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Simultaneous Determination of Amoxicillin Trihydrate and Potassium Clavulanate Levels in Combination Drug Preparations Using Video-Densitometric Thin Layer Chromatography Method

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ABSTRACT: Amoxicillin trihydrate - potassium clavulanate is a combination antibiotic to treat bacteria that can destroy β-lactams. The study aimed to validate the analytical method of amoxicillin trihydrate and potassium clavulanate determination using the thin layer chromatography (TLC) video densitometry method. The stationary phase used was silica gel plate GF254, the mobile phase was ethyl acetate - glacial acetic acid-water (4: 1.5: 0.5 v/v). Spots were detected and recorded using a mirrorless camera under UV light at 254 nm and analyzed using ImageJ software to obtain the chromatogram and AUC values. The validation results showed that the Limit of Detection, Limit of Quantitation, accuracy, precision, and intermediate precision of amoxicillin trihydrate were 39.48 g/mL, 131.60 g/mL, 99.8 %, 0.515%, and 0.691%, respectively. While for potassium clavulanate were 10.18 g/mL, 33.95 g/mL, 100.1%, 0.459% and 0.561%, respectively. Amoxicillin trihydrate and potassium clavulanate levels in sample A were 99.82% and 99.13%, while for sample B were 99.35% and 99.0%, respectively. It can be concluded that the TLC video densitometry method can determine the levels of amoxicillin trihydrate and potassium clavulanate simultaneously.

KEYWORDS: Amoxicillin trihydrate, Potassium Clavulanate, Drug Combination, TLC Video Densitometry

I. INTRODUCTION

Antibiotics are antibacterial substances produced by various species of microorganisms that can suppress the growth and or kill other microorganisms. The effectiveness of a drug must reach its therapy; when it does not reach therapy and its use in the long term, it can cause resistance, so to increase the therapeutic effect, a combination of drugs can be used (1). Amoxicillin is an antibiotic often used as the first choice of infection therapy. Because amoxicillin is a broad-spectrum antibiotic that can kill gram-positive and gram-negative bacteria. Associated with a large number of cases of amoxicillin resistance, there is a combination of amoxicillin and clavulanate preparations. This combination is an antibacterial combination consisting of -lactam derivatives and -lactamase inhibitors. This combination is given to treat -lactam-producing (-lactamase-producing) bacteria, with the aim of increasing its spectrum of action against -lactamase-producing bacteria that are resistant to amoxicillin antibiotics (2,3).

Thin layer chromatography combined with densitometry can simultaneously determine the levels of several active ingredients in a combination preparation (4,5). Densitometry is an instrumental analytical method based on the interaction of electromagnetic radiation with the analyte in the form of spots on a TLC plate. Densitometry is a method of determining the concentration of a compound on a TLC plate using a TLC scanner instrument. This measurement is carried out by measuring the absorption of the analyte where the light measured can be reflected or transmitted light. Electromagnetic radiation that comes to the plate is absorbed by the analyte and produces emission in the form of fluorescence and phosphorescence. The presence of an analyte in the spot will block the emission of the stationary phase (adsorbent). This tool is equipped with a photometer detector whose wavelength can be adjusted from 200-700 nm (6).

The video densitometry technique was brought to the densitometric evaluation of thin-layer chromatograms. Several features of TV-type multichannel detectors are not seen in commercially available densitometers. The fundamental distinction stems from the scanning principle: instead of using mechanical movement of the specimen and/or light source, the scanning is done electronically. The scanning is extremely quick, and

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the geometry is unlike that of typical densitometers. These additional qualities necessitated a unique technical solution in instrumentation design, allowing densitometric evaluation to be applied to new application fields (7). Video densitometry is a method with the working principle of optical scanning that takes place electronically using a computer with a digital camera, a light source to illuminate the plate and focus the image. The advantages of this method in thin layer chromatography are fast and simultaneous data acquisition, simple instrument design, and compatibility with data analysts. The disadvantage of this method is that if there is a problem with layer lighting during image acquisition, it can be overcome with proper lighting to increase image contrast and resolution (8).

II. METHODOLOGY

This research was divided into several stages: the search for optimum conditions for separation using TLC plates, camera settings, system suitability testing, analytical method validation, sample preparation, and determination of amoxicillin trihydrate and potassium clavulanate levels in the combination drug sample. The solvent used was chosen, which can dissolve both analytes well, while the mobile phase selected must produce good separation with an Rf range between 0.2 to 0.8. Visualization of TLC spots using 254 nm UV lamp, then recorded using a SONY MIRRORLESS camera with settings that produce a constant intensity throughout the measurement. The recorded images were then analyzed using ImageJ software to create a chromatogram and calculate the area under the curve of each peak as a measuring parameter. Furthermore, validation of the analytical method was carried out with selectivity parameters, linearity, the limit of detection, the limit of quantitation, accuracy, and precision (9). Determination of amoxicillin trihydrate and potassium clavulanate levels in a combination drug sample using a validated chromatographic system.

III. RESULT AND DISCUSSION

TLC optimization

Aquadest was chosen as the solvent because it can dissolve both amoxicillin trihydrate and potassium clavulanate (10). The combination of ethyl acetate, glacial acetic acid, methanol, and water (4: 1.5: 0.5v/v) as the mobile phase resulted in the retention factor (Rf) value of amoxicillin trihydrate and potassium clavulanate 0.56 and 0.72, respectively. The composition produced the appropriate mobile phase polarity to optimally separate the amoxicillin trihydrate and potassium clavulanate compounds in the sample.

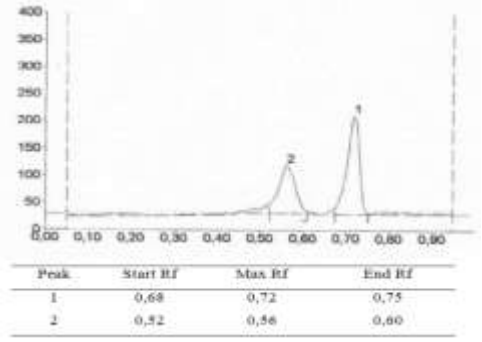
Camera settings

A Sony mirrorless camera equipped with an 18-50mm lens was used to record the TLC plate. The ISO sensitivity was set at 100, shutter speed at 1/6, aperture at f/5.6 at 50mm focal point to produce a constant intensity throughout the measurement.

The ISO value determines the level of sensitivity of the sensor to light. The higher the ISO value, the more sensitive the sensor will be to light, but it will also increase the noise in the image. Shutter functions to adjust how long the light will hit the sensor, expressed in units of seconds. The shorter the shutter speed, the less light enters, and vice versa. Aperture serves to adjust the amount of light that enters the lens by increasing or decreasing the size of the diaphragm, expressed in terms of the f value in the form of a fractional scale from the largest to the smallest (example: f/2.8; f/3.5; f/8, etc.). A small f value indicates a large aperture, while a large f value indicates a small aperture.

System suitability test

A suitable chromatographic system is indicated with a resolution value (Rs) of more than 1.5.



Peak	Start Rf	Max Rf	End Rf
1	0,68	0,72	0,75
2	0,52	0,56	0,60

Figure 1: Chromatogram of amoxicillin trihydrate-potassium clavulanate mixture standard solution

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Resolution (Rs) $= \frac{2\,(\mathrm{dr\,A} - \mathrm{drB})}{(\mathrm{WA} + \mathrm{WB})} = \frac{2\,(0.72-0.56)}{(0.07+0.08)}$
= 2.13

Silica gel GF254 stationary phase, ethyl acetate, glacial acetic acid, methanol, and water (4: 1.5: 0.5v/v) mobile phase with spot detection under 254 nm UV lamp resulted in a suitable chromatographic system with a resolution of 2.13.

Analytical method validation

Selectivity

The selectivity factor (α) was measured by comparing the Rf values of the two analytes in the mixed standard solution.

Figure 2: Chromatogram of selectivity test

(1) potassium clavulanate standard solution; (2) amoxicillin trihydrate standard solution; (3) sample; (4) sample and potassium clavulanate standard solution; (5) sample and amoxicillin trihydrate standard solution

Selectivity factor (α)= $\frac{\mathrm{drA}}{\mathrm{drB}} = \frac{0.72}{0.56} = 1.28$

This method is selective because it can provide a selective response to amoxicillin trihydrate and potassium clavulanate with an α value of more than 1.0.

Thus it can be stated that the TLC video densitometry method meets the selectivity parameters in determining the levels of amoxicillin trihydrate and potassium clavulanate.

Linearity

Linearity is the ability of an analytical method to show a proportional response to the analyte concentration in the sample and within the concentration range used. The linearity of the analytical method is determined by making a series of analyte concentrations to create a calibration curve. The parameters measured are the correlation coefficient (r) and the regression function coefficient (Vx0) (9).

Figure 3 and 4 showed that based on the calibration curve data from a series of standard solutions, it was found that the values of r and Vx0 for amoxicillin trihydrate were 0.9992 and 0.20%, respectively, while for potassium clavulanate were 0.9994 and 0.2%, respectively. Thus, both meet the linearity requirements: r is more than 0.99, and Vx0 is less than 2.0%.

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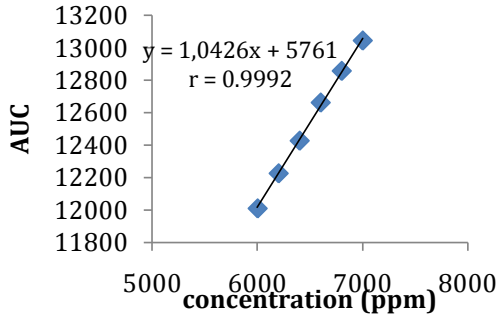

Figure 3: Calibration curve of amoxicillin trihydrate

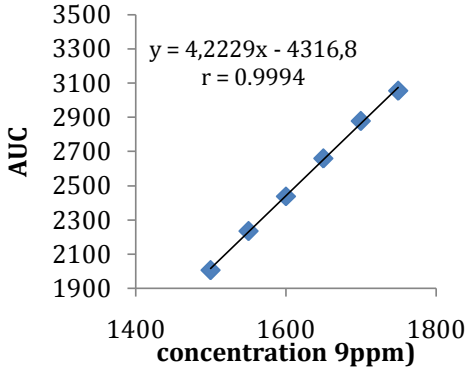

Figure 3: Calibration curve ofpotassium clavulanate

Sensitivity

The limit of detection (LOD) is calculated to determine the lowest analyte concentration detected by the analytical method used at a good level of accuracy and precision. Meanwhile, the limit of quantitation (LOQ) is calculated to determine the lowest analyte concentration that can be determined by the analytical method used at a good level of accuracy and precision.

The results showed that the LOD and LOQ values of amoxicillin trihydrate were 39.48µg/mL and 131.69 g/mL, respectively, while for potassium clavulanate were 10.18µg/mL and 33.95 g/mL, respectively. This shows that the levels of amoxicillin trihydrate and potassium clavulanate in the samples, which can still be adequately determined, are not less than 131.69 g/mL and 33.95 g/mL, respectively. Thus, the range of standard solutions of amoxicillin trihydrate and potassium clavulanate must be above this value.

Accuracy and precision

The recovery test was carried out using the spiked-placebo recovery with levels ranging from 80%, 100%, and 120% of the dosage.

Table 1: The recovery test results

Sample	Theoretical concentration (µg / mL)	Calculated concentration (µg / mL)	% recovery
Amoxicillin trihydrate	5200	5181	99.634
		5168	99.384
		5173	99.480

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	Average		**99.5**
	6500	6499	99.984
		6478	99.661
		6484	99.753
	Average		**99.8**
	7800	7758	99.461
		7741	99.243
		7769	99.602
	Average		**99.435**
Potassium clavulanate	1300	1293	99.461
		1289	99.153
		1291	99.307
	Average		**99.307**
	1650	1624	99.938
		1636	100.308
		1627	100.123
	Average		**100.123**
	1950	1940	99.487
		1944	99.692
		1941	99.538
	Average		**99.572**

The data in Table 1 showed that the recovery for amoxicillin trihydrate at 80%, 100%, and 120% of the dosage were 99.5%, 99.8%, and 99.4%, respectively, and potassium clavulanate were 99.3%, 100.1%, and 99.5%, respectively. Thus the accuracy meets the requirements, which is in the range of 98-102%.

Intermediate precision (also known as within-laboratory or within-device precision) is a measure of precision under a set of parameters that include the same measurement process, same measuring apparatus, same location, and replicate measurements on the same or comparable objects over an extended period.

The calculation of intermediate precision was carried out by calculating the levels of amoxicillin trihydrate and potassium clavulanate in the simulated sample for three consecutive days with six replications per day.

Table 2: The precision test results

Sample	Day	Replication	AUC	Concentration µg/mL (X')	SD	RSD
Amoxicillin trihydrate	1	1	12537	6499	44.608	0.69%
		2	12515	6478		
		3	12522	6484		
		4	12471	6435		
		5	12537	6499		
		6	12456	6421		
	2	1	12477	6441		
		2	12540	6502		

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		3	12487	6451	
		4	12440	6406	
		5	12429	6395	
		6	12416	6383	
	3	1	12559	6520	
		2	12543	6504	
		3	12444	6410	
		4	12493	6457	
		5	12419	6385	
		6	12476	6440	
Potassium clavulanate	1	1	2544	1624	9.105 0.561
		2	2567	1630	
		3	2553	1627	
		4	2577	1632	
		5	2519	1619	
		6	2489	1612	
	2	1	2494	1613	
		2	2561	1628	
		3	2499	1614	
		4	2484	1610	
		5	2570	1631	
		6	2583	1633	
	3	1	2491	1612	
		2	2571	1631	
		3	2505	1616	
		4	2564	1629	
		5	2561	1628	
		6	2549	1625	

The results in Table 2 showed that the relative standard deviation (RSD) values for amoxicillin trihydrate and potassium clavulanate were 0.691 % and 0.561%, respectively. Thus the precision meets the requirements, which is the RSD is less than 2.0%.

Determination of amoxicillin trihydrate and potassium clavulanate

The determination of amoxicillin trihydrate and potassium clavulanate levels was carried out on sample A and sample B that were taken from the market. Both contain 500 mg of amoxicillin trihydrate and 125 potassium clavulanate per tablet.

Table 3: The results of amoxicillin trihydrate and potassium clavulanate levels calculation

Sample	Amoxicillin trihydrate level (mg/tablet)	Potassium clavulanate level (mg/tablet)
A	499.15	124.54
	498.05	124.35

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	500.15	122.85
	498.2	124.46
B	497.45	123.95
	494.6	122.85

The comparison of the results of the determination of amoxicillin trihydrate and potassium clavulanate in Table 3 with the levels listed on the label showed that the average levels of amoxicillin trihydrate and potassium clavulanate in sample A were 99.82% and 99.13%, respectively, while in sample B were 99.35% and 99.0%, respectively. From the recovery, it is known that the tablets in the trade meet the content requirements of 90–120%.

IV. CONCLUSION

The TLC video-densitometry method can be used to determine the concentration of amoxicillin trihydrate and potassium clavulanate simultaneously with silica gel GF254 as stationary phase, ethyl acetate, glacial acetic acid, methanol, and water (4: 1.5: 0.5v/v) as mobile phase. Retention factor (Rf) value of amoxicillin trihydrate and potassium clavulanate 0.56 and 0.72, respectively.

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