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Research article

Development and validation of ultraviolet spectrophotometric method for estimated mixture of paracetamol, acetosal and caffeine in tablet dosage form

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Key words: Paracetamol, Acetosal, Caffeine, Derivative Spectrophotometry.

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Abstract

Objective: The aim of this research was to develop and test the validation of derivative spectrophotometry method to determine the level of paracetamol, acetosal, and caffeine in tablet dosage form without prior separation. **Method:** The study was conducted with tablet mixtures of paracetamol, acetosal, and caffeine using derivative spectrophotometry with a zero-crossing technique. Ethanol was used as a solvent for the analysis. **Result:** The results showed that the application of derivative spectrophotometry method on the determination of paracetamol level carried out on the third derivative at λ 267.6 nm (Δλ2), determination of acetosal level performed on the third derivative at λ 236.2 nm (Δλ2), and determination of caffeine level performed on the second derivative at λ 235.8 nm (Δλ 16) resulted in the level of 101.198%, 105.78%, and 107.74% respectively for paracetamol, acetosal, and caffeine. Therefore, it was suggested that result of determination of paracetamol, acetosal and caffeine mixture in tablet is the requirement of united states Pharmacopeial (USP 32 NF 27, 2008). **Conclusion:** The derivative spectrophotometric method fulfilled the requirements of accuracy and precision, so it can be used to determinate the level of paracetamol, acetosal, and caffeine in tablets.

Introduction

Paracetamol, acetosal, and caffeine are often combined as antipyretic and analgesic drugs. This mixture aims to improve the therapeutic effect, and it is easy to use [1-2]. Derivative spectrophotometric method or derivative curve method is one of the spectrophotometric methods that can be used to analyze a mixture of several substances directly without previous separation, even with adjacent wavelengths. Some advantages of the derivative spectrum are that derivative spectrum provides an overview of the detailed structure of the absorption spectrum, and this description becomes clearer from the first derivative to the fourth derivative spectra [3].

Various studies have been conducted on derivative spectrophotometry. Hajian and Soltaninezhad, (2012) investigated the multi-component spectrophotometry of a ternary mixture of paracetamol, aspirin, and caffeine using double divisor–ratio spectra derivative method [4]. Furthermore, another study also ivestigated a multivariate calibration technique of paracetamol, aspirin, and caffeine in pharmaceutical formulations using classical least squares (CLS) and inverse least squares (ILS) methods on spectrophotometric devices with 0.1M HCl [5]. The study by Ali *et al.* (2012) examined the validated spectrophotometric and spectrodensitometric method in the determination of a ternary mixture of analgesic drugs in different doses (paracetamol, aspirin, caffeine). The spectrophotometric method used the application of ratio spectra derivative with methanol solvent [6]. Another study by Wijaningtyas (2015) investigated a combination of UV spectrophotometry and multivariate calibration for the analysis of paracetamol, acetosal, and caffeine in the tablet dosage form. The study evaluated the ability of the UV spectrophotometric method combined with partial least square (PLS) multivariate calibration using ethanol solvent [7].

The present study conducted the derivative spectrophotometry to determine the level of the mixture of paracetamol, acetosal, and caffeine using the zero-crossing method. This method was simple, accurate, precise, and quickly developed for the estimated mixture of paracetamol, acetosal, and caffeine in the tablet dosage form. The method was validated for the mixture of paracetamol, acetosal, and caffeine in the tablet dosage form and must fulfill the validation requirements of the analytical method. Thus, the validation of the analytical method was aimed to ensure that the analytical method has met the specifications that can be accepted in accordance to the expected objectives [8-9]. It is necessary to determine the level of paracetamol (PAR), acetosal (ACE), and caffeine (CAF) in the mixture on tablet preparations using the derivative

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spectrophotometric method with the zero-crossing method.

Materials and methods

Instruments

UV-Visible 1800 spectrophotometer (Shimadzu) and a personal computer (PC) equipped with 2.42 UV-Probe software, cuvette 1 cm, oven, glassware, mortar and pestle, analytical balance (Boeco), and a sonicator (Branson 1510).

Materials

All reagents used in the study were analytical grade unless stated otherwise. Paracetamol, Acetosal, Caffeine purchase from Brataco Indonesia, Ethanol Pruchase form E-Merck, Distilled water purchased from PT. IkaPharmindo, Whatman filter paper No. 41, and parchment paper.

Sampling

Purposive sampling was used in the study, and the samples were PoldanMig® tablets containing 400 mg of paracetamol, 250 mg of acetosal, and 65 mg of caffeine.

Preparation of stock solutions

Stock solutions which contained 50 µg/mL PAR, 50 µg/mL ACE, and 50 µg/mL CAF were prepared by using ethanol solvent. Further dilution was carried out using ethanol which was explained in the preparation of standard solutions.

Preparation of standard solutions

Standard solutions of PAR, ACE, and CAF were prepared, each of which was put in a 10-mL volumetric flask. The standard solution was dissolved with ethanol solvent, so the concentrations obtained for PAR were 2.5, 3.5, 4.5, 5.5, and 6.5, the concentrations obtained for ACE were 5, 7, 9, 11, and 13, and the concentrations obtained for CAF were 5, 7.5, 10, 12.5, and 15. The absorptions at the wavelengths of 200-400 nm were measured. The absorption spectrum of each solution was explained in the preparation of the calibration graph.

Determination of the wavelength of analysis

The solution of 4.5 µg/mL PAR, 9 µg/mL ACE, and 10 µg/mL CAF and the mixture solution of PAR, ACE, and CAF (4.5 µg/mL, 9µg/mL, and 10 µg/mL) were prepared. The solutions were then measured at the wavelengths of 200-400 nm. Subsequently, the solutions were transformed into the first, second, third, and fourth derivative absorption spectra with Δλ 2, 4, 8 and 16. The wavelength of analysis used in the study was a certain wavelength where a single absorption of the two compounds gave a zero-value while a single absorption of another compound and the ternary mixture gave a similar or completely the same value.

Determination of PAR, ACE, and CAF levels in tablet dosage form

Twenty tablets were weighed and crushed homogeneously. The powder was weighed equal to 100 mg of paracetamol, and the equivalent weight of acetosal and caffeine contained in it was also calculated. The powder was put into a 100 mL volumetric flask and filled with ethanol solvent until it reached the marked line. The solution was homogenized using a sonicator for 15 minutes and filtered (±25 ml of the first filtrate was removed whereas the next filtrate was collected). After that, 0.1 mL of the filtrate solution was put into a 25 mL volumetric flask using a pipette and filled with ethanol until it reached the marked line. The absorbance was then measured at the wavelength of analysis that has been determined using the zero-crossing method.

Validation of the method

The method performed has been widely validated in terms of linearity, accuracy, and precision. The accuracy of the method was determined by calculating the recovery of PAR, ACE, and CAF by adding raw materials.

Results and discussion

The overlay of the absorption spectrum of paracetamol, acetosal, and caffeine with the absorption spectrum of the mixture of paracetamol, acetosal, and caffeine can be seen in Figure 1.

In this case, the standard solutions of PAR, ACE, and CAF were prepared in the concentration range expressed in the calibration curve.

The determination for paracetamol obtained from the standard solution of PAR, ACE, CAF, and the mixture solution was measured in the absorbance on the third derivative (Δλ 2 nm) at the analysis wavelength of 267.6 nm where the absorbance of ACE and CAF gave zero value while the absorbance of another compound and the ternary mixture was nearly or completely the same (Figure 2). An analysis of the relationship between concentration and absorption value was then calculated to obtain a linear regression equation (zero- crossing point of ACE and CAF).

The determination for acetosal obtained from the standard solution of PAR, ACE, CAF, and the mixture solution was measured in the absorbance on the third derivative (Δλ 2 nm) at the analysis wavelength of 236.2 nm where the absorbance of PAR and CAF gave zero value while the absorbance of another compound and the ternary mixture was nearly or completely the same (Figure 2). The relationship analysis between concentration and absorption value was also calculated to obtain a linear regression equation (zero-crossing point of PAR and CAF).

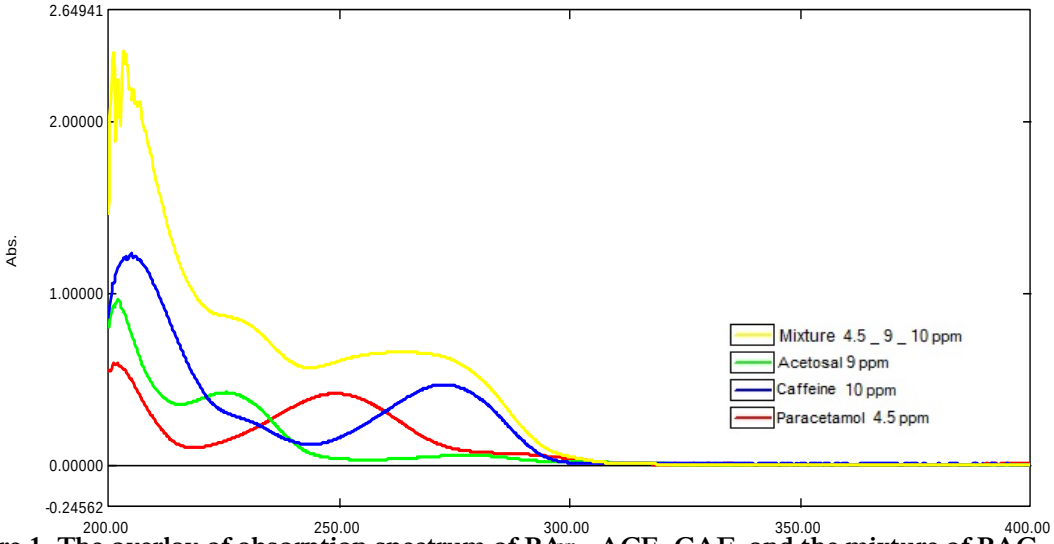

Figure 1. The overlay of absorption spectrum of PAnm., ACE, CAF, and the mixture of PAC.

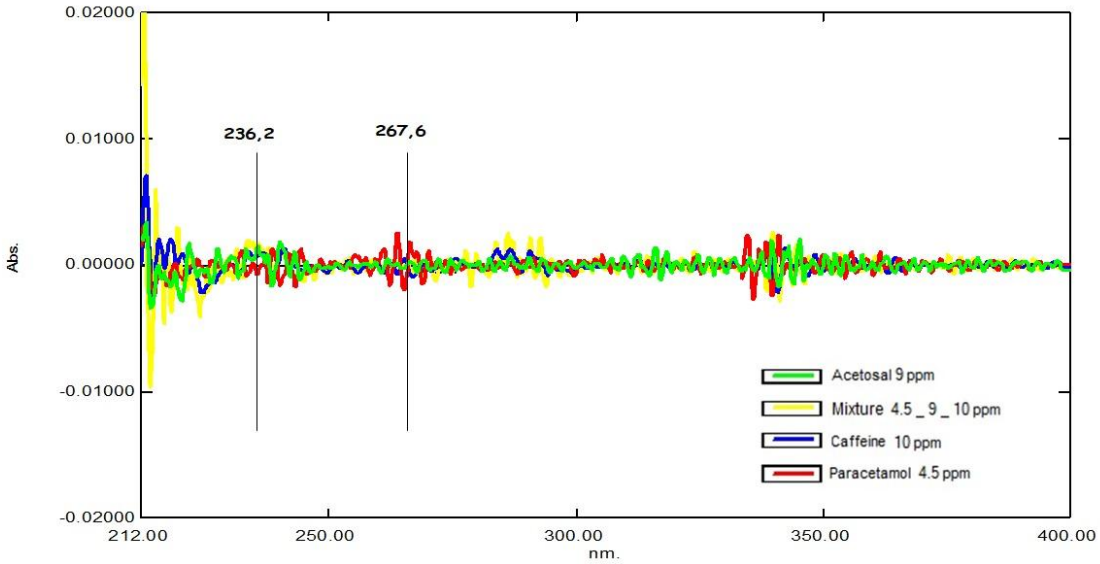

Figure 2. The wavelength of analysis of PAR at 267.6 nm and ACE at 236.2 nm.

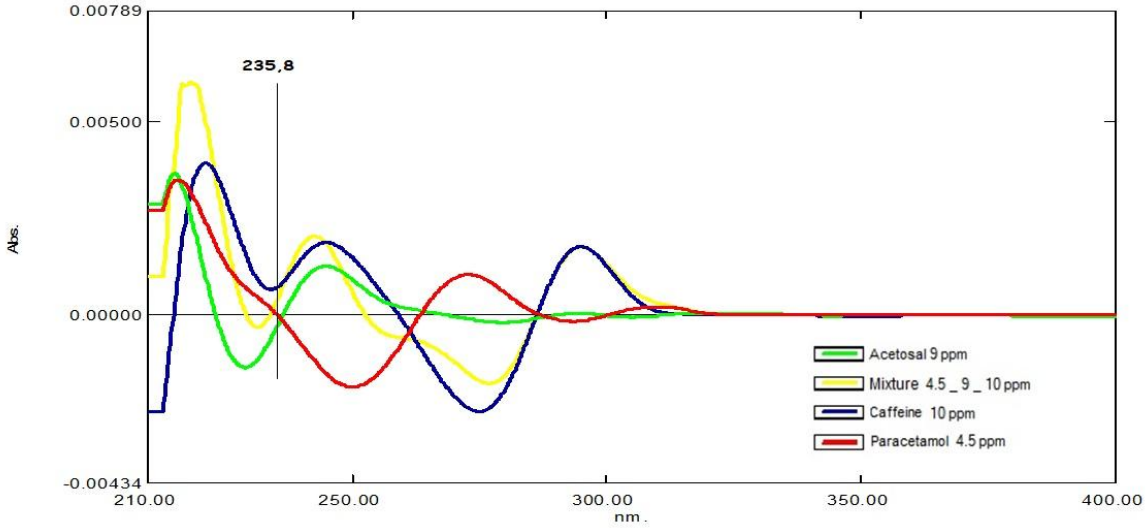

Figure 3. The wavelength of analysis (Δλ 16 nm) of CAF at 235.8 nm.

The determination for caffeine obtained from the standard solution of PAR, ACE, CAF, and the mixture solution was measured in the absorbance on the second derivative (Δλ 16 nm) at the analysis wavelength of 235.8 nm where the absorbance of PAR and ACE gave zero value while the absorbance of another compound and the ternary mixture was nearly or completely the same (Figure 3). The relationship analysis between concentration and absorption value was calculated to obtain a linear regression equation (zero-crossing point of PAR and ACE).

The wavelengths of 267.6 nm, 236.2 nm, for ACE and PAR are used derivative 3 Δλ 2, and the wavelength 235.8 nm for caffeine is used derivative R Δλ 16.

Validation method

Table 1 shows that the zero-crossing method met the validation requirements for the parameter of linearity, limit of detection (LOD), and limit of quantitation (LOQ). The linearity value was illustrated by almost all of the correlation coefficients for the zero-crossing method were close to one which indicated that there was an immensely good relationship or correlation between concentration and absorbance according to the Lambert-Beer law. The test was performed according to the treatment stated previously.

Determination of the mixture of PAR, ACE, and CAF level in the tablet dosage form

The results of the level determination in the tablets containing 400 mg of PAR, 250 mg of ACE, and 65 mg of CAF with the zero-crossing method can be seen in Table 2.

In table 2 shows that parameter of PAR, ACE and CAF in mixture were analyzed for the level of PAC are 100.19%, ACE are 105.78%, and CAF are 107.74%. Therefore, the mixture tablet has qualified the requirements where the level at 98-110% in accordance with the united states Pharmacopeial [10].

Linearity was assessed for PAR, ACE and CAF by plotting calibration curves of the absorbance versus the concentration over the concentration.

Accuracy was assessed by the determination of the recovery of the method by addition standard drug. To the prequantified sample preparation of PAR, ACE and CAF at 3 different concentration level 80, 100, 120%. The % recovery was found that the method has qualified validation method at 98 – 102%.

Table 1. Validation parameter for the zero-crossing method.

Sr. No.	Parameter	Zero-crossing Method		
		Paracetamol	**Acetosal**	**Caffeine**
1	Linearity (R^2)	0.9996	0.9954	0.9963
2	LOD	0.2320	1.63484	1.16673
3	LOQ	0.7031	4.95405	3.53556

Table 2. Parameter of PAR, ACE, and CAF in the tablet dosage form.

Sr. No.	Parameter	Zero-crossing Method		
		Paracetamol	**Acetosal**	**Caffeine**
1	Level (%)	100.19%	105.78%	107.74%
2	Claim on the Label (mg)	400	250	65
4.	Precision (% RSD)	0.7389	1.0071	0.9283

Table 3. Statistical data of PAR, ACE, and CAF for the zero-crossing method.

Sr. No.	Parameters	Zero Crossing Method		
		Paracetamol	**Acetosal**	**Caffeine**
1	Linear range	2.5-6.5 mcg/ml	5 – 13 mcg/ml	5-15 mcg/ml
2	Slope	0.05720	0.02623	0.02606
3	Intercept	-0.0015	0.000124	- 0.01010
4	Regression coefficient (r2)	0.9996	0.9954	0.9963

Table 4. Accuracy data of PAR, ACE and CAF for the zero-crossing method.

Drug	Level	Amount taken (total) mcg/ml	Amount added (mcg/ml)	Amount recovered (mcg/ml)	% Recovery ± SD
PAR	80 %	24	79.3250	55.7128	98.61 ± 0.498
	100 %	30	100.7575	70.9164	99.77 ± 0.424
	120 %	36	120.41	85.0072	98.64 ± 0.629
ACE	80 %	15	51.8352	36.7275	100.76 ± 0.796
	100 %	18.75	64.9405	46.3296	99.31 ± 0.866
	120 %	22.5	78.0456	55.5445	100.05 ± 1.044
CAF	80 %	3.9	13.6402	9.7261	100.43 ± 0.958
	100 %	4.875	17.0845	12.6689	98.85 ± 0.576
	120 %	5.85	20.5110	14.7092	99.24 ± 0.351

Conclusion

The zero-crossing derivative spectrophotometric method can determine the level of the mixtures of PAR, ACE, and CAF in the tablet dosage form that meets the requirements for dosage levels. The development of the zero-crossing derivative spectrophotometry conducted has met the requirements of method validation so that this method can be applied to the laboratory quality test in the dosage form.

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