

Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Donepezil Hydrochloride and Ursolic Acid in Bulk Drug Mixture

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ABSTRACT

Objective: The aim is to develop simple, rapid, economical and simultaneous UV spectrophotometric method for determination of Donepezil Hydrochloride (DNP) and Ursolic Acid (UA) in the bulk binary mixture.

Methods: The analytical solvent used was methanol and UV spectra were taken in the range 200-400 nm. The DNP absorption maximum was found at 230 nm and the UA absorption maximum at 268 nm with an iso-absorptive point at 255 nm. The linearity was assessed by covering concentrations of 1–5 µg/mL for DNP and 200–1000 µg/mL for UA. Binary mixtures were used with 230 and 268 nm as analytical wavelengths for the simultaneous equation method. The method was tested for linearity, accuracy, precision, intermediate precision, inter-day precision, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

Results: The method showed good linearity, with R² values of 0.9994 and 0.9992 for DNP at 230 and 268 nm, respectively, and 0.9995 and 0.9997 for UA at 230 and 268 nm, respectively. In binary-mixture accuracy studies at 80%, 100%, and 120% levels, DNP recovery ranged from 99.90% to 100.33% (mean 100.06%), while UA recovery ranged from 100.03% to 100.37% (mean 100.20%). The %RSD values obtained in precision and robustness studies were lower than 2%. LOD/LOQ values were 0.124/0.375 µg/mL for DNP at 230 nm and 52.14/158 µg/mL for UA at 268 nm.

Conclusion: The validated simultaneous equation method allowed for accurate, precise, and robust estimation of DNP and UA in bulk binary mixtures within the investigated concentration ranges.

Keywords: Donepezil Hydrochloride, Ursolic Acid, UV Spectrophotometry, Method Validation, International Council for Harmonisation (ICH) guidelines.

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INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder that primarily affects memory and cognitive function. Its complex pathophysiology has led to continued research into therapeutic approaches involving drugs with complementary pharmacological properties [1, 2]. Donepezil hydrochloride (DNP) is an acetylcholinesterase inhibitor widely used for the symptomatic management of cognitive impairment associated with alzheimer's disease [3, 4]. Ursolic acid (UA) is a naturally occurring pentacyclic triterpenoid that

has been investigated for its antioxidant, anti-inflammatory, and neuroprotective activities (Fig. 1) [5]. The distinct pharmacological properties of these compounds have generated interest in their combined investigation in pharmaceutical research related to neurodegenerative disorders.

Accurate and reliable analytical methods are required for the estimation of individual components in drug combinations, particularly during formulation development and quality assessment [6]. Chromatographic

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methods provide high selectivity; however, their application may involve sophisticated instrumentation, relatively higher solvent consumption, and more elaborate sample preparation. UV spectrophotometry offers a simpler and economical alternative when the absorption characteristics of the analytes allow their individual contributions to be distinguished [7, 8].

The simultaneous-equation method is based on the Beer–Lambert law and allows the concentrations of two components in a mixture to be determined from their absorbance values at two selected wavelengths. The concentration of each component is calculated by solving

the simultaneous equations using the respective absorptivity coefficients [8, 9].

In the present investigation, a simultaneous-equation UV spectrophotometric method was developed for the estimation of DNP and UA using 230 and 268 nm as analytical wavelengths. The developed procedure was subsequently evaluated for linearity, accuracy, precision, intermediate precision, inter-day precision, reproducibility, robustness, limit of detection (LOD), and limit of quantitation (LOQ) in accordance with the principles described in the ICH guideline for validation of analytical procedures [10].

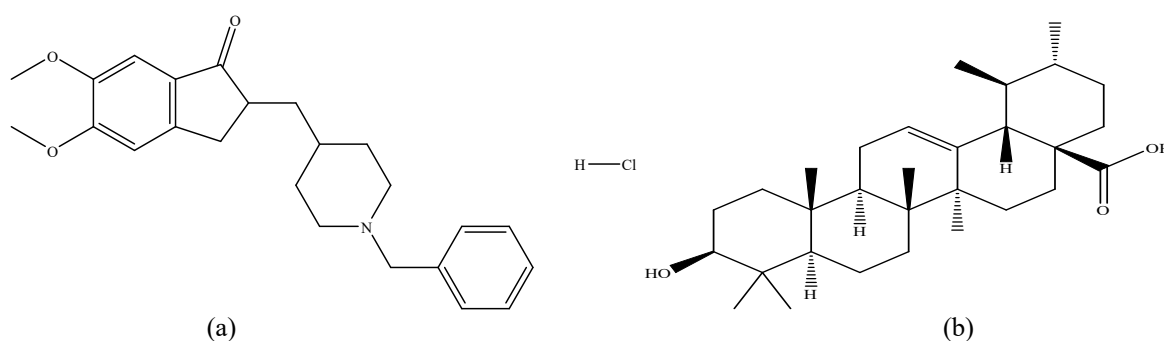


Fig. 1: Chemical Structure of (a) Donepezil HCl and (b) Ursolic Acid [4, 5].

MATERIALS AND METHODS

Chemical and Reagents

Donepezil hydrochloride and ursolic acid reference standards were procured from SM Pharma and Chemical Pvt. Ltd., Maharashtra, India. Methanol of analytical-reagent grade was also procured from SM Pharma and Chemical Pvt. Ltd., Maharashtra, India and used for preparation of standard and working solutions. All other chemicals and reagents used in the study were of analytical grade and were obtained from the same commercial source.

Instrumentation

The UV absorbance measurements were carried out using a Labtronics LT-2900 UV–Visible spectrophotometer (Labtronics, India). Accurate weighing of Donepezil hydrochloride and Ursolic acid standards was performed using a precision digital balance (WENSAR PGB200, Wensar Weighing Scales Ltd., India).

Preparation of Standard Solutions

A weighed amount of DNP was added to a 100 mL volumetric flask. The drug was dissolved in methanol and thus the volume was brought to the mark using methanol and sonicated to prepare the stock solution. This stock solution was diluted as appropriate to provide working concentrations for analysis.

Accurately weighed amount of UA was transferred to a 100 mL volumetric flask. The drug was dissolved in methanol and the volume of solution brought to the mark with methanol. The required working concentrations were made by further diluting the stock solution.

Selection of Analytical Wavelengths

Standard solutions of DNP and UA were scanned with blank (methanol) in the wavelength range of 200–400 nm. The absorption spectra obtained were carefully analysed for the wavelengths showing maximum absorbance.

Determination of the Iso-Absorptive Point

Two solutions of DNP (1.5 µg/mL) and UA (200 µg/mL) were prepared in methanol, and scanned for the wavelength range from 200 to 400 nm to find a common analytical wavelength. Each of the resulting spectra was then overlaid, and the wavelength at which both the drugs showed the same absorptivity was noted.

Linearity of DNP

A series of standard solutions of DNP at concentration levels of 1, 2, 3, 4, and 5 µg/mL was prepared by appropriate dilution of the stock solution. The absorbance of each solution was measured against methanol at 230 and 268 nm. Calibration curves were constructed by plotting absorbance against concentration, and linear regression analysis was performed at each wavelength.

Linearity of UA

Standard solutions of UA containing 200, 400, 600, 800, and 1000 µg/mL were prepared from the stock solution. Absorbance was measured at 230 and 268 nm using methanol as the blank. Calibration curves were constructed by plotting absorbance against concentration, and linear regression analysis was performed at each wavelength.

Simultaneous Equation Method

The simultaneous equation method was employed for the simultaneous estimation of DNP and UA in the binary drug mixture. The method is based on the Beer–Lambert law, according to which the total absorbance of a mixture at a particular wavelength is the sum of the absorbances contributed by the individual components [10].

The absorbance of the binary mixture at two selected wavelengths was expressed as:

$$A_1 = a_{x1}C_x + a_{y1}C_y$$

$$A_2 = a_{x2}C_x + a_{y2}C_y$$

Where A_1 and A_2 are the absorbances of the mixture at wavelengths λ_1 and λ_2 , respectively; a_{x1} and a_{x2} are the absorptivity coefficients of Drug X at λ_1 and λ_2 ; a_{y1} and a_{y2} are the absorptivity coefficients of Drug Y at λ_1 and λ_2 ; and C_x and C_y represent the concentrations of Drug X and Drug Y, respectively.

The concentration of Drug X was calculated using:

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

The concentration of Drug Y was calculated using:

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

For the present study, DNP was designated as Drug X and UA as Drug Y. The analytical wavelengths (λ_1 and λ_2) were selected as 230 nm and 268 nm, corresponding to the maximum absorption wavelengths of DNP and UA, respectively.

Method Validation

The developed analytical method was validated in accordance with the International Council for Harmonisation (ICH) guidelines for the validation of analytical procedures [10].

Linearity

The linearity of the method was determined from a set of standard solutions within the chosen concentration range. Linear regression analysis was used to create and test calibration curves. The correlation coefficient, slope, and intercept were determined to find the relationship between concentration and absorbance [11].

Accuracy as Recovery

Binary mixtures of DNP and UA were prepared at 80%, 100%, and 120% concentration levels in methanol. The prepared solutions were analyzed at 230 and 268 nm, and the concentrations of DNP and UA were calculated using the simultaneous equations. Three replicate determinations were performed at each level. Percentage recovery and %RSD were calculated to assess the accuracy and precision of the method.[12].

Precision

Repeatability

Repeatability was measured by its standard deviation calculated from six independent replicates of the same concentration using the same experimental conditions. The results were expressed as %RSD [13].

Intermediate Precision

Intermediate precision was evaluated by analysing selected concentration levels of each analyte under changed analytical conditions, including different analysts and different days. The variation in absorbance was expressed as percentage relative standard deviation (%RSD) [14].

Inter-Day Precision

Inter-day precision was assessed by analysing the selected drug solutions on different days. The consistency of the analytical response over time was evaluated using %RSD [14].

Robustness

Robustness was evaluated by analyzing DNP (3 µg/mL) and UA (600 µg/mL) solutions at the selected analytical wavelengths under deliberately varied wavelength conditions. Measurements were performed in triplicate, and the effect on analytical response was assessed using mean assay and %RSD. The method was considered robust when the variations produced no significant change in the assay results [15].

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD represents the lowest concentration of analyte that can be reliably detected, whereas the LOQ represents the lowest concentration that can be quantitatively determined with suitable precision and accuracy. Both parameters were calculated from the standard deviation (σ) of the analytical response and the slope (S) of the corresponding calibration curve using the following equations [16]:

$$LOD = \frac{3.3 \sigma}{S}$$

$$LOQ = \frac{10 \sigma}{S}$$

where σ is the standard deviation and S is the slope of the corresponding calibration curve.

Specificity

Specificity was evaluated using methanol blank, individual DNP and UA standard solutions, and the binary mixture at 230 and 268 nm. Methanol showed negligible absorbance, with no significant interference observed at the selected wavelengths. The method was therefore considered specific for simultaneous estimation of DNP and UA.

Statistical Analysis

All experiments were repeated three times, unless indicated otherwise. Results are presented as mean $\pm$ SD. A linear regression analysis was used to obtain calibration characteristics and the precision was determined by percentage relative standard deviation. The statistical

calculations were performed with the aid of Microsoft Excel as well as the software program related to the UV–visible spectrophotometer.

RESULT AND DISCUSSION

Selection of Wavelength of Maximum Absorbance (λ_{max})

UV absorption spectra were recorded in methanol over 200–400 nm. DNP showed λ_{max} at 230 nm, while UA showed λ_{max} at 268 nm. The different absorptivity values reflect the distinct chromophoric properties of the two analytes (Fig. 2).

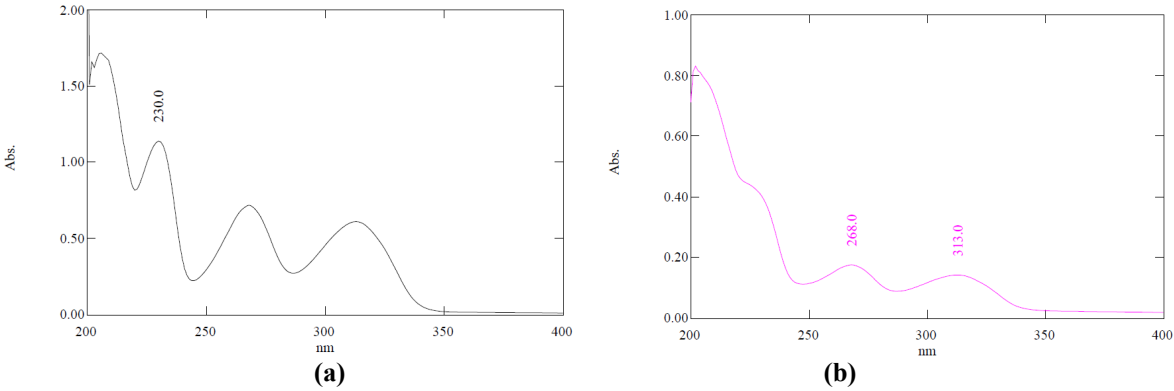


Fig. 2: UV absorption spectrum of (a) DNP (b) UA.

Determination of Iso-Absorptive Point

Overlay of UV absorption spectra of DNP (1.5 $\mu\text{g/mL}$) and UA (200 $\mu\text{g/mL}$) in methanol. The iso-absorptive point at 255 nm was identified as a common wavelength

where both drugs showed equal absorptivity. Simultaneous estimation using this point requires additional validation. (Fig. 3).

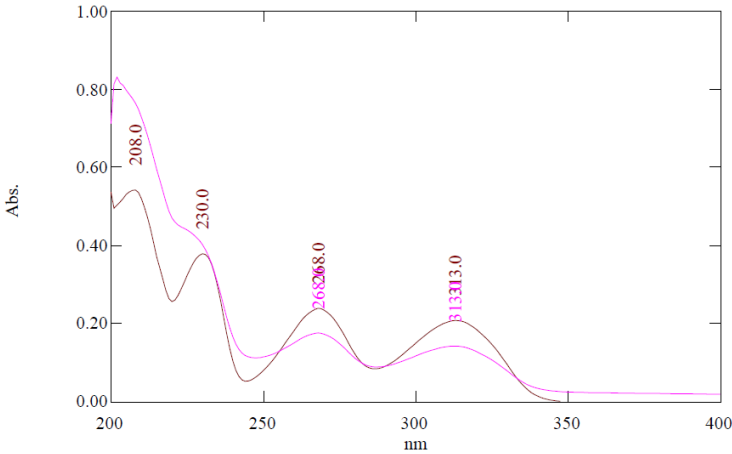


Fig. 3: Overlay UV spectra of DNP and UA.

Linearity

Calibration curves for DNP were constructed by plotting absorbance vs. concentration. Each point represents the mean of three replicates. R^2 values of 0.9994 (230 nm) and

0.9992 (268 nm) indicate excellent linearity. The equations were: At 230 nm: $y = 0.1816x + 0.1918$; At 268 nm: $y = 0.1129x + 0.1221$ (Fig. 4).

Table 1. Linearity characteristics of DNP and UA.

Analyte	Wavelength	Range	Regression equation	R^2
DNP	230 nm	1–5 $\mu\text{g/mL}$	$y = 0.1816x + 0.1918$	0.9994
DNP	268 nm	1–5 $\mu\text{g/mL}$	$y = 0.1129x + 0.1221$	0.9992
UA	230 nm	200–1000 $\mu\text{g/mL}$	$y = 0.0002x + 0.0142$	0.9995
UA	268 nm	200–1000 $\mu\text{g/mL}$	$y = 0.0001x + 0.0025$	0.9997

DNP: Donepezil hydrochloride; **UA:** Ursolic acid; **R²:** coefficient of determination. The calibration range represents the concentration range investigated for each

analyte. Regression equations were obtained by plotting absorbance against the corresponding analyte concentration.

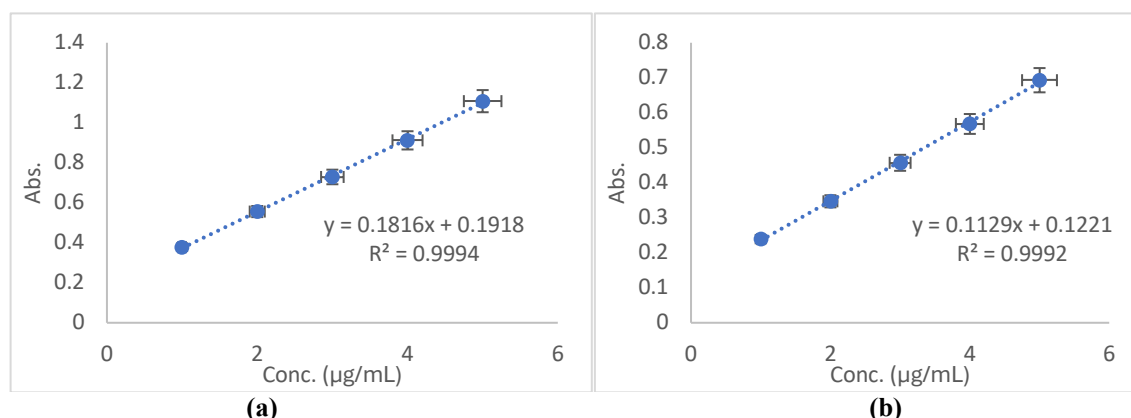


Fig. 4: Calibration curve of DNP at (a) 230 nm (b) 268 nm.

Calibration curves for UA were constructed by plotting absorbance vs. concentration. Each point represents the mean of three replicates. R^2 values of 0.9995 (230 nm) and

0.9997 (268 nm) indicate satisfactory linearity. The lower absorbance values at 268 nm compared to 230 nm reflect the absorption characteristics of UA (Fig. 5).

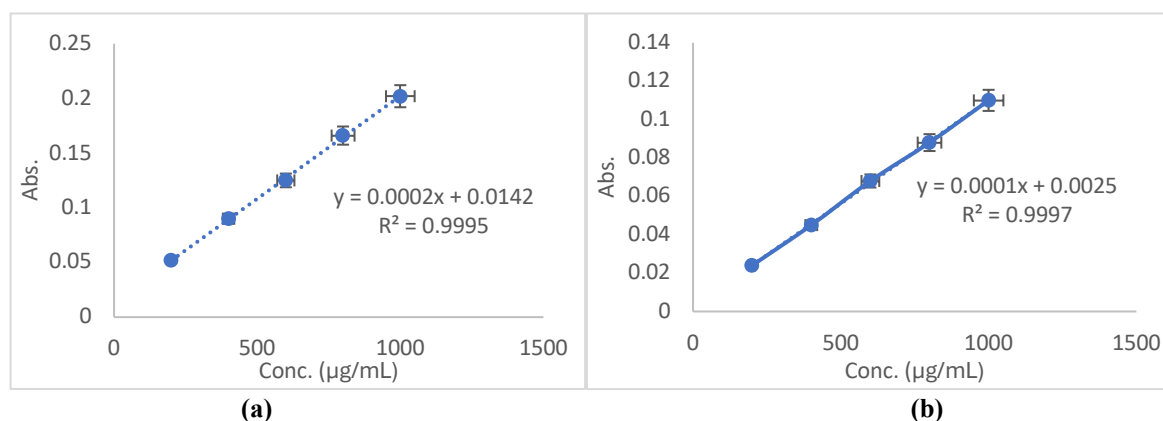


Fig. 5: Calibration curve of UA at (a) 230 nm (b) 268 nm.

Accuracy as Recovery

The binary-mixture study demonstrated satisfactory accuracy of the proposed simultaneous equation method for the simultaneous estimation of DNP and UA. DNP showed recoveries ranging from 99.90% to 100.33%, with an average recovery of 100.06% and %RSD values of 0.151–0.325%. UA exhibited recoveries ranging from 100.03% to 100.37%, with an average recovery of

100.20% and %RSD values of 0.300–0.695%. The low %RSD values indicate good agreement among replicate determinations and minimal variability during analysis. The close agreement between the added and recovered concentrations at all three concentration levels further demonstrates that the simultaneous equation approach can accurately resolve DNP and UA in their binary mixtures (Table 2).

Table 2: Accuracy and recovery study of DNP and UA in the binary drug mixture.

Drug	Level (%)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	%RSD	Average % Recovery
DNP	80	2.4	2.408 ± 0.004	100.33	0.151	100.06
	100	3	2.997 ± 0.008	99.9	0.254	
	120	3.6	3.598 ± 0.012	99.94	0.325	

UA	80	480	481.76 ± 2.86	100.37	0.695	100.2
	100	600	601.17 ± 1.80	100.2	0.3	
	120	720	720.19 ± 4.26	100.03	0.592	

Repeatability

The repeatability of the developed UV spectrophotometric method was assessed by six replicate measurements of DNP and UA under identical analytical conditions. DNP showed %RSD values of 0.274 and 0.219% at 230 and 268 nm, respectively. For UA, the corresponding %RSD values were 1.099 and 1.087%. The data are expressed in Table 3.

DNP: Donepezil hydrochloride; UA: Ursolic acid; S.; standard deviation; %RSD: percentage relative standard deviation. Recovery studies were performed at 80%, 100%, and 120% concentration levels. Values are expressed as mean $\pm$ SD (n = 3).

Precision

Table 3: Repeatability data for DNP and UA at 230 nm and 268 nm.

Drugs	DNP		UA	
	3 μ g/mL	3 μ g/mL	400 μ g/mL	400 μ g/mL
S. No.	Abs. at 230 nm	Abs. at 268 nm	Abs. at 230 nm	Abs. at 268 nm
1	0.730	0.456	0.091	0.045
2	0.731	0.455	0.092	0.046
3	0.733	0.456	0.090	0.046
4	0.730	0.458	0.089	0.046
5	0.729	0.457	0.090	0.045
6	0.727	0.456	0.091	0.046
Mean $\pm$ SD	0.730 $\pm$ 0.0020	0.456 $\pm$ 0.0010	0.091 $\pm$ 0.0010	0.046 $\pm$ 0.0005
% RSD	0.274	0.219	1.099	1.087

DNP: Donepezil; UA: Ursolic Acid; Abs.: Absorbance; Conc.: Concentration; % RSD: Percentage relative standard deviation; SD: Standard deviation. Repeatability was assessed by six replicate measurements (n = 6) under identical experimental conditions. %RSD < 2% indicates acceptable repeatability.

Intermediate precision

Intermediate precision was evaluated by analysing DNP and UA by two different analysts under identical

experimental conditions. For DNP at 230 nm, the %RSD values ranged from 0.206 to 0.383%, while at 268 nm, values ranged from 0.102 to 0.328% (Table 4 and Table 5). The close agreement between the results obtained by both analysts indicated good intermediate precision of the method. For UA, %RSD values ranged from 0.368 to 1.099% at 230 nm and from 0.674 to 1.333% at 268 nm. The data are presented in Table 6 and Table 7.

Table 4: Intermediate precision for DNP at 230 nm.

Analyst	Analyst 1			Analyst 2		
Conc.	2 μ g/mL	3 μ g/mL	4 μ g/mL	2 μ g/mL	3 μ g/mL	4 μ g/mL
Abs.	0.556	0.728	0.912	0.557	0.727	0.915
	0.554	0.727	0.915	0.556	0.725	0.917
	0.552	0.725	0.919	0.553	0.728	0.913
Mean	0.554	0.727	0.915	0.555	0.727	0.915
SD	0.0020	0.0015	0.0035	0.0021	0.0015	0.0020
%RSD	0.361	0.206	0.383	0.378	0.206	0.219

DNP: Donepezil; Abs.: Absorbance; Conc.: Concentration; by two different analysts (n = 3 per analyst). %RSD values < 2% confirm acceptable intermediate precision. % RSD: Percentage relative standard deviation; SD: Standard deviation. Intermediate precision was evaluated

Table 5: Intermediate precision for DNP at 268 nm.

Analyst	Analyst 1			Analyst 2		
Conc.	2µg/mL	3µg/mL	4µg/mL	2µg/mL	3µg/mL	4µg/mL
Abs.	0.347	0.457	0.568	0.348	0.458	0.567
	0.348	0.456	0.567	0.349	0.455	0.569
	0.348	0.458	0.568	0.348	0.457	0.568
Mean	0.348	0.457	0.568	0.348	0.457	0.568
SD	0.0006	0.0010	0.0006	0.0006	0.0015	0.0010
%RSD	0.172	0.219	0.102	0.172	0.328	0.176

DNP: Donepezil; Abs.: Absorbance; Conc.: Concentration; % RSD: Percentage relative standard deviation; SD: Standard deviation. Similar %RSD values between analysts indicate good method precision

Table 6: Intermediate precision for UA at 230 nm.

Analyst	Analyst 1			Analyst 2		
Conc.	400µg/mL	600µg/mL	800µg/mL	400µg/mL	600µg/mL	800µg/mL
Abs.	0.090	0.123	0.162	0.090	0.122	0.163
	0.090	0.122	0.164	0.091	0.124	0.164
	0.091	0.123	0.163	0.092	0.123	0.163
Mean	0.090	0.123	0.163	0.091	0.123	0.163
SD	0.0006	0.0006	0.0010	0.0010	0.0010	0.0006
%RSD	0.667	0.488	0.613	1.099	0.813	0.368

UA: Ursolic Acid; Abs.: Absorbance; Conc.: Concentration; % RSD: Percentage relative standard deviation; SD: Standard deviation. Low %RSD values confirm method consistency across analysts.

Table 7: Intermediate precision for UA at 268 nm.

Analyst	Analyst 1			Analyst 2		
Conc.	400µg/mL	600µg/mL	800µg/mL	400µg/mL	600µg/mL	800µg/mL
Abs.	0.046	0.067	0.089	0.045	0.067	0.089
	0.046	0.068	0.089	0.046	0.068	0.088
	0.045	0.067	0.088	0.045	0.068	0.089
Mean	0.046	0.067	0.089	0.045	0.068	0.089
SD	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
%RSD	1.304	0.896	0.674	1.333	0.882	0.674

UA: Ursolic Acid; Abs.: Absorbance; Conc.: Concentration; % RSD: Percentage relative standard deviation; SD: Standard deviation. %RSD values below 2% indicate satisfactory intermediate precision.

Inter-day precision

Inter-day precision was assessed by analysing DNP and UA on two different days. For DNP, %RSD values were 0.215 and 0.179% at 230 nm and 0.173 and 0.344% at 268 nm on Days 1 and 2, respectively. UA showed %RSD

values of 0.659–1.099% at 230 nm and 1.250–1.277% at 268 nm. All %RSD values were below 2%, confirming satisfactory inter-day precision and reproducibility of the method (Table 8 and Table 9).

Table 8: Inter-day precision for DNP and UA (Day 1).

Drug	DNP		UA	
Wavelength	230 nm	268 nm	230 nm	268 nm
Absorbance (Day 1)	0.556	0.347	0.090	0.048
	0.558	0.346	0.091	0.047
	0.556	0.346	0.091	0.048
Mean	0.557	0.346	0.091	0.048
SD	0.0012	0.0006	0.0006	0.0006
% RSD	0.215	0.173	0.659	1.250

DNP: Donepezil; UA: Ursolic Acid; % RSD: Percentage relative standard deviation; SD: Standard deviation. Inter-day precision was assessed on two different days (n = 3 per day)

Table 9: Inter-day precision for DNP and UA (Day 2).

Drug	DNP		UA	
Wavelength	230 nm	268 nm	230 nm	268 nm
Absorbance (Day 2)	0.558	0.348	0.092	0.047
	0.559	0.350	0.090	0.047
	0.557	0.348	0.091	0.048
Mean	0.558	0.349	0.091	0.047
SD	0.0010	0.0012	0.0010	0.0006
% RSD	0.179	0.344	1.099	1.277

DNP: Donepezil; UA: Ursolic Acid; % RSD: Percentage relative standard deviation; SD: Standard deviation. Consistent %RSD values between days indicate acceptable inter-day precision.

Robustness

For DNP, %RSD values ranged from 0.137 to 0.138% around 230 nm and from 0.218 to 0.328% around 268 nm (Table 10). Similarly, UA exhibited %RSD values of 0.441–0.806% around 230 nm and 0.849–1.408% around 268 nm (Table 11).

Table 10: The robustness of DNP at different wavelengths.

Conc.	230 nm	228nm	232nm	268 nm	266nm	270nm
3 µg/mL	0.728	0.726	0.729	0.455	0.454	0.458
3 µg/mL	0.727	0.725	0.730	0.457	0.453	0.459
3 µg/mL	0.729	0.724	0.731	0.458	0.455	0.460
Mean	0.728	0.725	0.730	0.457	0.454	0.459
SD	0.0010	0.0010	0.0010	0.0015	0.0010	0.0010

% RSD	0.137	0.138	0.137	0.328	0.220	0.218
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DNP: Donepezil, Conc.: Concentration, % RSD: Percentage relative standard deviation, SD: Standard deviation, Mean $\pm$ SD, n=3

Table 11: The robustness of UA at different wavelengths.

Conc.	230 nm	228nm	232nm	268 nm	266nm	270nm
600 $\mu\text{g/mL}$	0.125	0.122	0.130	0.068	0.063	0.071
600 $\mu\text{g/mL}$	0.124	0.121	0.131	0.068	0.062	0.070
600 $\mu\text{g/mL}$	0.123	0.122	0.131	0.067	0.063	0.072
Mean	0.124	0.122	0.131	0.068	0.063	0.071
SD	0.0010	0.0006	0.0006	0.0006	0.0006	0.0010
% RSD	0.806	0.473	0.441	0.849	0.917	1.408

UA: Ursolic Acid; Conc.: Concentration; % RSD: Percentage relative standard deviation; SD: Standard deviation. %RSD values < 2% indicate method robustness to wavelength variation.

LOD and LOQ

For DNP, the LOD and LOQ were 0.124 and 0.375 $\mu\text{g/mL}$ at 230 nm and 0.235 and 0.710 $\mu\text{g/mL}$ at 268 nm, respectively, as shown in Table 18. For UA, the LOD and LOQ were found to be 39.27 and 119 $\mu\text{g/mL}$ at 230 nm and 52.14 and 158 $\mu\text{g/mL}$ at 268 nm, respectively (Table 12).

Table 12: LOD and LOQ of DNP and UA at the selected analytical wavelengths.

Analyte	Wavelength (nm)	Slope (S)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
DNP	230	0.1816	0.124	0.375
DNP	268	0.1129	0.235	0.710
UA	230	0.0001	39.27	119
UA	268	0.0002	52.14	158

DNP: Donepezil hydrochloride; UA: Ursolic acid; LOD: limit of detection; LOQ: limit of quantitation; S: slope of the calibration curve.

Table 13: Specificity data of DNP and UA at selected wavelengths.

Drug	Selected Wavelength	Blank Absorbance	Standard Absorbance	Interference Observed	Specificity Result
DNP	230 nm	0.000–0.005	Clear absorbance at 230 nm	No significant interference	Specific
DNP	268 nm	0.000–0.005	Clear absorbance at 268 nm	No significant interference	Specific
UA	268 nm	0.000–0.005	Clear absorbance at 268 nm	No significant interference	Specific
UA	230 nm	0.000–0.005	Clear absorbance at 230 nm	No significant interference	Specific

DNP: Donepezil Hydrochloride; UA: Ursolic Acid; Abs.: Absorbance. Blank absorbance represents the response of methanol at the selected wavelength. No significant interference was observed.

CONCLUSION

The simultaneous equation UV spectrophotometric method was developed and validated for the estimation of DNP and UA in bulk binary mixtures. The method

demonstrated good linearity over the investigated concentration ranges, satisfactory accuracy at 80%, 100%, and 120% levels, acceptable repeatability and intermediate/inter-day precision, robustness, and measurable sensitivity based on LOD and LOQ. The recovery results and low %RSD values indicate that the method can simultaneously estimate DNP and UA in the investigated binary mixtures. Owing to its simple instrumentation, limited sample preparation, rapid analysis, and low operational cost, the method may be

useful for preliminary formulation-development and bulk-drug analytical studies.

ABBREVIATIONS

Abbreviation Full form

DNP	Donepezil Hydrochloride
UA	Ursolic Acid
UV	Ultraviolet
ICH	International Council for Harmonisation
LOD	Limit of Detection
LOQ	Limit of Quantitation
R²	Coefficient of Determination
RSD	Relative Standard Deviation
SD	Standard Deviation
nm	Nanometre
µg/mL	Microgram per Millilitre
Abs.	Absorbance
Conc.	Concentration
S	Slope of the Calibration Curve
HCl	Hydrochloric Acid
λ_{max}	Wavelength of Maximum Absorption

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AUTHOR CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The data supporting the findings of this study are available within the article.

AI USE STATEMENT

No AI tools were used in the preparation of this manuscript.

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