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RESEARCH ARTICLE

Development and validation of a dissolution test for andrographolide dispersible tablets using UV-Vis spectrophotometry

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Abstract

Background: Andrographolide, a terpenoid contained in an herbal plant, potentially has antidiabetic properties. Dispersible tablets are one of the pharmaceutical dosage forms used to improve acceptability. It is necessary to evaluate the dissolution profile to ensure the drug's efficacy. **Objective:** This study aimed to determine the optimum condition of a dissolution test and its analysis for andrographolide dispersible tablets. **Method:** The optimisation process involves varying solvents, pH, and mole ratio of ARS-Cu(II)-andrographolide to determine the best conditions for the dissolution test of andrographolide dispersible tablets. Validation and analysis are conducted using spectrophotometry UV-Vis. **Results:** The optimal condition for the dissolution test was obtained with type II at 75 rpm, 900ml of citrate buffer medium with pH 3.1, and a temperature of 37±0.5ºC. The optimal analysis condition for the aliquot was obtained with Cu(II) metal and ARS reagents at pH 7, mole ratio 6:1:1.8, and a 15-minute optimum time. The maximum wavelength was 518nm using a UV-Vis spectrophotometer. The validation method has met the requirements. **Conclusion:** A dissolution test and its analytical method of andrographolide dispersible tablet have been validated.

Introduction

About 80% of the global population prefers herbal medicines, according to the World Health Organisation 2022 data (WHO Media Team, 2022). This high rate encourages the pharmaceutical industry to increase the production and development of herbal medicine formulations. Andrographolide, a bioactive compound present in *Andrographis paniculata* (Burm.f.) Wall. ex Nees, is a diterpene lactone with various pharmacological activities, e.g. anti-hyperglycemic and hepatoprotective effects (Dai *et al.,* 2018), and could be potentially used as an active pharmaceutical ingredient (Patil & Jain, 2021).

Dissolution, a crucial parameter in pharmaceutical dosage form development, involves releasing the solute active substance of a drug into a solvent at a specific volume (Shargel *et al.,* 2005). This process addresses quality control issues, equivalence, and relationship batch to batch (Hasan *et al.,* 2017). Selecting the dissolution media that simulates best in vivo conditions is critical (Mirasol, 2020). Typically, the pH for dissolution test media ranges between 1.2 and 6.8 (Depkes RI, 2020), allowing for evaluation of the drug's performance and dissolution profile under physiological conditions. The dissolution test provides insights into the drug's active substance levels (Food and Drug Administration, 1997), with analysis often conducted using instruments such as HPLC, UHPLC, and UV-Vis spectrophotometer. Among these, the UV-Vis spectrophotometer is widely favoured because of its high accuracy, precision, and low cost (Rohmah *et al.,* 2021).

Today, herbal medicine is often formulated in synthetic forms, such as tablets, to enhance acceptability and tackle economic aspects. Andrographolide dispersible tablets are a solid dosage form with andrographolide as the active pharmaceutical ingredient. It is essential to

evaluate the dissolution profile to ensure its efficacy (Yun *et al.,* 2015). Therefore, this study examined the dissolution test and analytical methods for andrographolide dispersible tablets.

Methods

Preparation of the ARS-Cu-andrographolide complex solution

Precise volumes of three stock solutions were transferred into a 10 mL volumetric flask: 0.285 mL of ARS (1000 ppm), 0.318 mL of Cu(II) (1000 ppm), and 0.292 mL of andrographolide (1000 ppm). Next, NaOH 2N was added incrementally until the desired pH was reached. The flask was then filled to the mark with a buffer solution and shaken until homogenised.

Optimisation of solvent and pH

ARS-Cu and ARS-Cu-andrographolide complex solutions were prepared using citrate buffer at pH 3.1, phosphate buffer at pH 4.0, and acetic buffer at pH 4.6. Then, NaOH 2N was added until pH 7 and 8 were reached. The complex solution was scanned at a 200-800 nm wavelength.

Optimisation of mole ratio

The ARS-Cu-andrographolide complex solution was prepared with a mole ratio of ARS to Cu(II) set at 1:6. Various andrographolide ratios were considered during the process: 1:2, 1:4, 1:6, 1.8, 2:0, and 2:2. The absorbance of each solution was then measured using a UV-Vis spectrophotometer.

Validation methods

• *Specificity:* ARS, ARS-Cu(II), ARS-Cu(II)-placebo, and ARS-Cu(II)-andrographolide solutions were prepared and measured at a maximum wavelength of 200-800 nm using a UV-Vis spectrophotometer.

• *Linearity:* Six different concentrations (20.3, 23.3, 26.3, 29.3, and 32.3 ppm) of andrographolide were incorporated into ARS-Cu(II)-andrographolide complex solutions. The absorbance of each solution was measured with a UV-Vis spectrophotometer at the maximum wavelength. Values were used to construct a calibration curve.

• *Limit of detection (LOD) and limit of quantification (LOQ):* Six different concentrations (20.3, 23.3, 26.3, 29.3, and 32.3 ppm) of andrographolide were incorporated into ARS-Cu(II)-andrographolide complex solutions. The absorbance of each solution was measured with a UV-Vis spectrophotometer at the maximum wavelength. The LOD and LOQ were calculated based on the constructed calibration curve.

• *Intraday and interday precision:* Six replicates of andrographolide within ARS-Cu(II)-andrographolide complex solutions (26.3 ppm) were prepared, and the absorbance of each solution was measured at the maximum wavelength using a UV-Vis spectrophotometer. Intraday precision was assessed by calculating the Relative Standard Deviation (RSD) based on the absorbance results from the six replications within the same day. For interday precision, six additional replicates of andrographolide within ARS-Cu(II)-andrographolide complex solutions (26.3 ppm) were prepared, and their absorbance was measured on different days at a maximum wavelength using a UV-Vis spectrophotometer. The RSD was then calculated using the intraday and interday precision results.

• *Accuracy:* Three replicates of each concentration (23.3, 26.3, and 29.3 ppm) of andrographolide within ARS-Cu(II)-andrographolide complex solutions were prepared. A volume of 0.741 mL of placebo stock solution was added to each complex solution, and their absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer to calculate the recovery of each concentration.

• *Dissolution test:* Dissolution testing was conducted on six prepared andrographolide dispersible tablets using Apparatus Type 2 (paddle) and Apparatus 6. The test used media of citrate buffer at pH 3.1, with the paddle rotating at 75 rpm and temperature maintained at 37±0.5ºC. Sampling was performed after 30 minutes and then filtrated. The absorbance of each sample was measured at the maximum wavelength using a UV-Vis spectrophotometer. The dissolution levels of andrographolide in the samples were then calculated.

Results

Optimisation of solvents and pH

Solvent optimisation was conducted using three different buffers, i.e., phosphate buffer at pH 4.0, acetate buffer at pH 4.6, and citrate buffer at pH 3.1. The pH was adjusted using NaOH 2N solution until the desired pH of 7 and 8. Table I shows the wavelength shift between ARS-Cu(II) and ARS-Cu(II)-andrographolide complex solutions using various solvents and pH conditions.

Optimisation of mole ratios

Mole ratio optimisation was done using ARS:Cu(II) of 1:6 and various andrographolide ratios (1:2, 1:4, 1:6, 1:8, 2:0, and 2:2). The ARS and Cu(II) ratio was

determined from prior research (Jannatin *et al.,* 2017). Figure 1 shows an increase in the curve, indicating that andrographolide can still react with the ARS-Cu(II) complex. The optimal ARS:Cu(II):andrographolide mole ratio is 1:6:1.8.

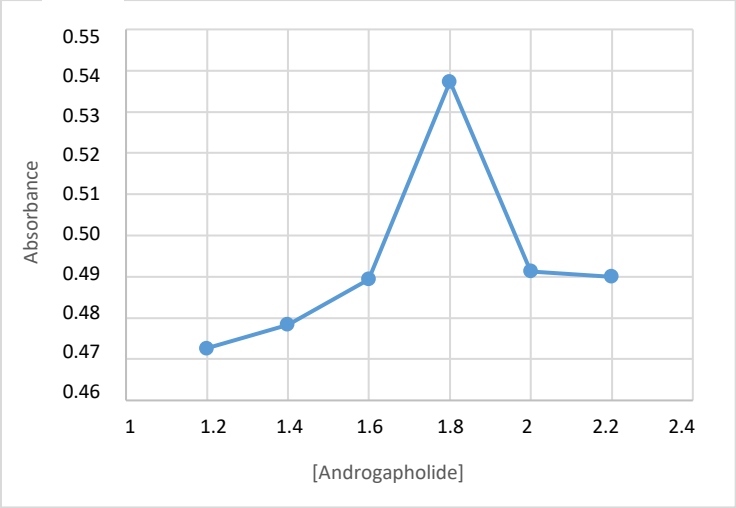


Figure 1: Plotting the molar ratio of andrographolide

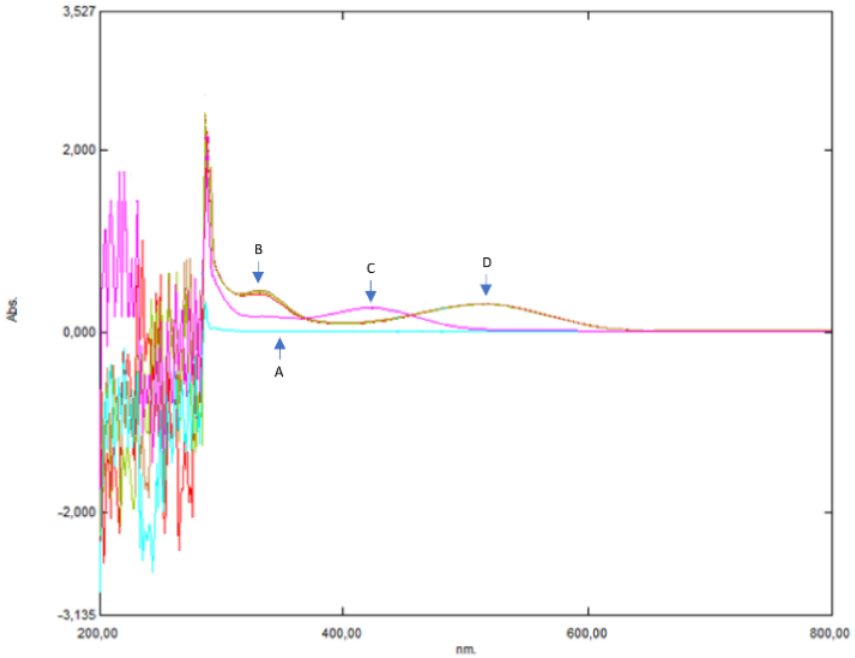
Table I: Wavelength shift of each complex at different conditions

Solvent	pH	Complex solution	Maximum wavelength (nm)	Wavelength shift (nm)
Citrate buffer pH 3.1	7	Cu(II)-ARS	514	4
		Cu(II)-ARS-andrographolide	518	
	8	Cu(II)-ARS	520	0
		Cu(II)-ARS-andrographolide	520	
Phosphate buffer pH 4.0	7	Cu(II)-ARS	520	0
		Cu(II)-ARS-andrographolide	520	
	8	Cu(II)-ARS	518	1
		Cu(II)-ARS-andrographolide	517	
Acetate buffer pH 4.6	7	Cu(II)-ARS	535	2
		Cu(II)-ARS-andrographolide	533	
	8	Cu(II)-ARS	533	2
		Cu(II)-ARS-andrographolide	535	

Validation method

The specificity test was conducted to determine the effect of the matrix during sample measurement. It involved scanning solvents, ARS, ARS-Cu(II), ARS-Cu(II)-placebo, and ARS-Cu(II)-andrographolide solutions at a 200-800 nm wavelength. Figure 2 shows that none of the other solutions overlapped at the maximum wavelength of ARS-Cu(II)-andrographolide (518 nm). Therefore, the method was considered specific.

The linearity test aimed to determine the correlation between concentrations and responses. In Figure 3, the linear regression equation is represented as y = 0.0092x + 0.0551, with an R2 value of 0.98. The results were acceptable, as they met the required coefficient correlation of ≥ 0.98 (Depkes RI, 2020).



(A: solvent, B:ARS-Cu(II)-placebo, C= ARS-Cu(II), D: ARS-Cu(II)-Andrographolide)

Figure 2: Absorption spectra of andrographolide

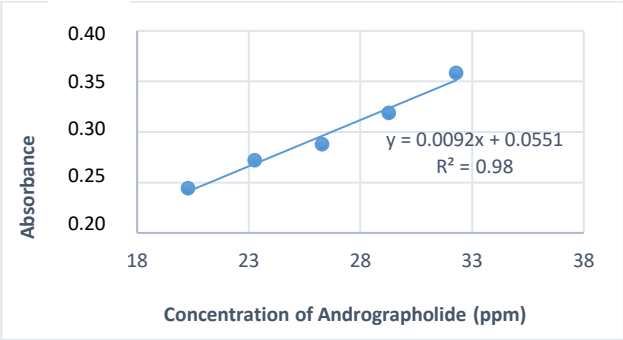


Figure 3: Calibration curve to determine andrographolide concentration

The LOD and LOQ were calculated from the linear regression equation, resulting in values of 2.367 and 7.890 ppm, respectively. These values were compared to those of another analysis of andrographolide bulk powder using UV-Vis spectrophotometry. LOD and LOQ were 14.71 and 44.57 ppm (Pancham *et al.,* 2019), indicating a lower sensitivity. Therefore, the method is considered sensitive.

Precision was done intraday and interday to determine the differences in results from replicating measurements. Tables II & III show that the RSD values of intraday and interday precision are 0.871% and 1.050%, lower than the required < 2%. Thus, the results are considered acceptable (Depkes RI, 2020).

Table II: Intraday precision results

Sample	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance average
1	0.286	0.286	0.286	0.286
2	0.286	0.286	0.286	0.286
3	0.291	0.290	0.290	0.290
4	0.286	0.286	0.286	0.286
5	0.291	0.291	0.291	0.291
6	0.286	0.286	0.286	0.286
			Average	0.287
			SD	0.002
			% RSD	0.871

Table III: Interday precision results

Sample	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance average
1	0.286	0.286	0.286	0.286
2	0.286	0.286	0.286	0.286
3	0.291	0.290	0.290	0.290
4	0.286	0.286	0.286	0.286
5	0.291	0.291	0.291	0.291
6	0.286	0.286	0.286	0.286
7	0.286	0.286	0.286	0.286
8	0.285	0.284	0.284	0.284
9	0.282	0.282	0.284	0.284
10	0.281	0.281	0.281	0.281
11	0.285	0.285	0.285	0.285
12	0.282	0.282	0.282	0.282
			Average	0.285
			SD	0.003
			% RSD	1.050

Accuracy testing aimed to determine the similarity between the measured and theoretical values using the spiked placebo method at three concentrations (23.3, 26.3, and 29.3 ppm). Table IV shows that the recovery for each concentration falls within the required recovery range of 95-105% (Depkes RI, 2020), confirming the accuracy of the method used to measure andrographolide concentrations.

Table IV: Accuracy validation results

Concentration (ppm)	Absorbance average	Measured concentration (ppm)	% Recovery
23.3	0.268	23.2	99.5
26.3	0.296	26.2	99.4
29.3	0.329	29.8	101.7

Dissolution test

The dissolution test used Apparatus Type 2 (paddle) with a 900 mL citrate buffer at pH 3.1 as the medium and the paddle rotating at 75 rpm. Sampling was performed at 30 min. The medium was selected based on the optimal solvent results, ensuring it falls within the physiological pH range of 1.2 to 6.8 (Depkes RI, 2020). Apparatus Type 2 was chosen for its proven ability to achieve a faster dissolution rate (Ghosh et al., 2012). Table V shows that the mean levels of andrographolide dissolution are 84.35%.

Table V: Dissolution test results

Chamber	Absorbance	Sample concentration (ppm)	Andrographolide content (mg)	% Dissolution
1	0.299	4.014	3.612	85.801
2	0.297	3.742	3.368	79.992
3	0.300	4.149	3.735	88.706
4	0.299	4.014	3.612	85.801
5	0.300	4.149	3.735	88.706
6	0.296	3.606	3.245	77.087
			Average	84.35
			SD	4.36
			CV	0.05

Discussion

Andrographolide is hard to analyse by UV-Vis spectrophotometry, and most compound analyses are carried out using HPLC (Villedieu-Percheron *et al.,* 2019). The addition of reagents, such as alizarin red S (ARS), and metals, specifically copper, facilitates the analysis. This addition aims to incorporate the chromophore group of andrographolide to form a complex. ARS acts as a bidentate ligand, donating two atoms, leading to the complexation of ARS and Cu(II), occurring at the carbonyl and hydroxyl groups and forming a coordination bond (Justino *et al.,* 2023). Cu(II) then binds to the hydroxyl group of andrographolide, forming an ARS-Cu(II)-andrographolide complex. The successful formation of complex molecules relies on optimal conditions, including solvent, pH, and mole ratio. The optimal solvent and corresponding pH were citrate buffer at pH 7, exhibiting the most substantial shift (4 nm). A wavelength shift beyond 2.5 nm suggests the extension of chromophore groups (Spangenberg *et al.,* 2011), indicating the formation of the ARS-Cu(II)-andrographolide complex (Suhartati, 2017). At a pH higher than 7, the OH ion from NaOH reacts with Cu(II) to precipitate into Cu(OH)2 (Jannatin *et al.,* 2019).

Mole ratio optimisation was achieved using ARS:Cu(II) set at 1:6 and various andrographolide ratios. The increasing curve suggests that andrographolide can react with the ARS-Cu(II) complex. An increase in the absorbance plot indicates the formation of a stable complex (Mabrouk *et al.,* 2018), while a decrease occurs with an excessive amount of andrographolide, which is considered a weak acid, leading to the formation of non-ionised ARS. This form prevents ARS from binding to Cu(II), thus decreasing the absorbance of the complex (Fain *et al.,* 2004). Hence, the optimal ARS:Cu(II): andrographolide mole ratio is 1:6:1.8.

Dissolution Apparatus Type 2 was chosen due to its established capacity to yield faster dissolution rates (Ghosh *et al.,* 2012). Typically, dissolution tests for immediate-release tablets last approximately 30 to 60 minutes. Dispersible tablets, on the other hand, are consumed by diluting them in water beforehand. Given that the gastric emptying rate for liquids is around 30 minutes (Farrell, 2019) and that andrographolide absorption occurs at the gastric level, the sampling test was conducted at 30 minutes. The mean levels of dissolved andrographolide reached 84,35%. While no specific monograph requirements exist for herbal drug product dissolution tests, a dissolution level exceeding 75% is generally considered satisfactory. Therefore, the dissolution test method was deemed acceptable.

Conclusion

A dissolution test for andrographolide dispersible tablets has been successfully optimised and validated. The analysis applied UV-Vis spectrophotometry, using complexation of ARS-Cu(II)-andrographolide at pH 7. The optimal conditions included a mole ratio for ARS:Cu(II):andrographolide of 1:6:1.8, an operating time of 15 minutes, and measurement at the maximum wavelength of 518 nm. The validation process, encompassing specificity, linearity, LOD, LOQ, precision, and accuracy, has met the predefined requirements.

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