RESEARCH PAPER

Development And Validation Of A New Ultraviolet Spectrophotometric Method For Quantification Of Donepezil In Nanoparticles And Biological Samples

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Received: 5th Jan, 2026; Revised: 25th Jan 2026; Accepted: 20th Feb, 2026; Available Online: 10th Mar, 2026

Abstract

Quality control processes influence the cost of pharmaceutical product manufacturing; hence, a simple, economical, and validated analytical method is required. Thus, this research aimed to develop a sensitive, precise and cost-effective ultraviolet spectrophotometric method for quantification of donepezil hydrochloride in bulk and within biological samples.

The method used methanol as a solvent and was validated for linearity, robustness, accuracy, precision, limit of detection/quantification. The results showed a regression coefficient value (approx. 0.999) at 271 nm. The method showed high sensitivity with a limit of detection (1.031 µg/mL) and quantification (3.123 µg/mL). The method indicated excellent accuracy and precision with a relative standard deviation (< 1%). The *in-vivo* samples showed significant enhancement of drug levels in the brain with intranasal administration as compared to the oral route within 2 hours.

The developed method was found to be simple, robust and accurate for routine analysis in bulk, pharmaceutical formulations and biological samples.

Keywords: Ultraviolet spectrophotometry; Donepezil Hydrochloride, Biological Samples; Nanoparticles; Intranasal.

How to cite this article: Garg Y, Bhatia A, Development And Validation Of A New Ultraviolet Spectrophotometric Method For Quantification Of Donepezil In Nanoparticles And Biological Samples. Int J Drug Deliv Technol. 2026;16(2): 66-72. DOI: 10.25258/ijddt.16.2.08

Source of support: Nil.

Conflict of interest: None

Introduction

Quantification of active pharmaceutical ingredients (APIs) is essential in the development of pharmaceutical formulations (Bhattycharyya et al., 2005). The method used for quantifying APIs should not only be validated but also economical, as it directly affects the product's cost (Almeida et al., 2020). Thus, a simple, accurate and sensitive ultraviolet (UV) spectrophotometric method was developed for quantifying donepezil hydrochloride (DH) (Khan et al., 2025). The DH is known as 5,6-dimethoxy-2,3-dihydro-1H-inden-1-one hydrochloride (Fig. 1) and is classified as a piperidine derivative (Tang et al., 2025; Gupta et al., 2023). It has been found to exhibit neurocognitive-enhancing properties. This compound functions as an inhibitor of acetylcholinesterase, impeding the enzymatic hydrolysis of acetylcholine into its constituent parts, acetate, and choline (Colovic et al., 2013; Tougu, 2001). Its therapeutic application lies in the management of various neurological disorders, including Alzheimer's disease (Khan et al., 2025).

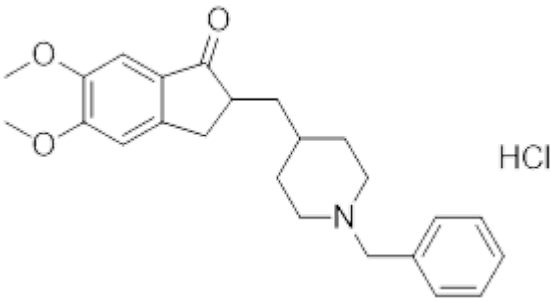


Fig. 1. Structure of Donepezil hydrochloride.

Materials

The DH was obtained as a gift sample from Sun Pharma Laboratories, Sikkim, India. Methanol, double-distilled water, and all required chemicals were bought from Loba Chemie Pvt Ltd, Mumbai.

Spectrophotometric analytical method development

Preparation of stock solution

The stock solution of DH was formulated by precisely measuring 100 mg of DH and subsequently dissolving it in 10 mL of methanol to get a concentration of 10 mg/mL (K. Singh et al., 2021). A secondary stock solution was prepared by diluting the primary stock solution with methanol to obtain a concentration of 100 µg/mL (Guo et al., 2009).

Finding the wavelength maxima

From the secondary stock solution, 3.3 mL was taken, and a spectrum was recorded on a Shimadzu 1800 UV spectrophotometer from 200 to 400 nm to determine the drug-specific wavelength (λ_{max}) for DH.

Preparation of sample solutions for the calibration curve

From the secondary stock solution, calibration samples (ranging from 5 to 50 µg/mL) were prepared. Methanol was used as a diluent (Kiseleva et al., 2020).

Linearity

The calibration curve (analyte concentration vs absorbance) was prepared to check linearity (Moosavi & Ghassabian, 2018). The readings were recorded in triplicate (Munoz et al., 2006). The mean values ± standard deviation (SD) [n = 3] were recorded (Van Loco et al., 2002; Wenzl et al., 2016).

Limit of detection & quantification

The lowest amount of the analyte in the sample that can be detected, but it was not necessarily quantified as an exact value, i.e., limit of detection (Uhrovčík, 2014) and the lowest amount of analyte in the sample that can be quantified with an acceptable precision and accuracy under the stated conditions of the test, i.e., limit of quantification (Yuwono & Indrayanto, 2005) were determined from linear regression equations by using the following equations:

$$LoQ = 10 \times SD / S \quad \text{Eq.....1}$$

$$LoD = 3.3 \times SD / S \text{ (Thakur et al., 2022)} \quad \text{Eq.....2}$$

Accuracy & precision

Accuracy and precision were assessed at three distinct concentration levels (n = 3) (Betz et al., 2011), as per the following equations:

$$\text{Accuracy} = \frac{\text{Observed concentration} \times 100}{\text{True concentration}} \quad \text{Eq.....3}$$

$$\text{Precision (\% CV)} = SD/\text{Mean} \times 100 \quad \text{Eq.....4}$$

Robustness

Robustness was checked to ensure the method's stability with a small change in conditions. It was carried out by analyzing the percentage deviation (% SD) among different analysts and different λ_{max}, keeping the rest of the parameters constant (Ferreira et al., 2017).

Formulation of nanoparticles

The nanoparticles were prepared using ionotropic gelation method. First, a chitosan solution (0.2% w/v) was prepared in a 1% v/v glacial acetic acid solution by stirring for 16 hours, followed by sonication (Thangavelu et al., 2004). Secondly, the sodium tripolyphosphate (0.2% w/v) solution was prepared in DDW, followed by sonication. The drug was incorporated into a chitosan solution. Then, the NPs were formed by slowly adding the sodium tripolyphosphate solution to the chitosan solution using a 24-gauge syringe while continuously stirring (Garg et al., 2024).

Drug assay

For the DH assay, 1 mL of NPs was dissolved in 10 mL of 1% v/v acetic acid glacial. Then, a 10-factor dilution was prepared using methanol as a diluent. The resultant solution is then quantified by UV spectroscopy by using a plain NPs solution as a blank.

Estimation of the drug in the brain homogenate

The experiment was conducted on Wistar rats weighing 200 - 250 g (M. Singh et al., 2018). The animals were housed in polyacrylic cages and under standard housing conditions (room temperature 22 ± 5 °C and relative humidity of 60 - 65 %) with a 12-hour light/dark reverse cycle (Ali et al., 2020). The food in the form of dry pellets and water was made available ad libitum (Tripathy et al., 2020). Experimental protocols were approved by the Institutional Animal Ethics Committee of MRSPTU, Bathinda (Approval No. MRSPTU/IAEC/2023/11) and were conducted as

per the CPCSEA guidelines for animal experimentation and care.

The animals were sacrificed after two hours of administration (as per Table 1) by the cervical dislocation method. Then, the brains were isolated and transferred to PBS 7.4 (10 mL). The brains were removed and rinsed with 0.9 % ice-cold normal saline, followed by homogenization with ice-cold 0.1 mol PBS 7.4 10 times. The homogenates were centrifuged for 20 min at 10,640 g at 4 °C (Kaur et al., 2016). The supernatant (PBS 7.4) was separated and diluted 10 times with methanol. The mixture was then put on a bath shaker for 24 Hours, followed by centrifugation. The supernatant (methanol with DH) was removed, and the drug content was estimated by UV.

Table 1. Animal protocol

Sr. no.	Group	Treatment	Volume administered (µL)
1	1	DH solution, p.o.	40
2	2	DH solution, IN	40
3	3	DH NPs, IN	40

(n = 3) for each group

Results and Discussion.,

UV spectrum of DH

The UV spectrum of DH in methanol is shown in Fig. 2. The λ_{max} was found to be 271 nm.

Linearity

The calibration curves for DH were developed by plotting the mean absorbance compared to the corresponding concentrations (µg/mL), illustrated in Fig. 3 (A, B & C). Both analytes exhibited excellent linearity across their respective concentration ranges. For DH, the calibration curve demonstrated a strong linear correlation within the range of 5 – 50 µg/mL, as indicated by the regression equation and a correlation coefficient (R^2), confirming the high precision of the method (Ghassabian et al., 2014).

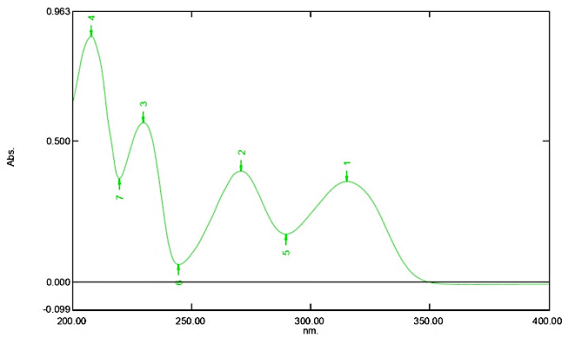
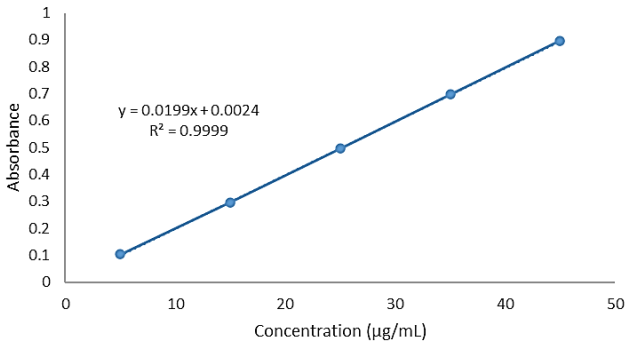
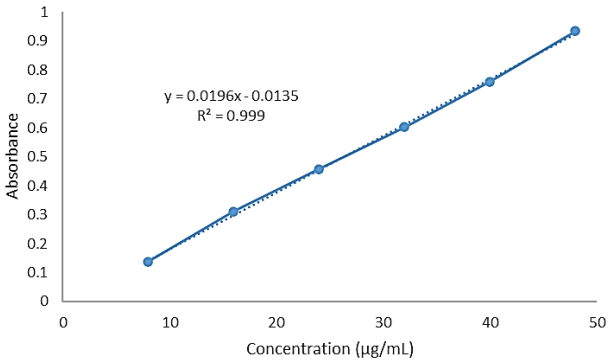


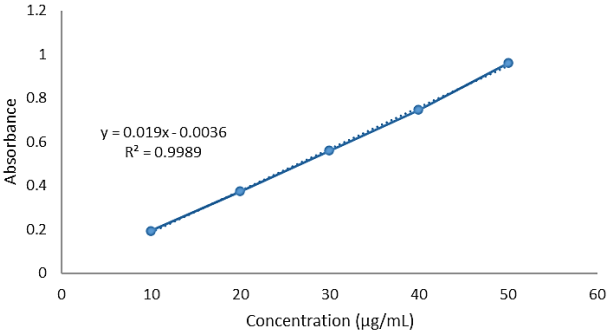
Fig. 2. UV spectrum of DH.



(A)



(B)



(C)

Fig. 2. Regression calibration curve of DH.

Limit of detection and quantification

The results of LoD & LoQ showed that the method is sensitive with LoD of 1.031 µg/mL and LoQ of 3.123 µg/mL (He et al., 2024).

Accuracy and precision

The accuracy results for DH ranged from 98.91 – 100.88 % (Table 1), indicating that the technique provides acceptable recovery values close to 100 %. Precision, expressed as % RSD, was found to be less than 1 %, demonstrating excellent repeatability and consistency of the method (Lakew et al., 2022). All values were well within the acceptable limits (≤ 1 % RSD), confirming that the method is both accurate and precise, as listed in Tables 2 & 3.

Table 1. Accuracy data of DH.

Conc. (µg/mL)	Cal. conc. (µg/mL)	% Accuracy
10	9.89	98.91
	9.98	99.81
	10.05	100.5
30	30.06	100.21
	30.26	100.88
	29.92	99.75
50	49.51	99.03
	49.99	99.99
	50.3	100.6

Table 2. Intraday precision data of DH.

Conc. (µg/mL)	Absorbance	% CV
10	0.1946	0.691
	0.1959	
	0.1932	
30	0.5712	0.766
	0.5628	
	0.5651	
50	0.9484	0.665
	0.9553	
	0.9611	

Table 3. Interday precision data of DH.

Conc. (µg/mL)	Absorbance	% CV
10	0.1914	0.888
	0.1898	
	0.1932	
30	0.5712	0.683
	0.5671	
	0.5749	
50	0.9476	0.813
	0.9545	
	0.9631	

Robustness

The observed concentrations for all variables were close to the true concentration (30 µg/mL), with % RSD values as low as (≤0.822 %), indicating good precision and minimal effect of variables on quantification (Lakew et al., 2022) (Table 4).

Drug assay

The assay for the DH was determined by using the developed UV method and found to be 99.24 %.

Table 4. Robustness study data of DH

Parameter	Absorbance	% RSD
λ_{max} (nm)		
270	0.5715	0.822
271	0.5667	
272	0.5761	
Analyst		
0.8	0.5653	0.771
1.0	0.567	
1.2	0.5736	

Estimation of the drug in the brain homogenate

The amount of DH in the supernatant was quantified by UV, and the current results showed that DH intranasally increased the amount of DH in the brain compared to oral administration within a 2-hour time frame (Fig. 3). While 2 hours following administration of DH solution shows significant availability of DH in the brain as compared to oral administration. However, the in solution of DH showed high blood clearance. On the contrary, oral administration of DH resulted in high blood levels, but did not reach quantifiable levels in the brain as determined by UV analysis (LoQ = 3.123 µg/mL).

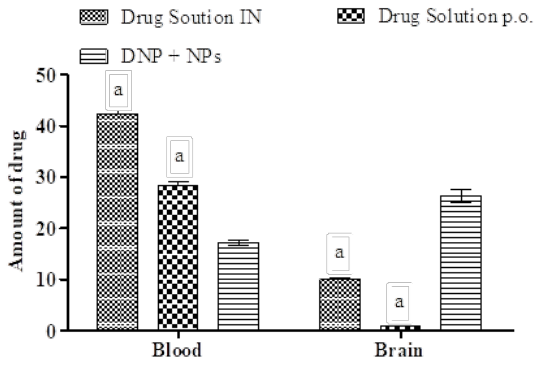


Fig. 3. Graph comparing the amount of drug in the blood and brain.

Conclusion

The UV spectrophotometric method for quantitative determination of DH was successfully designed & validated for its application in bulk drug analysis & pharmaceutical preparations (tablets, nanoparticles, etc.). High linearity was observed for various samples of DH in methanol. The study's results revealed that the % RSD was within the ICH Q2 (R1) guidelines' acceptance criteria (≤1 %), indicating that the method is accurate, consistent, robust, and simple. The *in-vivo* results showed the acceptability of developed method for the quantification of DH *in-vivo*, also. The technique was found to be suitable for commercial applications.

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