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Comparative Study of Densitometry and Videodensitometry for Quantitating the Active Pharmaceutical Ingredients Using Thin Layer Chromatography – Systematic Review

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Abstract

***Background**: Chromatography is one of the analytical techniques widely used for the quality control process in the pharmaceutical industry. One of the analytical methods used in drug analysis is Thin Layer Chromatography (TLC). The analysis process of TLC can be performed using densitometry (scanner) or videodensitometry (videoscan). The principal analysis of densitometry (scanner) is based on the density measured from each spot on the TLC plate using a specific wavelength range, and videodensitometry (videoscan) is performed by taking pictures of the plate using a Visualizer at a specific wavelength. **Objective**: This review article discusses the application of densitometry and videodensitometry methods for quantitative analysis of pharmaceutical products. **Methods**: This study was conducted using a systematic review method using the PRISMA statement from January to April 2023. Four databases were searched: PubMed, ScienceDirect, Scopus, and Google Scholar with inclusion criteria: studies on thin layer chromatography analysis using densitometry and videodensitometry. **Results**: Based on the ten articles in this study, it is known that the active ingredient concentrations in pharmaceutical products can be determined using densitometry and videodensitometry. The statistical analysis results show no significant difference between the two methods' chemical concentrations of active ingredients in pharmaceutical products. **Conclusion**: TLC densitometry and videodensitometry is a valid methods analysis that can be used for quantitating the active pharmaceutical ingredient concentration in finished pharmaceutical products.*

***Keywords**: densitometry, medicine, thin layer chromatography, videodensitometry*

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INTRODUCTION

Chemical compound analysis methods have been widely developed using various methods and analytical instruments. The analytical process aimed to identify the components (qualitative) or determine the concentration of the active ingredients (quantitative). Chemical compound analysis methods are mainly applied in several important sectors, such as quality control in industry, monitoring and control of contaminants, clinical and biological tests, and geological tests (Ege, 2021).

Quality control is important for guaranteeing a product's safety, efficacy, and effectiveness. For example, in the pharmaceutical, conventional, and herbal medicine industries, quality control is one of the critical points of the quality of a drug product produced. The selection of analytical techniques is essential for qualitative and quantitative analyses to ensure product quality. Qualitative analysis identifies certain compounds or groups of compounds, whereas quantitative analysis determines the concentration of compounds present in raw materials or products (Bandaranayake, 2006; Balekundri & Mannur, 2020)

Chromatography is an analytical technique that is widely used in quality control processes in the pharmaceutical industry. Thin-layer chromatography (TLC) is one of the analytical methods used in drug analysis. The analysis can be performed using densitometry (scanner) or videodensitometry (Videoscan). The principle of chromatography is the separation of chemical compounds based on their affinity for the stationary phase (solid or liquid) and mobile phase (liquid or gas) (Bittner *et al*., 2016). The choice of the analytical instrument used is relatively dependent on the type and characteristics of the sample to be analyzed. For example, TLC instruments are widely used to analyze chemical compounds in plants because they are simple, fast, and relatively inexpensive (Lucio-Gutiérrez, Coello & Maspoch, 2012).

TLC is the most frequently used method for qualitative and quantitative analysis of chemical compounds using densitometry (Liang *et al*., 2004). The analysis can be performed using densitometry (scanner) or videodensitometry (Videoscan). However, in compendial, the analysis of natural ingredients still uses TLC densitometry; however, video-densitometry is rarely used (Alaerts *et al.*, 2012). The videodensitometry method is a simple analytical method that uses images from the visualizer to be converted into a chromatogram profile. However, this method has a major weakness in that spectral analysis of each spot on the TLC plate cannot be performed. In addition, this method requires software that supports image analysis of the TLC plate to be converted into a chromatogram profile. Therefore, this method is rarely used for both qualitative and quantitative analysis. However, the videodensitometry method is advantageous because it can be used for the analysis of unstable samples on TLC plates, samples that require a reagent to detect the analyte, and through a derivatization process (Hahn, 2018).

Densitometric analysis was based on the density measured from each spot on the TLC plate using a specific wavelength range. Videodensitometry analysis was performed by taking pictures of the plate using a Visualizer at a specific wavelength, generally UV 254 or 366 nm, and then scanning it with videoscan software to obtain a chromatogram profile. The principle of videodensitometry is to group image pixels on each track on the TLC plate based on the value of the visual intensity of the color formed, consisting of *Red*, *Green*, and *Blue* (RGB) (Reich & Schibli, 2014). RGB values are one of the parameters used to describe colors precisely using mathematical models. This value was used to determine the intensity of the color formed from each spot on the TLC plate. The videodensitometry method utilizes images of visualization results from TLC plates, and the color intensity formed from each spot can be changed into RGB values. The conversion process from color to chromatogram profiles through a mathematical model approach requires particular software, such as videoscan from CAMAG. All image pixels from each spot on each track with the same Retardation Factor (Rf) were averaged and plotted as a distance function to produce an analog chromatogram curve. The principal analysis of both methods is explained in Figure 1. This analog chromatogram curve is the chromatogram profile of each track that has been analyzed; therefore, this technique can also be performed qualitatively and quantitatively (Srivastava, 2011; Fichou & Morlock, 2018).

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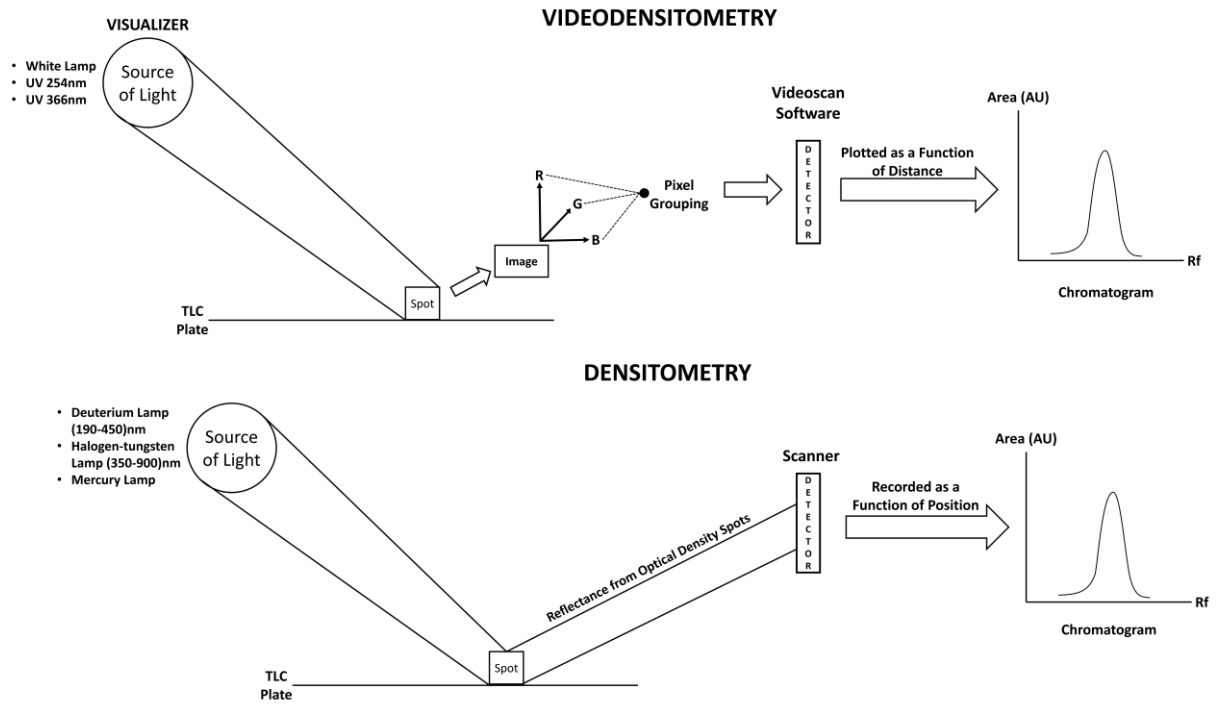

Figure 1. The different principle analysis of videodensitometry and videodensitometry

This study reviews articles related to TLC analysis using densitometry and videodensitometry instruments. Therefore, the development of an analytical process must be validated. According to the USP guidelines for method validation, reviewed articles show the results of several method validation parameters, including precision, accuracy, linearity, detection limit, and quantitation limit (USP, 2021). This study conducted a systematic review using the PRISMA statement to collect articles from several databases, including Google Scholar, PubMed, Science Direct, and Scopus (Page *et al.*, 2021). The review results are expected to provide new insights into the development of thin-layer chromatography for quantifying active ingredient concentrations using densitometry and videodensitometry.

METHODS

Eligibility criteria

The eligibility criteria in this systematic review were determined based on the research questions compiled in the following PICO (population, intervention, comparator, outcome) format.

- *Population*: Analysis using Thin Layer Chromatography
- *Intervention*: Densitometry and Videodensitometry
- *Comparison*: -
- *Outcome*: Quantitating Thin Layer Chromatographic Spots

In this systematic review, the PICO framework was used to develop literature search strategies to ensure comprehensive and bias-free searches. Generally, the PICO Framework is used in evidence-based practice, especially in evidence-based medicine, to provide solutions or formulate clinical or health care-related questions related to the research problem (Methley *et al.*, 2014). In this study, the PICO format was used to specify articles collecting data from several databases.

The inclusion criteria for this study were studies related to TLC analysis using densitometry and videodensitometry instruments, as well as articles containing comparative analysis and results from both methods from 2000 to 2023. The exclusion criteria were articles in languages other than English and systematic reviews, review articles, conference abstracts, case reports, editorials, proceedings, and letters to the editor.

Article selection and screening process

Searching and collecting article data were conducted online from January to April 2023 using the keywords "Thin Layer Chromatography AND densitometry AND videodensitometry" in several online databases, such as PubMed, Google Scholar, ScienceDirect, and Scopus. Furthermore, each article collected was screened using EndNote X9.3.3. The first

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screening stage was performed by checking for duplicates from the article search results, and then separating the duplicate articles found. After the article separation process, the sorting process continued, including the suitability of the title and abstract for this research topic, namely TLC analysis using densitometry and videodensitometry instruments. Furthermore, an eligibility test was carried out by reading the entire content of the article to determine its suitability with the inclusion criteria that had been previously set. The overall process of collecting and sorting articles was carried out by five people and double-checked by two others, and the risk of bias assessment of each article was determined using the PRISMA checklist. A research flow diagram is shown in Figure 2.

Data analysis

Data analysis of the articles obtained was carried out descriptively by comparing the data obtained from each article, including data on the validation of analytical methods and determination of concentrations from densitometry and videodensitometry methods. The data are presented in Table 1.

RESULTS AND DISCUSSION

A total of 319 articles were found in the four databases. Three hundred and nineteen articles were obtained using the search strategy:14 from PubMed, 120 from ScienceDirect, 164 from Scopus, and 21 from Google Scholar. After removing duplicate articles from the four databases, 107 articles were selected for systematic review. We excluded 81 articles because they listed the categories of book chapters, books, conference papers, and reviews that violated the eligibility criteria. Therefore, 26 full-text articles were reviewed according to the systematic review guidelines. After reading the full-text articles, 16 were excluded because they were irrelevant to the research question (Figure. 1).

TLC/HPTLC analysis methods using densitometry and videodensitometry for analyzing pharmaceutical products in various dosage forms have been widely developed (Srivastava, 2011). Generally, TLC analysis is performed using densitometry (scanner), but in several publications, TLC analysis methods were developed using videodensitometry. This systematic review aimed to compare the results of TLC analysis using densitometry and videodensitometry to determine active pharmaceutical ingredient concentrations, and to show that qualitative and quantitative TLC analysis can also be performed using videodensitometry. The results of this review are summarized in Table 1.

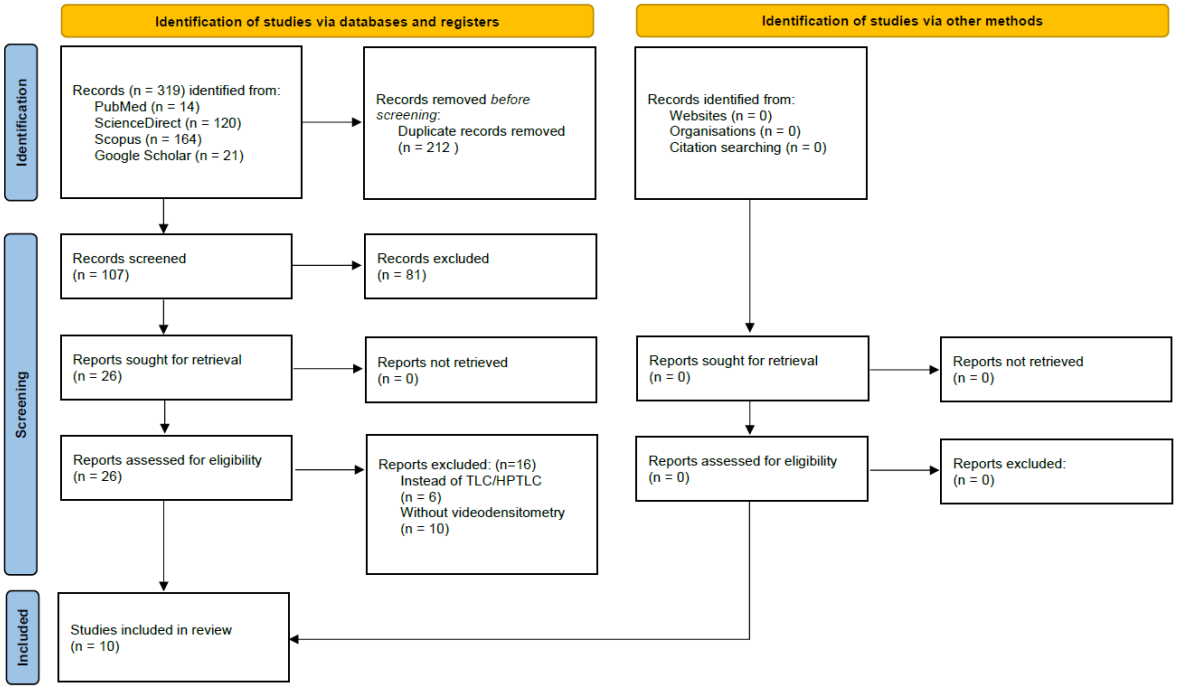

Figure 2. The research PRISMA 2020 flow diagram

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Table 1. Densitometry and videodensitometry applications in pharmaceutical products included in the review

Sample	Method Validation										Active Ingredient Concentration (mg)		References
	LOD (µg/spot)		LOQ (µg/spot)		Accuracy (%Recovery)		Precision (%RSD)		Linearity (r)				
	D	V	D	V	D	V	D	V	D	V	D	V	
Apo-Nadol® 80mg (Nadolol)	0.05	0.2	0.1	1	99.71 – 102.05	97.15 – 101.57	1.14	0.68	0.9972	0.9961	80.81 ± 0.86	80.28 ± 1.22	(Gumieniczek *et al*., 2002)
Apo-Pindol® 10mg (Pindolol)	0.05	0.05	0.1	0.1	98.41 – 101.57	97.37 – 101.14	0.74	0.79	0.9921	0.9960	9.93 ± 0.08	9.96 ± 0.06	
Betoptic 0.5% (Betaxolol)	2.0	1.9	2.3	2.2	95.36 – 96.36	94.02 – 98.21	1.51	1.82	0.9935	0.9857	5.32 ± 0.04	5.35 ± 0.05	(Hopkała *et al*., 2003)
Timohexal 0.1% (Timolol)	0.5	0.5	0.9	1	97.08 – 102.2	94.89 – 103.6	1.32	1.39	0.9966	0.9905	1.35 ± 0.04	1.31 ± 0.07	
Fluoxetine 20mg	0.05	0.15	N/A	N/A	101.00 – 106.66	96.25 – 100.75	1.10	1.59	0.9945	0.9997	21.02 ± 0.86	20.86 ± 0.64	(Skibiński, Misztal & Kudrzycki, 2003)
Paroxetine 20mg	0.02	0.15	N/A	N/A	103.00 – 106.50	98.50 – 107.00	1.04	1.78	0.9909	0.9996	19.83 ± 0.84	19.64 ± 0.96	
Fevarine 50mg (Fluvoxamine)	0.05	0.06	N/A	N/A	98.50 – 101.83	100.83 – 108.00	1.93	1.60	0.9983	0.9912	50.60 ± 1.26	49.48 ± 2.52	(Skibiński & Misztal, 2004)
Aurorix 150mg (Moclobemide)	0.02	0.07	N/A	N/A	98.30 – 107.67	96.50 – 108.50	1.25	1.87	0.9939	0.9843	151.72 ± 2.92	148.21 ± 5.91	
Cipamil® 20mg (Citalopram)	0.09	0.1	N/A	N/A	102.17 – 104.44	100.74 – 104.01	1.83	1.97	0.9946	0.9998	20.26 ± 0.37	19.89 ± 0.39	(Skibiński & Misztal, 2005)
Bezamidin 200mg (Bezafibrate)	9.39	9.81	24.21	24.85	97.17 – 103.61	91.88 – 100.45	0.50	1.06	0.9962	0.9934	200.78 ± 0.42	192.33 ± 1.17	(Misztal & Komsta, 2005)
Lipanor 100mg (Ciprofibrate)	11.65	11.89	23.55	23.15	95.41 – 100.93	95.03 – 100.57	1.91	1.91	0.9742	0.9678	98.01 ± 1.24	97.80 ± 1.13	
Liprox 20mg (Lovastatin)	N/A	N/A	N/A	N/A	97.90 – 104.2	95.80 – 104.6	1.88	1.49	0.9780	0.9650	20.82 ± 1.07	20.44 ± 1.32	(Komsta et al., 2007)
Simvahexal 20mg (Simvastatin)	N/A	N/A	N/A	N/A	97.20 – 102.2	96.80 – 106.2	1.43	1.49	0.9880	0.9671	19.72 ± 0.49	19.59 ± 0.87	
Seroquel 25mg (Quetiapine)	0.02	0.04	0.06	0.12	99.55 – 106.28	98.07 – 107.41	1.19	1.02	0.9862	0.9916	24.82 ± 1.26	24.84 ± 1.76	(Skibiński *et al*., 2008)
Fenoratio 100mg (Fenofibrate)	N/A	N/A	N/A	N/A	93.35 – 109.51	91.62 – 113.84	1.42	1.30	0.9770	0.9872	101.42 ± 0.87	102.73 ± 0.65	(Komsta & Misztal, 2005)
Gemfibral 450mg (Gemfibrozil)	N/A	N/A	N/A	N/A	93.80 – 107.13	95.29 – 102.35	1.95	1.41	0.9795	0.9790	452.12 ± 1.04	444.56 ± 1.21	
Atacand 16mg (Candesartan)	0.08	0.13	0.27	0.44	99.06 – 100.56	98.87 – 100.06	1.80	0.42	0.9997	0.9981	15.97 ± 0.79	15.91 ± 0.71	(Gumieniczek *et al*., 2011)
Xartan 50mg (Losartan)	0.12	0.11	0.39	0.37	99.80 – 101.12	99.00 – 100.04	1.38	0.81	0.9986	0.9982	50.23 ± 1.93	49.76 ± 1.59	

Notes: N/A Not evaluated; D: Densitometry; V: Videodensitometry

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Densitometry is a technique used to measure the density of a substance from each spot on a TLC plate. The principle of densitometry is based on the Kubelka-Munk theory, which provides a quantitative description of the absorption, reflectance, and scattering of light in a medium such as a TLC plate. In TLC analysis, the Kubelka-Munk theory can be used to quantitatively determine the concentration of components on a TLC plate by measuring their absorption, reflectance, and scattering properties. Analyzing absorption or reflectance data using the Kubelka-Munk equation makes it possible to obtain quantitative information (Reich & Schibli, 2014).

On the other hand, videodensitometry is a technique used in chromatography to quantitatively analyze and visualize the results of separated compounds from a TLC plate. The analysis process requires a video camera and image analysis software to capture and analyze the chromatograms. In videodensitometry, the chromatogram is illuminated with UV or visible light, and the video camera captures the image of the bands or spots on the TLC plate. The captured images were then analyzed using specialized software to identify and quantify the separate components in the plate based on their UV absorbance or color. The detection process for videodensitometry can be performed using several software, packagssuch as, ImageJ (U.S. National Institute of Health, Bethesda, MD, USA), Videoscan (CAMAG, Muttenz, Switzerland), Sorbfil TLC Videodensitometry (Jsc Sorbpolymer, Krasnodar, Russia), Macherey Nagel TLC scanner (Macherey Nagel, Düren, Germany), JustTLC (Sweday, Sodra Sandby, Sweden), TLC Analyzer (Amber, 2007), qtlc (cran.r-project), TLSee (AlfaTech, Genova, Italy), Matlab's imaging processing toolbox (MathWorks, Natick, MA, USA), and quanTLC (Fichou & Morlock, 2018).

Some of the above software, such as the TLC analyzer, qTLC, and MATLAB, are free and can be used for plate visualization, but data quantification requires additional software. qTLC has limited capabilities because data processing must be performed through a command prompt, which means that it is difficult for non-programmers. Other non-free software (VideoScan, Sorbfil TLC Videodensitometer, Macherey Nagel TLC scanner, JustTLC, TLSee, QuanTLC, and ImageJ) can be used for plate visualization and quantification of separated compound profiles on TLC plates. Hence, it is essential to select an analysis software for videodensitometry is essential to consider (Campus, 2011). Therefore, videodensitometry is a suitable method for analyzing the results of chromatographic separation and can provide important information regarding the quality and quantity of the TLC method. However, videodensitometry has some limitations, such as the need for high-quality video cameras and specialized software with the potential for analysis (Srivastava, 2011).

Based on the selected articles, we found that both densitometry and videodensitometry can be used to quantitatively analyze a sample's active pharmaceutical ingredients (API). The API concentrations obtained from these methods were not significantly different ($P>0.05$) when subjected to statistical analysis using *Student t-test*. Additionally, both methods met the requirements for method validation parameters, such as precision, accuracy, linearity, limit of quantification (LOQ), and limit of detection (LOD).

The linearity of the densitometry and videodensitometry methods was evaluated by analyzing a series of different concentrations of each standard. The linearity of the analytical method is the ability to provide results directly proportional to the concentration or amount of analyte measured over a specified range. The correlation coefficient 'r' was the acceptance criteria in linearity that indicate the linear relationship response between variables with the concentration or amount of the analyte. Based on this review article, the densitometry method has linearity over the correlation coefficient range from 0.9742 to 0.9972, while videodensitometry has a value of 0.9650–0.9998. A high 'r' value, close to 1, indicates good linearity (Harron, 2013). There was a linear correlation between the API concentration (µg/spot) and the peak area chromatogram. A comparison of the results obtained using the two methods using an independent sample t-test showed no statistical difference ($P>0.05$).

The limit of quantification (LOQ) and limit of detection (LOD) were also determined. LOD and LOQ are important parameters used to assess the sensitivity and reliability of analytical methods. Generally, the LOD and LOQ are often determined as the concentration or amount corresponding to a specified signal-to-noise ratio (S/N), but they can also be determined based on visual evaluation, standard deviation (SD), and slope response (Little, 2016). The LOD and LOQ were determined in the selected articles based on the standard deviation response and slope

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values in the linear regression. However, the LOD and LOQ values have not been determined in several articles. According to the USP guidelines for method validation, analytical procedures for determining the API concentration of major components in finished pharmaceutical products are classified in category I, where the detection limit and quantitation limit parameters are not necessarily required (USP, 2021). However, both of these parameters performed better during the validation process for the quantitative analysis. LOD was found to be 0.02 to 11.65 µg/spot for densitometry assay and 0.05 to11.89 µg/spot for videodensitometry assay, while the LOQ was found to be 0.06 to 24.21 µg/spot and 0.10 to 24.85 µg/spot for densitometry and videodensitometry assays, respectively. Moreover, no statistically significant differences were observed in the LOD and LOQ parameters (*P>0.05*). The sample matrix may affect the LOD and LOQ values, especially in complex matrices, as it may interfere with or alter the analytical signal of the target compounds (Yuwono & Indrayanto, 2005).

Accuracy is often assessed by calculating the percentage recovery, which compares the measured value obtained by the method to the known or reference value. Commonly used acceptance ranges for percent recovery include 80-120%, 90-110%, or tighter ranges defined by specific guidelines or compendials (European Medicines Agency, 2022). All selected studies used spiking concentrations of 80%, 100%, and 120% of the standard. The percent recovery achieved was 93.35 to 109.51 on densitometry and 91.62 to 109.51 on videodensitometry. This indicates that both methodologies accurately measure API concentrations in pharmaceutical products.

Precision refers to the degree of agreement or reproducibility between individual test results obtained from the analysis using a specific analytical method. Precision determination comprises three main categories: repeatability, intermediate precision, and reproducibility. Repeatability or intra-assay within-day precision methods from ten articles were analyzed using the lowest and highest standard concentrations of API in triplicate replications and determined as relative standard deviation (RSD). The densitometry results were 0.5 to 1.95%, and videodensitometry had a 0.42 to 1.91% RSD value. The results showed that the RSD did not exceed 2% for all API concentrations, indicating that the proposed densitometry and videodensitometry methods can be considered precise.

The densitometry and videodensitometry methods showed no statistical differences for all parameter validation. Both methods fulfilled the linearity parameters, precision, accuracy, LOD, and LOQ. The API concentrations obtained from the two methods were compared and then subjected to statistical analysis using the *Snedecor F test* and *Student t-test*. Through these statistical tests, it was found that there was no significant difference in the chemical content of API between the densitometry and videodensitometry methods (*P>0.05*).

Overall, when reviewed in more detail, it is known that the peak area from the videodensitometry method tends to be greater than that from the densitometry method. For example, according to Gumieniczek *et al*. (2011), the peak area from densitometry was 1376 AU, and videodensitometry was 3429 AU in Candesartan tablets (Gumieniczek *et al*., 2011). These conditions are caused by differences in the analytical principles of the two methods, in which the density of the substance in each spot is measured, whereas videodensitometry measures the intensity of the color formed in each spot on the TLC plate (Reich & Schibli, 2014). In addition, the results of the densitometry analysis depend on the wavelength of the analysis compound, which can be previously set on the application in the scanner instrument to measure the levels in the maximum wavelength region of the substance in each spot. In videodensitometry, the quality of the TLC plate image obtained determines the analysis results because the color intensity of the substance in each formed spot varies greatly depending on the type and quality of the image produced (Lucio-Gutiérrez *et al*., 2012).

Recent research on developing TLC methods using videodensitometry can be performed using a smartphone (TLC-smartphone analysis). These data were excluded from the research criteria of the articles reviewed because they were only analyzed using videodensitometry, but this data is used as additional information about videodensitometry analysis, which is rarely known. Ibrahim *et al.* (2022) developed a qualitative and quantitative analysis method for determining loperamide (Immodium) and bisacodyl (Dulcolax) using a TLC smartphone based on the principle of videodensitometry analysis. The analysis was performed by taking pictures of TLC plates using a smartphone, and then analyzing using *Color Picker free software version 5.0.6*. Qualitative analysis is based on visual detection of the Rf values, whereas quantitative analysis is based on the color intensity of

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the compound spots. Methods evaluation supported by analytical method validation data included precision, accuracy, linearity, LOD, and LOQ. The intraday precision validation results have an RSD value of 0.76-1.78% and interday precision of 1.10-1.55% with triplicate replications. The method accuracy was calculated using the total recovery, with a 99.93-100.04% value. The linearity of the method from five different concentrations resulted in an r-value of 0.9996-0.9999, as well as LOD values of loperamide 0.57 µg/mL and bisacodyl 0.10 µg/mL and LOQ of loperamide 1.73 µg/mL and bisacodyl 0.30 µg/mL, calculated through the SD value and slope of the regression equation. Furthermore, determining loperamide concentration in immodium tablets using the TLC-smartphone method had a recovery value of 98.63 ± 1.68, and bisacodyl concentration in Dulcolax tablets was 100.23 ± 1.57. The results showed that the TLC-smartphone method can qualitatively and quantitatively determine the concentrations of loperamide and bisacodyl compounds (Ibrahim *et al.*, 2022).

However, densitometry and videodensitometry are challenging for analyzing natural products. Because raw materials contain many multicomponent components that work synergistically to cause pharmacological activity, this content will significantly affect the analysis process, starting from sample preparation techniques that must separate each component well. In addition, there is no reference standard for specific compounds that can be used for comparison (Xie *et al*., 2006; Renger *et al*., 2011). The variability factor in the composition of chemical compounds, influenced by environmental and plant genetic factors, also affects the analysis results (Kusumawati, 2021).

CONCLUSION

This systematic review shows that TLC densitometry and videodensitometry methods can be used for quantitative and qualitative analyses to determine the concentrations of active ingredients in pharmaceutical products. Through this review article, we intend to provide a new perspective that qualitative and quantitative TLC analyses can be performed using densitometry and videodensitometry.

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AUTHOR CONTRIBUTIONS

Conceptualization, I. K.; Methodology, I. K., R. P.; Software, F. A. R., S. R.; Validation, I. K., R. P.; Formal Analysis, F. A. R.; Investigation, F. A. R., H. R. P.; Resources, F. A. R., F. J. S.; Data Curation, F. A. R.; Writing - Original Draft, F. A. R.; Writing - Review & Editing, I. K., R. P.; Visualization, S. R.; Supervision, . K., R. P.; Project Administration, I. K., R., F. A. R.; Funding Acquisition, I. K.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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