and caffeine in tablet formulation without any interference from the excipients. The results of our study indicate that these methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of acetominophen and caffeine,
2. The levels of acetominophen and caffeine in tablet that determined with multiple wavelengths by spectrophotometry fulfilled the requirements in the Indonesian Pharmacopoeia (2014) which the results levels are 91.75% ± 0.39% and 99.49% ± 0.34% for each acetominophen and caffeine.

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